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Doctor Thesis

**Evaluation of the soundness of river water environment based
on bacterial community analysis using DNA microarrays**

(DNA マイクロアレイによる微生物群集解析に基づいた
河川環境の健全性の評価)

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2011

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Graduate School of Engineering
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PREFACE

This thesis contains my research work on the evaluation of soundness of river water environment performed at the Division of Sustainable Energy and Environmental Engineering, Osaka University, Osaka, Japan. This thesis is divided into six chapters describing general introduction, materials and methods, results of microarray analyses employing three types of microarrays which target bacterial genes related to various environmental functions, eubacteria and pathogenic bacteria, followed by general conclusions. Apart from these six chapters, there are two appendices: describing a case study performed to evaluate the quality of landfill leachates and list of DNA probes spotted onto the microarrays used in this study, and heat maps, showing the abundance of eubacteria in different river water samples.

Several parts of this thesis were previously published in different journals or presented in international conferences. A list of relevant references in chronological order is given below.

Oral/Poster presentation in international conferences

1. Detection of pathogens persistent in river water environment by an oligonucleotide DNA microarray. Upadhye R.R., Inoue D., Inaba M., Sei K. and Ike M. IWA 14th International Symposium on Health-Related Water Microbiology, Abstract, 193, 2007 (Sept. 9-15, Tokyo, Japan) [ID: P1052].
2. Development of DNA microarray for the evaluation of environmental functions. Sei K., Inaba M., Upadhye R.R., Inoue D. and Ike M. 2nd IWA-ASPIRE Conference and Exhibition, Proceedings, 2007 (full paper in USB memory) (Oct. 28-Nov. 1, Perth, Australia) [ID: 060]

Published articles

1. DNA microarray analysis of temporal and spatial variation of bacterial communities in Japanese rivers. R. Upadhye, D. Inoue, M. Inaba, K. Sei and M. Ike. *Japanese Journal of Water Treatment Biology* 109-120, Vol. 44 (no.2), 2008.
2. Microarray analysis of eubacterial community and bacterial pathogens in leachate from three different landfills of Japan. R. Upadhye, D. Inoue, T. Ishigaki, K. Sei and M.

Ike. *Environmental Engineering Research* (環境工学研究論文集) 195-202, Vol. 45, 2008.

3. Development of DNA microarray for the evaluation of environmental functions. K. Sei, M. Inaba, R. Upadhye, D. Inoue and M. Ike. *Water Science and Technology* 97-107, Vol. 59 (no.1), 2009.

4. Multiple detection of occurrence of bacterial pathogens in two rivers in the Kinki district of Japan with a DNA microarray. D. Inoue, M. Inaba, R. Upadhye, K. Sei, M. Ike. *Japanese Journal of Water Treatment Biology* 31-43, Vol. 45 (no.1), 2009.

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Rahul R. Upadhye
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ABBREVIATIONS AND SYMBOLS

BOD	– Biochemical Oxygen Demand
BSL	– Bio Safety Level
C12O	– Catechol 1,2-dioxygenase
C23O	– Catechol 2,3-dioxygenase
CGY	– Casitone-glycerol-yeast extract agar
CHB	– Culturable Heterotrophic Bacteria
DO	– Dissolved Oxygen
DOC	– Dissolved Organic Carbon
DNA	– Deoxyribonucleic Acid
EPI	– Environmental Performance Index
H'	– Shannon-Weaver diversity index
K1	– Sampling station 1 located in upstream of Kita River (Table 2-1)
K2	– Sampling station 2 located in downstream of Kita River (Table 2-1)
MPN	– Most Probable Number (for MPN-PCR)
PCA	– Principal Component Analysis
PCR	– Polymerase Chain Reaction
PHB	– Poly(3-hydroxybutyrate)
RNA	– Ribonucleic Acid
RT-PCR	– Reverse Transcription-Polymerase Chain Reaction
RSI	– Relative Signal Intensity
SSC	– Sodium Dodecyl Sulfate
T-N	– Total Nitrogen
T-P	– Total Phosphorus
Y1	– Sampling station 1 located in upstream of Yodo River (Table 2-1)
Y2	– Sampling station 2 located in midstream of Yodo River (Table 2-1)
Y3	– Sampling station 3 located in midstream of Yodo River (Table 2-1)
Y4	– Sampling station 4 located in downstream of Yodo River (Table 2-1)
WWTP	– Wastewater Treatment Plant

CHAPTER 1

General Introduction

1.1 Necessity of biological indicators for evaluating environmental soundness

Freshwater supports the livelihood of almost every terrestrial habitat. However, the freshwater resources are limited and can only be replenished by natural ways. Nevertheless, population growth and industrial development have led to the discharge of a variety of pollutants into the aquatic environment, which can result in adverse effects on the ecosystem such as reduction/loss of biodiversity, biological purification function and elemental/material cycling functions. Therefore, careful monitoring and management of the soundness of aquatic environment is quite important to conserve the aquatic biodiversity and functions from the disruption by anthropogenic pollutions.

Evaluation of aquatic environmental quality has been carried out, mainly based on physicochemical water quality indicators. Physiochemical indicators are used to measure various minerals, dissolved salts or suspended impurities present in water. Although these indicators are useful for understanding the status of environmental pollution, they cannot predict the influence of the pollution on the natural ecosystem.

Therefore, various countries and international organizations have recently tried to establish novel, comprehensive evaluating system for aquatic environment, focusing on the ecosystem and water circulation system. Among these trials, biological indicators have received a lot of attention. Table 1-1 shows various biological indicators suggested in Japan, USA and the European Union. These biological indicators include macrophytes, invertebrates and vertebrates. There has been no evaluating system focusing on microorganisms. Microorganisms play a vital role in the cycling of elements/materials and breaking down organic matters or other pollutants. In addition, several microorganisms are pathogenic and can pose health hazards to plants, animals and humans, which lead to serious ecological and economic damage. Therefore, microbial indicators would be helpful to understand/assess the soundness of whole ecosystem. Although several studies have very recently evaluated bacterial species as indicators for predicting water quality (Savichtcheva et al., 2006; Field et al., 2007; Pronk et al., 2007), more detailed and systematic study is needed to establish microbial/bacterial indicators for evaluating the soundness of aquatic environment.

Table 1-1 Biological indicators for evaluating aquatic environments in various countries

Country	Indicator organism	Measured parameters	Reference
Japan	Benthic invertebrates	Species richness, abundance, diversity	Ministry of Environment and Ministry of Land, Infrastructure, Transport, and Tourism Japan
	Fish	Species richness and composition, number of indicator species, abundance, condition	
	Macrophytes	Species richness and composition	
	Amphibia, reptile and mammal	Species richness	
U.S.A	Fish	Species richness and composition, number of indicator species, abundance, condition	U.S. Environmental Protection Agency
	Invertebrates	Composition, abundance, diversity, presence of sensitive taxa	
	Periphyton	Composition, abundance, diversity, presence of sensitive taxa	
	Macrophytes	Composition, abundance	
European Union	Benthic invertebrates	Composition, abundance, diversity, presence of sensitive taxa	European Environmental Agency
	Macrophytes	Composition and abundance, presence of sensitive taxa	
	Benthic algae	Composition and abundance, presence of sensitive taxa	
	Fish	Composition and abundance, sensitive species diversity, age structure	
	Phytoplankton	Composition, abundance and plankton booms, presence of sensitive taxa	

1.2 Microbial indicators targeted in this study

To establish microbial indicators for assessing the soundness of aquatic environment, this study focused on functional bacteria, total bacterial community diversity (composition) and pathogenic bacteria.

1.2.1 Functional bacteria The material/elemental cycles in nature (such as carbon, sulfur, nitrogen etc.) are greatly dependent on the role of microorganisms. Microorganisms transform/degrade pollutants to acquire energy and compounds to support their lifecycles. This in turn reduces the pollutant burden from the environment. Several studies have revealed that many bacteria with different characteristics perform a similar task of pollutant decomposition and coexist in harmony in an inter-dependent community (Wu et al., 2001; Taroncher-Oldenburg et al., 2003; Wilmes et al., 2006). Such bacterial communities are referred as functional bacteria in this thesis.

The material/elemental cycling functions of microorganisms are encoded on specific genes which transferred within different microorganisms during the process of evolution. Environmental pollution greatly affects the variety and abundance of microbial species, and consequently causes the shift of gene matrices. Thus, the variation in occurrence of those

genes which are useful in reducing environmental pollutant can be helpful to evaluate the soundness of the ecosystem

1.2.2 Total bacterial community Several researches have targeted the freshwater bacteria to understand the occurrence and fate of bacterial communities (Sigua et al., 2000; Traister et al., 2006; Winter et al., 2007). It has been known that total bacterial community and specific bacterial populations varies naturally and anthropologically (Wakelin et al., 2008). In particular, pollutants (nutrients, xenobiotics, etc.) influx from various anthropological activities into the river environment leads to a shift in the structure of the river's microbial community (Crump et al., 1999; Rubin et al., 2007).

1.2.3 Bacterial pathogens Health risks are often caused by pathogens which may be dormant and present in lower frequency in nature. Emerging and re-emerging diseases pose newer threats to mankind. Over the last decades, pathogen risks have been assessed based on several fecal indicators like (fecal) coliforms. However, these indicators are not necessarily useful to predict the risks from pathogens that cause emerging and re-emerging diseases. In addition, recent several systematic studies have revealed that the fate of several important pathogens present in the aquatic environment does not show correlation with indicator bacteria currently used as the hygienic indicator (Lemarchand et al., 2003; Hörman et al., 2004; Maynard et al., 2005; Dorner et al., 2007; Savichtcheva et al., 2007; Walters et al., 2007). Therefore, it is urgent to fully elucidate the kinds and numbers of pathogens present in surface waters to access the hygienic safety of aquatic environment.

1.3 DNA microarray for comprehensive microbial monitoring in aquatic environment

As the vast majority of environmental bacteria are unculturable (Amann et al., 1995), the use of culture-independent molecular techniques is helpful for improving our understanding of their fate in the environment. Several techniques based on polymerase chain reaction (PCR) such as PCR–denaturing/temperature gradient gel electrophoresis (Riesner et al., 1989; Muyzer et al., 1993), terminal restriction fragment length polymorphism (Liu et al., 1997) and DNA microarray analysis (Guschin et al., 1997) have been applied in the microbial ecology. Among them, DNA microarray technology is a powerful tool as it can simultaneously detect tens or hundreds of thousands of genes from a single sample (Gentry et al., 2006), thus enabling the detailed analysis of complex microbial communities

in the environment. In DNA microarray analysis, the working principle is based on the fact that DNA probes of the known bacterial species are mounted on special glass slides. The DNA extracted from a sample is amplified and fluorescently labeled through a PCR or reverse transcription-PCR, and the fluorescently labeled DNA is then hybridized onto the slide. After hybridization the glass slide is scanned for analyzing the signal intensities for each spot to determine the relative abundance of target species.

Since it was first reported by Schena et al. (1995), microarray analysis has become a huge success in every field of microbiology. Very recently, this method has been extensively applied to study microbial community in natural ecosystem such as river waters (DeSantis et al., 2005; Loy et al., 2005; Peplies et al., 2006; Winter et al., 2007). Some specific applications of DNA microarrays have been reported such as a DNA microarray to target the 1033 functional genes related to the nitrogen cycle and sulfur reduction in a quantitative way (Tiquia et al., 2004). Similarly, detection of pathogens by DNA microarrays have been reported in wastewater (Lee et al., 2008), river water (Maynard et al., 2005) and wastewater treatment plants (WWTPs) (Savichtcheva et al., 2007).

1.4 Objective of this thesis

As described above, microorganisms can be useful indicators for assessing and conserving the soundness of aquatic environment. However, to establish microbial indicators, normal variation of target microorganisms depending on the season and their shift in accordance with anthropogenic environmental burden should be understood in detail. Therefore, in this study, total bacterial community, specific functional bacterial genes and pathogenic bacteria in surface water samples collected seasonally from two rivers, Yodo River and Kita River, in Japan were analyzed using DNA microarray technique to obtain the basic knowledge (their distribution and variations in the natural aquatic environment) for evaluating the soundness of the aquatic environment.

Chapter 2 provides the details on riverine samples analyzed in this study and methods for DNA extraction, amplification of target genes and DNA microarray analysis.

In chapter 3, the seasonal occurrence and distribution of the functional bacterial genes in river water were analyzed using a DNA microarray that were mounted a total of 85 bacterial genes related to carbon cycle, chemical degradation, nitrogen cycle, sulfur cycle, metal metabolism, and energy flow. Based on the results, the genes were grouped

according to detection frequency to pick up the candidate indicators for assessing the environmental soundness.

In chapter 4, the bacterial species present in the river water are analyzed, and the impact of various factors (effect of water hold-up in a dam, effluents from WWTPs and seasonal variations) on the composition of bacterial community in river water were discussed in detail.

In chapter 5, the occurrence of bacterial pathogens in river water was analyzed using a DNA microarray targeting 1012 species/groups of bacterial pathogens all including Biosafety level (BSL) 2 and 3 pathogens and other opportunistic pathogens. In addition, the results from DNA microarray were compared with traditional hygienic indicators (total coliform count).

In chapter 6, the results obtained from DNA microarray analyses targeting total bacterial community, functional bacteria and bacterial pathogens were summarized and an overall understanding of the riverine microbial community was presented. Finally, an exclusive scheme for evaluating the soundness of river water environment based on microbial indicators was proposed.

CHAPTER 2

Materials and Methods

2.1 Sampling stations

River water samples analyzed in this study were collected from two rivers, Yodo River and Kita River, flowing in the Kinki district of Japan as shown in Fig. 2-1. Yodo River is the largest river within the Kinki district, with a mainstream length of 75 km and catchment area of 8240 km². Yodo River has 14 dams to partly control the water flow and divert its waters for municipal and agricultural activities. In addition, Yodo River receives large amount of wastewaters from industrial and agricultural activities and effluents from wastewater treatment plants (WWTPs). Thus, it represents a typical urban river characteristic. On the other hand, Kita River is a relatively small river with mainstream length of 30 km and catchment area of 211 km². Kita River has no dams along its stream and receives very less anthropologically polluted influents. As a result, the river exhibits a best water quality based on the biochemical oxygen demand (BOD) level.

Four sampling stations from Yodo River (one upstream: Y1, two midstream: Y2, Y3, and one downstream: Y4) and two sampling stations from Kita River (one upstream: K1 and one downstream: K2) were chosen to represent the overall spatial variation within the rivers. Further information on the sampling stations is provided in Table 2-1. In the Yodo River, station 1 was an upstream site near the mouth of the Lake Biwa. Stations 2 and 3 are the midstream sites, and several large WWTPs are located between these two stations. Station 4 is a downstream site which may receive urban wastewaters from Osaka Prefecture. In

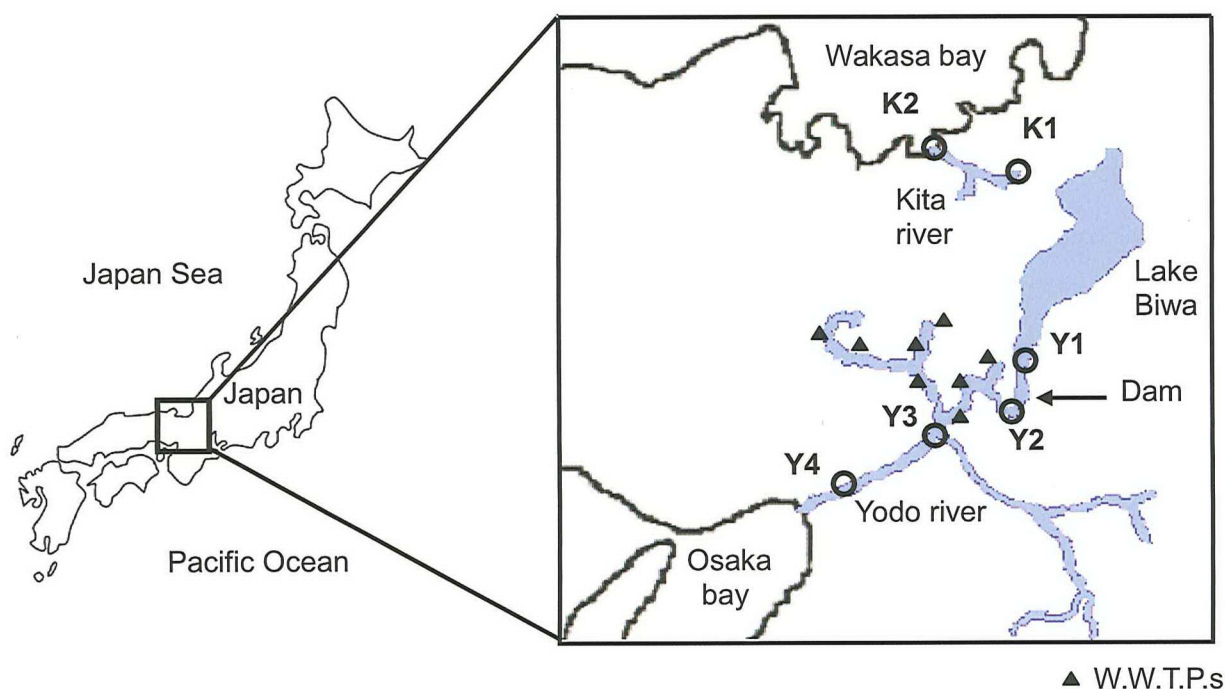


Fig. 2-1 Location of sampling stations

Table 2-1 Description of sampling stations

River	Sampling station	Location	Description
Yodo	Y1	Seta, Otsu, Shiga	Outflow of Lake Biwa
	Y2	Ujikanaido, Uji, Kyoto	Outflow of Amagase Dam
	Y3	Yawata, Kyoto	Downflow of wastewater treatment plants
	Y4	Ohniwa-cho, Moriguchi, Osaka	Urban-basin of Osaka
Kita	K1	Kumakawa, Wakasa, Fukui	Mountain-ringed region of Fukui
	K2	Nishitsubashi, Obama, Fukui	Brackish-water region

Kita River, station 1 is an upstream site near the origin of river surrounded by a mountainous region, whereas station 2 is a downstream site close to the Wakasa Bay and experiences a brackish environment caused due to the backflow of marine waters.

2.2 River water sampling and water quality measurements

2.2.1 River water samples

River water samples were collected in October 2005 (autumn), August 2006 (summer), January (winter) and May (spring) 2007. The samples were collected during fine weather conditions and sampling time varied from early morning till noon on the same day for every season. Samples were collected from 30- to 50-cm below the surface of river and transported on ice to the laboratory. Water quality analysis was done on the same day and DNA extraction for DNA microarray analysis was done within 12 hours of sample collection.

2.2.2 Water quality measurements

Water temperature, electrical conductivity, pH, and dissolved oxygen (DO) were recorded at the sampling site. Concentration of dissolved organic carbon (DOC), total nitrogen (T-N), total phosphorus (T-P), culturable heterotrophic bacteria (CHB), total coliforms and eubacterial 16S rDNA were analyzed in the laboratory. DOC was analyzed with a total organic carbon analyzer (TOC-5000A; Shimadzu, Kyoto, Japan). Concentrations of T-N and T-P were measured by the alkaline potassium peroxodisulfate decomposition – ultraviolet absorptiometry method and the potassium peroxodisulfate decomposition – molybdenum blue (ascorbic acid) absorptiometry method, respectively (Clesceri et al., 1998). Numbers of CHB were determined by plating 1/10 diluted water

samples on a casitone-glycerol-yeast extract agar (CGY) medium. The CGY medium was composed of casitone 5 g/L, glycerol 5 g/L, yeast extract 1 g/L and agar 13 g/L with final pH adjusted to 7.2 by NaOH, sterilized at 121°C for 15 min. The medium was inoculated with one drop of 1/10 diluted water sample and incubated at 22°C for 6 days (Pike et al., 1972). The CHB count was calculated as sum of the colonies formed after 6 days of incubation. Eubacterial 16S rDNA number was enumerated by most-probable-number (MPN)-PCR (Picard et al., 1992). DNA sample was serially diluted by a factor of 3 and subsequently PCR amplified. The number of amplifiable target was determined by MPN technique. PCR primers used for MPN-PCR were EUBf933 5'- GCA CAA GCG GTG GAG CAT GTG G -3' and EUBr1387 5'- GCC CGG GAA CGT ATT CAC CG -3' primer set (Iwamoto et al., 2000). Total coliforms were quantified by the MPN method using a slightly modified standard coliform medium (lactose 5 g/L, bonito extract 3 g/L, peptone 10 g/L, pH 7.0).

2.3 DNA microarrays

Three types of microarrays targeting: (1) functional bacterial genes, (2) eubacteria and (3) pathogens, were used in this study. Details on each of these arrays are described below and the probes used in making them are listed in *Appendix B*.

2.3.1 Functional bacterial gene microarray

For construction of functional bacterial gene microarray, 33 bacterial functions were selected and corresponding genes related to organic pollution degradation (5 genes), carbon cycle (9 genes, including 3 genes relevant to methane cycle and 9 genes related to energy flow), nitrogen cycle (11 genes), sulfur cycle (2 genes) and metal metabolism (6 genes) were used to evaluate the environmental functions. From these genes, totally 85 probes were designed (Table 2-2), and spotted on the DNA microarray. The size of all probes was set to 60 nucleotide bases. Construction of probes and spotting them on DNA microarray slides was entrusted to TakaraBio Inc., Japan. The probes and PCR primers which had specificity only for the target gene were selected from previously published reports. In case if such information was not available, then probes were designed for genes encoding the functions of interests (Table 2-2) by choosing specific sequences enough to detect only target gene and avoiding any false-positive hybridization, using blast analysis. It was reported (Kane et. al.), that about 50-mer oligonucleotide probe were specific enough for targeting the conserved region and it was shown that to avoid cross-hybridization, the

Table 2-2 Oligonucleotide probes designed for detecting functional bacterial genes

Target	Function	Sequence (5' – 3')	Accession No. used for design
C12O DNA	Catechol oxidation (<i>ortho</i>)	CACCTATTCGTACTTCGATTCGACCCAGTCCGAGTTCAACCTGCGCCGGCGTATCATCAC	D37783
		GATGGGAAGTACAGCGGTTTCCATGACGACATCCCGACTGATTTTATCGAGGGAAGCTC	D16356
		TGCACTTCAAGGTGCGCAAGGACGGTTTCGAACCGTTGACCACGCAATACTACTTCTGAAG	M16964
		TACTCCTTCTTTGATAAGAGCCAAACCCCTTACAATTTGCGCCGCACCATTATCGCTGAC	M57500
		CTCAATTTATTGGGTGACATGGTAACCGCCCTTCGCATGTTCACTACTTTGTCTCTGCG	Z36909
		CGAATGGAACCTCCGCGGCTCCATCATTGCCGATGACCAGGGCAACTTCCAGATCAACAC	M94318
C23O DNA	Catechol oxidation (<i>meta</i>)	GTCCTAACCTGGATGAAACTCTGAATTTGTTCCGTGATGTGCTCGGTTTTGACCTCGCTG	AB008831
		AGAGCCGAATCAAGAAAAAGGGAAGCTTCATCATCTTTGCTATTGGTATGGCATCCCGCA	X67860
		GCTAGCTCTAACTTGCGGGATTTTATGAAAGCTAAAGCTGAGCGATGCGAGTGAACGTCG	D37828
		GCCTAGATTTCTATTTAAGTGAGCAAGGTTTGGTTGGACCCGATGCATCCACTCAAGCGG	U01826
		TCGACTAGATCACCTCAACATCACAGCGAAGGACGTTGACGACGCCCGTAACTGGTATTC	X69504
		CCATGGGATCACACGCGGGCAGACGATCTACTTCTTCGACCCCTCGGGCAACCGGAACGA	U23375
		GACTCACGGCAAGACCATCTACTTCTTCGACCCTTCGGGCAACCGCAACGAGGTGTTCTG	X80765
		ACATCGTGCGCCAAAGAGCATCGCTAGAGAGAACAATGCAAACCAATAACAAGAGTTCGT	S78585
		CACATCGTGGTGATGGTTCGACGACTACGACGAGACGATGCGCTTTTACCGCGAGGTACTC	L77225
<i>alkB</i>	Alkane hydroxylation (Group I)	CACCGTGATGTTGCTACACCGATGGACCCTGCGACCTCACGTATGGGAGAAAATATATAT	AJ233397
		GTGCCCCAGGAACACTCCTTATGGGCAATGTAATTTATAAGATGGCAATGCGCGAGAGAG	U40233
<i>alkM</i>	Alkane hydroxylation (Group II)	CAAGGCTGGGGAATGAGTGCTGCTTTTCATGCTTCTATGGTGGGAATTTTGGTAAAAGT	AJ009585
		TATATCGAGCATTATGGTCTAAAACGCCAGAAAAGAGCGGATGGCAATTACGAACGTACC	AJ002316
		CCATGTTTGCGAAATTTGGTGCCCGTTTCGATTCCGTTTCAAGTTACTCAAGCTGCGTATG	AJ009582
		GATAATGAGTTATTGCAAGGCTGGACAATGACCGCAGCATTCCATTCTGTCTATGGTCGGG	AJ009584
<i>alkB</i> & <i>B1</i>	Alkane hydroxylation (Group III)	TATCTGCTCATTACAGGCGGTGGTCGGGTTTTCCCTGCTCGAGATCGTCAACTACATGGAG	AJ009587
		GTTTCGGCCAGTCGGTCTACCAGTTCCTGCCGCATGCCTACAAGTACAACCTTCCTCAGCG	AJ009580
		GTGATCCAGGGGATCTACGGCTTCTCTCTGCTGGAGGTGGTCAACTACGTGAGCACTAC	AJ009579
		GTGATCCAGGCCCTTCATCGGGTTCTCGTTACTCGAAGCGATCAACTACCTCGAGCACTAC	AJ009586
		GTAATTCAGGCGGTGTACGGCGCGTCTGCTGCTCGAAGTCGTGAACCTACGTGAGCACTAC	AJ293344
		GAGGACCCGGCGAGTTACGGTTCGGAGAAAGCTTCTGGACGTTTCTGCCCCGCGAGTGTC	AJ401611
<i>mmoX</i>	Methane oxidation	GGTCTACGAGGATTGGGGTGAATCTGGATCGGCCGTCTCGGCAAATATGGCGTCGAGAG	X55394
<i>pmoA</i>	Methane oxidation	GATCTGATCGGCCTTCACTACGTCCGCACGTGATGCCGGAATATATCCGCATGGTCGAG	U31651
		AACTTCTGGGGTTGGACTTACTTCCCAGTTAACTTTGCTTCCCATCTAACCTGCTGCCA	U31653
		AACTTCTGGGGATGGACTTACTTCCCAGTTAACTTCGTGTTCCCATCACAATTCGCTCCA	U31654

Table 2-2 –Continued

<i>mcrA</i>	Methanogenesis	TGGCTAGGATCTTACATGTCAGGTGGAGTAGGATTCACACAATATGCTACAGCATCGTAT	J03375
		GACGACTTCACCTACTTCGGTAAGGAGTACGTGGAGGACAAATATGGACTCTGTGAGGCA	U10036
		TACATGTCTGGTGGCGTAGGTTTCACACAATACGCTACAGCAGCATACACCGACGATATC	M16893
<i>amoA</i>	Ammonia oxidation	CGTACAGGTACACCCGAGTATGTTTCGTATATTGAGCAAGGTTCACTGCGTACCTTTGGT	L08050
<i>napA</i>	Nitrate reduction	TATGCCTTCATGCGCACCTTCGGCATGGACGAGCCGATGGGCTGCTATGACGATTTGAG	Z36773
<i>narG</i>	Nitrate reduction	GGACCTACTTCCACGCAGGTATCCTCGACAACACTGATCCGGACACCGCAGAGAAGATAC	AJ277440
		AAATGGCATCGATCTGTATAAAGAAGCTGAAAAAGCAGGGGCAGCAACACCTGAAGACGT	X91819
		CAGGAATTGCGCCAGGGCGACCTCTACGACCTTCAGGAACGCTATGTGCTGCTGTTTCGAC	Z26255
		CCATGAATTCTTCCTCAAGTATCTCTAGGCACGAAGAATGGCCTGTTTCGCCGAGGAAGA	AB096694
		CGGCAAAGGTCATGAATATTTTCTGAAGCACTTGCTCGGCACGACTAACGGACTGATGAA	AB087407
		CTGGCGTTCTAACCTGCTCGGTTCTTCGGGTAAAGGTCATGAGTTTATGCTCAAGTACCT	X16181
<i>nirK</i>	Dissimilatory nitrite reduction	CTCACCGCCAAGGTGCGCGAGACCGTGCTGCTGATCCACTCGCAGGCCAATCGCGACACC	AF051831
		TTACTACACGTTCCAGCAGCCCGGTATCTATGCGTATGTAAACCACAATCTGATCGAGGC	M97294
		CTATGACACCGTCTATTACATCGGCGAGAGCGACCACTACATCCCGAAGGACGAGGACGG	U62291
		TATATCCCCAAGGACAAGGACGGCCACTACAAGGACTACCCGGACCTGGCGTCCAGTTAC	Z21945
		CTGACGTACGACAAGATCTACTATGTGCGCGAGCAGGACTTCTACGTGCCGAAGGACGAG	Z48635
<i>nirS</i>	Dissimilatory nitrite reduction	GAACGTCAAGGAAACGGGCAAGATCCTGCTGGTGGACTATACCGACCTCGACAACCTCAA	AJ401462
		CAGATCATGCTGGTCGACTACACCGACATCAAGAACCTCAAGACCACCACCATCGAATCC	M80653
		GAGTTCATCGTCAACGTGAAGGAGACCGGCAAGGTCTGCTGGTCAACTACAAGGATATC	X16452
		CAAGATCCTGATGGTCAACTACTCGGACTTGTTCCAACCTGAAGACCACCACCATCGATTC	X91394
<i>qnorB</i>	Dissimilatory nitric oxide reduction	TATGAGTATGTCGACCTGGGCCGGCTGTGGCAGATCGGCAAGTTCGCCGGCATCCTGATC	AF002661
<i>cnorB</i>	Dissimilatory nitric oxide reduction	GATCTTCTCGAGCTTCGAGATCGTGCCCTTCTTCGCCATGATGTCATTGCGCTTCGTCAT	U28078
<i>nosZ</i>	Nitrous oxide reduction	GTCCACATGTCCTTTACCGAGGGCAAGTATGACGGCCGCTTCCTGTTTCATGAACGACAAG	AJ440508
		GGAAGGCACCTATGACGGGCGCTATCTCTACGCCAACGACAAGGCCAATACGCGTGTCTG	AJ440509
<i>nifH</i>	Nitrogen fixation	CGATGCCTATTTCGCGAAAAACAAGGCTCAGGAAATCTACATCGTCATGTCCGGTGAGATGA	V01215
<i>nrfA</i>	Nitrite reduction	GGAATATGAAACCTGGACAGCGGGCATTACGGTAAAAACAACGTGACCTGTATCGACTG	X72298
16S rDNA	Anammox	TGCATTGATAACCTGCCTTTGAGATGGGAATAACTGCGTTTCGAGCAATCGGAACCTACCG	AJ250882
<i>dsrAB</i>	Dissimilatory (bi)sulfate reduction	AAAGATGGCATTTCATATCTTCCGGGTACAATCCCGAAAAGCCGATGGCAAACCGTATCAC	U16723
<i>soxB</i>	Thiosulfate oxidation	ACTGCCTTCAAACTATCGGTCTGGAGTTCGAGAGAGGTGAGTGAATTCCGAGTGTAGA	Y16933
<i>ferA</i>	Iron reduction	CAACAGCATCAATGATCAGTACGACCTCTGCACCAGCTGCCACACCGTCAACACCATGAC	AY033095
<i>mofA</i>	Manganese oxidation	GTCTACGAGACGGTGCAGGATCCGAACCAGATGAACGGTTTCAACTCGGTTCGCCGCTGG	Z25774

Table 2-2 –Continued

<i>merA</i>	Mercuric reduction	CAGCAAGGTCACGGCCCTGGCGCGCAATACCTTGTTCTTCCGTGAAGACCCGGCCATCGG	AJ418049
		GTTGTGCACGGTGAGGCGCGCTTCAAGGACGACCAGAGCCTTACTGTCCGTTTGAACGAG	AJ418052
		AGTGCGGCCCGTGCCGCCGATTCCCGGACTGCAAGATACCCCTTTTGGAAGTCCGAAAAAG	AJ418056
		CATGGAAGGCATCCTGGAAGAGCTATGGCCATCACCATTTGCCCCGGCTACGCCCGTTTC	AJ418057
<i>tpm</i>	Selenium methylation	TTGAAGGCCGATTGGAGCGTATGGATGAGCACGTTTATGTGTTGGAACGTGTGTAAGTC	L49178
<i>cadA</i>	Cadmium resistance	ACTACACTCCGATCATTATGGTTATTGCAGCCTTGGTTGCAGTCGTTCCACCCCTATTCT	J04551
<i>pcoR</i>	Copper resistance	GATCCGTTCCGGGAAGAAGATCCATCTCACCGGTAAAGAATACGTTCTGCTTGAGTTGCT	X83541
<i>phaZ</i>	PHB depolymerization	TCTATCGCAACGGCAACAAGGCCAACGCGCTCCCGGTCTCTGCCACCAGCTATGTCGATG	U16275
		CCGTATCGGCGACGACGAAATCGGCCTTCACCTGTACTGCCACCACGGCCAGCAACTACG	J04223
		GTCACGGCCACCACCTACACCGACACAGGCCTGACCACCGGTACGGCCTACTCCTACACC	U58990
		CTATACCGACACCGGCCTGATTGCTGGTACCACCTACAGCTACACCGTAACCGAGATCGA	D25315
<i>apr</i>	Hydrolysis of peptidic compound	GGCAACGACACCCTGGACTTCTCCGGTTTCACCCAGAACCAGAAAGATCAACCTCAATGAG	AB013895
<i>npr</i>	Hydrolysis of peptidic compound	AACAATGCATTCTGGAACGGATCACAGATGGTATACGGAGATGGTGATGGTGTAAACGTTT	M83910
<i>sub</i>	Hydrolysis of peptidic compound	TACTGGTTCTACAGCGCTGAAAACAGTAGTTGATAAAGCGGTTTCCAGCGGTATCGTCGT	S51909_1
<i>sub</i>	Hydrolysis of peptidic compound	TTCAACAGGAAGCGGCCAATATAGCTGGATTATTAACGGCATTGAGTGGGCCATTTCCAA	S51909_2
<i>chiA</i>	Chitin depolymerization	GTGAAAGAGTTCTGCGACCTGGAAGTTCTTCGATGGCGTGGATATCGACTGGGAGTTC	AY040610
		CAGCAATTGGCGTTGGTTACGACAAAATTGAAGACGTTGATTACGCAGATGCTGTGCAGT	AF193498
		ATAACGGTATCCAACCTTCTTCTTGCTCAAGGCGTTCTGCGAACAAGCTAGTCCTTGGA	AB004935
		CTGAAACAGGCCGTACTTACGAACTGACATCGGCAGTAGGTGTAGGCTACGACAAGATTG	AF193500
		CATATGGGTTTGATGGCGTGGATTTAGACTGGGAATATCCGGGCGTTGAAACGATTCCTG	AY129671
		TAAACCGCAGATCGACGTTGAGGAAGAAATCCGCAGTCGCGTAGATTTCTGAAACAGTA	AB110082
		CTCCAACAGATGACATTACGCCTACACCTACACCTACTCTTGAACCAACGCCTGAACCAA	Z68924
		GGGCATTGATGGGCTGGTTGCAATGCAGATAATGATGGGAAAATGGTTGTTGAGAATGGT	BA000028

non-target region should be less than 75% similar to the sequence of probes. Thus following these guidelines designing of DNA microarray for evaluating functions of environment was accomplished.

2.3.2 Eubacterial microarray

Microarrays for detecting eubacteria were designed by Prof. Takayuki Ezaki, Department of Microbiology Regeneration and Advanced Medical Science, Graduate School of Medicine, Gifu University, and distributed from AMR Inc. Gifu. These microarrays were purchased as commercial products. Oligonucleotide probes for the 16S rRNA gene of the 1016 eubacterial species commonly present in the environment were spotted on the microarray slides. The spotted probe number at the phylum level was as follows: *Actinobacteria*, 152; *Bacteroidetes*, 48; *Cyanobacteria*, 39; *Firmicutes*, 226; *Proteobacteria*, 455 (*alpha* subclass, 123; *beta* subclass, 78; *gamma* subclass, 133; *delta* subclass, 108; *epsilon* subclass, 13); Others, 96 (*Acidobacteria*, 3; *Aquificae*, 5; *Chlamydia*, 8; *Chlorobi*, 4; *Chloroflexi*, 9; *Chrysiogenetes*, 1; *Deferribacteres*, 5; *Deinococcus-Thermus*, 3; *Dictyoglomi*, 1; *Fibrobacteres*, 1; *Fusobacteria*, 15; *Nitrospira*, 10; *Placentomycetes*, 4; *Spirochaetes*, 17; *Spiral bacteria*, 1; *Thermodesulfobacteria*, 1; *Thermomicrobia*, 1; *Thermotogae*, 5; *Verrucomicrobia*, 2). The balance of probe numbers in each phylum, e.g. higher probe numbers for *Proteobacteria*, *Firmicutes* and *Actinobacteria* seems to resemble the general bacterial population in aquatic samples reported previously (Crump et al., 1999; Brummer et al., 2000; Sekiguchi et al., 2002; Allgaier et al., 2006; Peplies et al., 2006; Winter et al., 2007). Thus, the microarray used here would be reasonable for investigating the riverine eubacterial community. Refer to *Appendix B* for list of probes.

2.3.3 Pathogen microarray

Microarrays for detecting bacterial pathogens were designed by Prof. Takayuki Ezaki, Department of Microbiology Regeneration and Advanced Medical Science, Graduate School of Medicine, Gifu University, and distributed from AMR Inc. Gifu. These microarrays were purchased as commercial products. Oligonucleotide probes for the 16S rRNA genes of 1012 bacterial pathogens infectious to humans, animals, plants, fish and shellfish are mounted on this array. The target pathogens included all biosafety level 2 and level 3 pathogens in the classification of Japanese Society of Bacteriology (2007) in addition to several opportunistic pathogens. The array covers both fecal and non-fecal pathogens.

This array was designed to be most comprehensive, accurate and rapid testing of the causative pathogens of infectious diseases, including emerging/reemerging diseases which are increasing in number and becoming more globalized (Yamada et al., 2007). Please refer to *Appendix B* for list of probes.

2.4 DNA microarray analysis

2.4.1 DNA extraction

DNA was extracted from 10ml of water sample by filtering it through a pore size 0.22µm Whatman GF/B filter (Whatman, Maidstone, Kent, UK). The filter-paper was then suspended in 500 µl of extraction buffer (50 mM Tris-HCl, 20 mM EDTA, 100 mM NaCl, pH 8.0) in a tube. Cells were then lysed by adding 50 µl of 2 mg/ml proteinase K and incubated at 37°C for 2 hours. After cell lysis, samples were extracted with equal volumes of phenol-chloroform twice. The aqueous phase was then collected and DNA was precipitated by adding 1/10th volume of 0.3 M sodium acetate and 2 volumes of chilled ethanol and precipitated by centrifugation at 10,000 × *g* for 10 min, followed by vacuum drying and stored at -20 °C until further use.

2.4.2 PCR amplification

All PCR amplifications were carried out with a Mastercycler Standard (Eppendorf, Tokyo, Japan).

For analyses with eubacterial and pathogen arrays, the conserved region of eubacterial 16S rDNA (ca. 510 base pairs) was amplified by PCR using an 8UA (5'-AGA GTT TGA TCM TGG CTC AG-3') and 519B (5'-GTA TTA CCG CGG CKG CTG-3') primer set. The 5'-end of the reverse primer was labeled with Cy3; thus PCR products were fluorescently labeled at the same time. PCR amplification was performed in a 20-µl PCR mixture containing 1× *Ex Taq* buffer (TaKaRa, Shiga, Japan), 200 µM dNTPs (TaKaRa), 20 pmol of forward and reverse primers, 0.6 U of *Taq* DNA polymerase (TaKaRa), and 4 µl of DNA template prepared in section 2.4.1. The thermal profile for PCR amplification included an initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 30 s; annealing at 55°C for 30 s, and extension at 72°C for 30 s; and a final extension step at 72°C for 7 min. Amplified products were purified by ethanol precipitation to get a final amount of 35ng of

labeled DNA.

The DNA template preparation for functional bacterial gene array was done by amplifying the community DNA with various primers in independent PCR reaction tubes as listed in Table 2-3. The 5'-end of each reverse primer was labeled with Cy3 to obtain Cy3 labeled PCR products. PCR amplification was performed in various 20- μ l PCR tubes containing 1 $\times$ *Ex Taq* buffer (TaKaRa, Shiga, Japan), 200 μ M dNTPs (TaKaRa), 20 pmol of forward and reverse primers, 0.6 U of *Taq* DNA polymerase (TaKaRa), and 4 μ l of DNA template prepared in section 2.4.1. The condition of PCR was denaturation in first step at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 1 min, a gradient annealing at 40°C, 45°C, 55°C, 60°C and 65°C for 1 min each, and extension at 72°C for 3 min; with extension in the final cycle at 72°C for 10 min. Amplified products were purified by ethanol precipitation to get a final amount of 35ng of labeled DNA.

2.4.3 Microarray hybridization

Microarray hybridization was performed as follows. The microarrays were prehybridized in prehybridization buffer (2 $\times$ SSC, 0.2% sodium dodecyl sulfate) for 15 min at room temperature and in freshly made prehybridization buffer for 5 min at 37°C. After the slides had been dipped in ultrapure water three times to remove excess prehybridization buffer, the arrays were dried by centrifugation (110 $\times$ g, 2 to 4 min). Cy3-labeled target DNA (35ng for eubacterial and pathogen microarrays or all DNA amplified for 33 target genes for functional bacterial gene microarray) was dissolved in a 50- μ l hybridization buffer (5 $\times$ SSC, 0.5% SDS), denatured at 90°C for 1 min, cooled down to 55°C, and deposited onto a glass cover-slip. Then, prehybridized array was placed on the cover-slip, and hybridization was carried out at 55°C for 16 h in a hybridization chamber (DNA Chip Research Inc., Kanagawa, Japan), where 150 μ l of 5 M NaCl was applied to avoid drying. Following hybridization, cover-slips were removed by immersion in 2 $\times$ SSC, 0.2% SDS at 37°C. Arrays were washed with 2 $\times$ SSC, 0.2% SDS and with 2 $\times$ SSC at room temperature for 1 min each, before being air dried in the dark.

2.4.4 Scanning

An arrayWoRx (GE Healthcare UK Ltd., Buckinghamshire, England) was used to scan fluorescent signals after hybridization on each spot in accordance with the manufacturer's

Table 2-3 Primers used to prepare template for functional bacterial gene array

Target gene	Forward primer	Reverse primer	Annealing Temp (°C)	Reference
C12O DNA	GCCAACGTCGACGTCTGGCA	CGCCTTCAAAGTTGATCTGCGTGGT	60	Sei <i>et al.</i> , 1999
C23O DNA	AAGAGGCATGGGGGCGCACCGGTTGATCA	CCAGCAAACACCTCGTTGCGGTTGCC	60	Sei <i>et al.</i> , 1999
<i>alkB</i>	CATAATAAAGGGCATCACCGT	GATTTCAATTCTCGAAACTCCAAAC	40	Kohno <i>et al.</i> , 2002
<i>alkM</i>	GAGACAAATCGTCTAAAACGTAA	TTGTTATTATTCCAACATGCTC	40	Kohno <i>et al.</i> , 2002
<i>alkB / alkB1</i>	TCGAGCACATCCGCGGCCACCA	CCGTAGTGCTCGACGTAGTT	40	Kohno <i>et al.</i> , 2002
<i>mmoX</i>	GGCTCCAAGTTCAAGGTCGAGC	TGGCACTCGTAGCGCTCCGGCTCG	55	McDonald <i>et al.</i> , 1995
<i>pmoA</i>	GGGGGAACCTTCTGGGGITGGAC	GGGGGRCIACGTCITTACCGAA	45	Cheng <i>et al.</i> , 1999
<i>mcrA</i>	TAYGAYCARATHHTGGYT	ACRTTCATNGCNGCRTARTT	45	Springer <i>et al.</i> , 1995
<i>amoA</i>	GGGGTTTCTACTGGTGGT	CCCCTCKGSAAAGCCTTCTTC	55	Rotthauwe <i>et al.</i> , 1997
<i>napA</i>	TAYTTYTNHSNAARATHATGTAYGG	DATNGGRTGCATYTCNGCCATRTT	45	Flanagan <i>et al.</i> , 1999
<i>narG</i>	TAYGTSGGSCARGARAA	TTYTCRTACCABGTBGC	60	Philippot <i>et al.</i> , 2002
<i>nirK</i>	GGSGCGGTCATGGTGCTGCC	TCGAAGGCCTCGATCAG	65	This study
<i>nirS</i>	TAYCACCCCGAGCCGCGCGT	CTTRAGYTYAGBTCTTGTCGTC	65	This study
<i>qnorB</i>	GGNCAYCARGGNTAYGA	ACCCANAGRTGNCANACCCACCA	55	Braker and Tiedje, 2003
<i>cnorB</i>	GACAAGNNNTACTGGTGGT	GAANCCCCANACNCCNGC	55	Braker and Tiedje, 2003
<i>nosZ</i>	CGGYTGGGGAMWKACCAA	ATRTCGATCARYTGNTCRTT	55	Nogales <i>et al.</i> , 2002
<i>nifH</i>	AAAGGYGGWATCGGYAARTCCACCAC	TTGTTSGCSGCRACATSGCCATCAT	60	Rösch <i>et al.</i> , 2002
<i>nrfA</i>	GCNTGYTGWSNTGYAA	TWNGGCATRTGRCARTC	45	Mohan <i>et al.</i> , 2004
16S rDNA (anammox)	GGATTAGGCATGCAAGTC	AAAACCCCTCTACTTAGTGCCC	60	Egli <i>et al.</i> , 2001
<i>dsrAB</i>	ATCGGWACCTGGAAGGAYGACATCAA	GGGCACATSGTGTAGCAGTTACCGCA	60	Karkhoff-Schweizer <i>et al.</i> , 1995
<i>soxB</i>	GAYGGNGGNGAYACNTGG	CATGTCNCCNCCRTGYTG	60	Petri <i>et al.</i> , 2001
<i>ferA</i>	ACARMARSGRTGTYGGSTGC	TGGATYMCSRARGTYGYAGTG	45	Neal <i>et al.</i> , 2004
<i>nofA</i>	GGCTTCACCGAGTTCACGCA	CCAGCGGGGTGTCCATCCAG	60	Siering and Ghiorse, 1997
<i>merA</i>	TTGGAGAACGTGC	ACGTCTTGGTGAAGGTCTG	55	Felske <i>et al.</i> , 2003
<i>tpm</i>	CAGTCAGAGGTCAATAAGG	GAGTTACACACGTTCCAACA	40	Courmoyer <i>et al.</i> , 1998
<i>cadA</i>	CAAAYTGYGCRGHAARTTYGA	AACTAATGCACAAGGACA	55	Oger <i>et al.</i> , 2001
<i>pcoR</i>	CAGGTCGTTACCTGCAGCAG	CTCTGATCTCCAGGACATATC	55	Trajanovska <i>et al.</i> , 1997
<i>phaZ</i>	CGTCTACCGCAACGGCACCAAGG	TGGGCGTAGTTGCTGGCCGT	55	Sei <i>et al.</i> , 2001
<i>apr</i>	TAYGGBTTCAAYTCCAAYAC	VGCGATSGAMACRTRCC	55	Bach <i>et al.</i> , 2001
<i>npr</i>	GTDGAYGCHCAYTAYTAYGC	ACMGCATGBGTYADYTCATG	55	Bach <i>et al.</i> , 2001
<i>sub</i>	ATGSAYRTTRYAAAYATGAG	GWGWHGCCATNGAYGTWC	55	Bach <i>et al.</i> , 2001
<i>sub</i>	GNACHCAYGTDGCHGGHAC	GWGWHGCCATNGAYGTWC	55	Bach <i>et al.</i> , 2001
<i>chiA</i>	GGIGGITGGACIYTIWSIGAYCCITT	ATRTCICCRTTRTCIGCRTC	40	LeClerc <i>et al.</i> , 2004

instructions. Oligonucleotide probes were mounted on the glass slide, and Cy3 dye labeled DNA sample was applied on the array for hybridization experiments. Scanned images were then processed with Array Vision ver. 8.0 (GE Healthcare UK Ltd.). There were 12 Cy3 positive control spots on each of pathogen and eubacterial microarray and the mean value of these spot intensities was used as the positive control intensity. After subtracting the background noise, the signal intensities of the spots were normalized relative to the positive Cy3 spot for eubacterial and pathogen arrays. For eubacterial array, test spots whose relative signal intensities (RSI) exceeded 0.1 were considered to be valid and used for further analysis. For pathogen array, test spots whose RSI exceeded 0.25 were considered to be valid and used for further analysis. In contrast, for functional bacterial gene array, there was no standard positive control spot, hence test spots whose signal intensities exceeded 2500 fluorescent units were considered positive. This criteria for cut-off was assumed for the reason that often cross-hybridization might occur between the oligonucleotide probes and the template DNA and thus to avoid the false-positive results the criteria of cut-off of signal intensities was considered.

2.5 Statistical analysis

Heat map was generated for eubacterial microarray data. Each RSI was converted into a color gradient by using macros for Microsoft Excel 2002 (Microsoft Corporation, Redmond, WA, USA). The heat map macro, developed by Dr. Yukihiro Yabuta at the Riken Center for Developmental Biology, Japan, is a freely available tool from his website: <http://homepage.mac.com/yabyab/program/heatmap.html> (accessed February 2011).

The Shannon-Weaver diversity index (Shannon et al., 1963) was calculated using natural logarithm by the following equations:

$$H' = -\sum P_i \cdot \ln P_i$$

$$P_i = n_i / N$$

where n_i is the RSI of spot i , and N is the summation of the normalized signal intensities in a sample.

Principal components analysis (PCA) and multivariate analysis were performed with the statistical analysis tool SPSS ver. 15.0 for Windows (SPSS inc., Chicago, IL, USA). PCA was performed against the occurrence pattern (presence/absence) of target species/genes for data of pathogen and functional bacterial gene microarray analyses, a value zero was assigned to the probes (undetected species/genes) which showed signal intensity <0.25 for

pathogen array and <2500 florescence unit for genes related to functions of environment, and a value one was assigned to the detected probes (species/genes). For the eubacterial microarray, data analysis was performed against RSI of test spots (RSI was assigned a value zero if a species is under the detection limit (<0.1)). RSI value was used in eubacterial array results so as to accurately predict the community structure and variation of bacterial species according to the sampling stations. However in case of pathogen array or the genes related to bacterial functions array the objective was to identify the presence or absence pattern of the species/genes. In addition, correlation analysis between the RSI of each pathogen and the total coliform count relative to the total number of heterotrophic bacteria was performed with Microsoft Excel 2002 (Microsoft Corporation, Redmond, WA, USA).

CHAPTER 3

Distribution of Bacterial Functions in Rivers

3.1 Background

In the aquatic ecosystem, microorganisms play pivotal role to degrade/detoxify pollutants, or elemental and material recirculation. In opposition to eukaryotes; prokaryotes often possess a particular gene related to an environmental function which is distributed over various *taxa* (classes or even *phyla*) of prokaryotes. For example, the function of nitrogen fixing is carried out in the environment by various *taxa* of bacteria or even certain *archaea* utilizing the *nifH* gene and such species belong to broad *taxa* including *Actinobacteria*, *Archaea* (*methanogens*), *Cyanobacteria*, *Firmicutes* and some classes of *Proteobacteria* (Ueda *et al.*, 1995). Evaluation of such bacterial genes could not be done based on 16S rDNA analysis due to the differences in individual lineages of different bacteria. Thus, evaluation of microbial functions based on evaluating such broad-spectrum genes can provide the overall understanding on the status of the environment. However, studies in multidisciplinary microbiological functions in the environment are often limited by the experimental format, generally involving independent evaluation of each function. For example, nitrogen cycle includes nitrogen fixation, ammonium oxidation, nitrite oxidation, nitrate reduction, nitrite reduction, nitric oxide reduction, nitrous oxide reduction, and anaerobic ammonium oxidation (*anammox*) functions; however it is too cumbersome to evaluate each function individually even if molecular microbiological methods are applied.

3.3.1 Terminology of Functional array

In environmental studies and particularly in this thesis, the term functional array exclusively refers to the DNA microarray which is used to detect the presence (or absence) of a target environmental function. Function itself is defined as the ability of microorganisms to carry out biotransformation of chemical compounds into environmentally beneficial or less toxic substances. The functional array is thus used to evaluate the potential of environmental function and to assess the soundness of river waters.

In this chapter, environmental functions in river waters were evaluated using a DNA microarray. There were total 85 genes which targeted 33 functions in environment, these were distributed as functions related to circulation of: carbon (3 functions and 7 genes), nitrogen (11 functions and 24 genes) and sulfur (2 functions and 2 genes); chemical degradation (5 functions and 27 genes), metal metabolism (6 functions and 9 genes), and energy flow (6 functions and 16 genes) for more details please refer chapter 2.3.1.

3.2 Spatiotemporal variations of bacterial functions

Among 85 targeted genes, 11 to 68 functional bacterial genes were detected in river water samples (Fig. 3-1). The numbers of detected genes were highest in summer, except station K1. The detected gene numbers were greatly different in autumn depending on the sampling station. In contrast, similar numbers of functional bacterial genes were detected in spring and winter.

In Kita River, the number of detected functional bacterial genes increased along the course of flow of the river in summer, autumn and winter. This might be due to the brackish environment, where freshwater and seawater microbes coexist and generally highly diverse community is formed (Crump et al., 1999), at station K2.

In Yodo River, functional bacterial gene number always decreased from station Y1 to station Y2. A dam is positioned just upstream of station Y2, and thus hydraulic retention time is longer there. This might result in the change of microbial community composition and decrease of function. By contrast, station Y3 always had higher functional bacterial gene numbers with little seasonal variations. Activated sludge in WWTP contains a wide variety of microorganisms with a high density. Thus, release of WWTP effluents would cause the increase of functional genes at station Y3.

PCA was performed based on the occurrence pattern of bacterial genes. As shown in Fig. 3-2, samples were roughly distributed depending on the season. Summer samples except a sample from station K1 were largely separated from the samples in the other seasons. This suggested that microbial functions in summer were greatly different from those in the other seasons. In addition, it was revealed that PC1 was strongly correlated

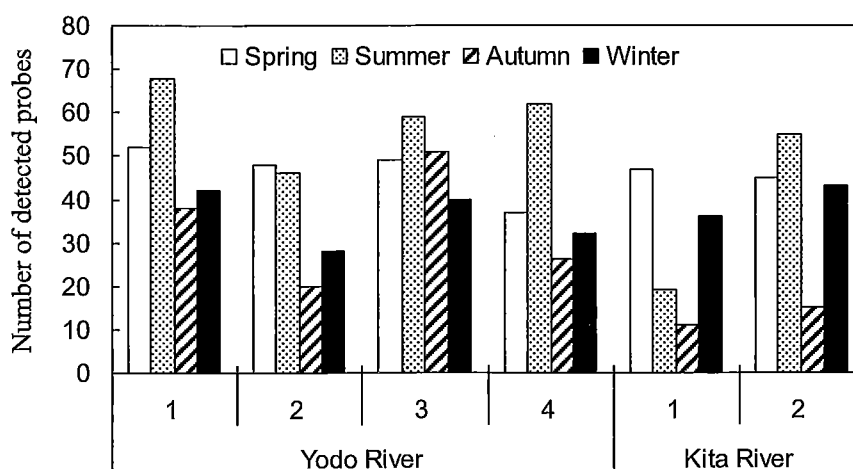


Fig. 3-1 Positive number of genes detected in river water samples collected from the Yodo and Kita Rivers. Probe with signal intensity ≥ 2500 was judged as positive.

with the number of detected genes ($r^2 = 0.85$) (Fig. 3-3), and that PC2 was weakly correlated with water temperature ($r^2 = 0.44$) and not with the other water quality parameters. Thus, it is suggested that water temperature would be a factor relevant to the change of the microbial function of riverine water. It was previously reported that ammonia oxidizing bacteria rapidly decreases with falling water temperatures (Urakawa et al., 2008). Similarly, Yang et al. (2007) have reported that the denitrification activity is highest at 28°C-30°C and decrease in temperatures drastically lowers the activity. Therefore, water temperature higher than 28°C in summer in 5 stations except for station K1 resulted in the

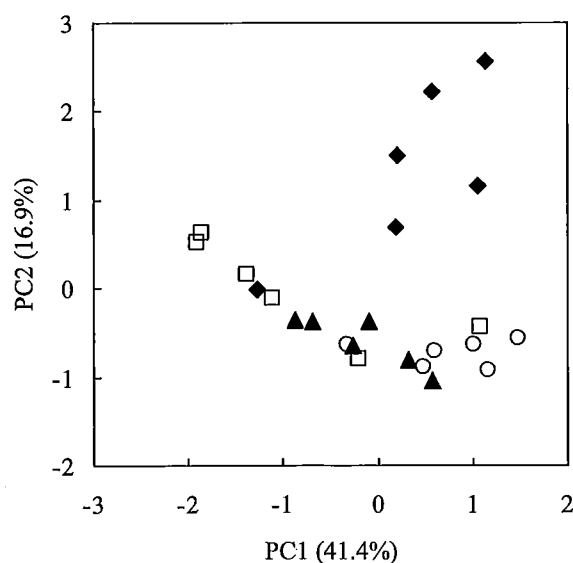


Fig. 3-2 Ordination produced from PCA based on gene profiles in river water samples obtained from Yodo and Kita Rivers in spring (open circle), summer (closed diamond), autumn (open square), and winter (closed triangle).

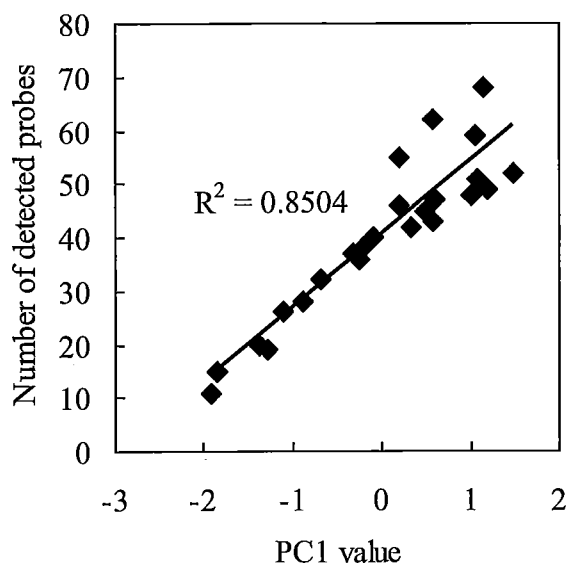


Fig. 3-3 Correlation between the value of principal component analysis factor 1 (PC1) and the number of detected gene probes in river water samples.

highly different functional gene profiles in these 5 stations compared with the other seasons.

3.3 Characteristics of individual and overall bacterial functions

The occurrence of each functional bacterial gene is summarized in Table 3-1.

3.3.1 Functions related to chemical degradation

Among the genes related to aromatic compounds degradation, catechol 1,2-dioxygenase (C12O) DNA and catechol 2,3-dioxygenase (C23O) DNA, C23O DNA was detected in all samples, while C12O DNA was detected in several samples and no relationship between its occurrence and station/season was observed. It has been reported that although C12O DNA is normally dominated in seawater, under high concentrations of aromatics C23O DNA increase (Sei et al., 2004). Thus, common occurrence of C23O in this study might indicate that river water have been polluted by aromatics from human activities. Detection of C23O even at station K1 with low pollution level may suggest the presence of chemical contamination that cannot be predicted by physicochemical water quality parameters.

Among alkane degradation genes, *alkB*, *alkB* & *alkB1* were detected in all samples. On the other hand, *alkM* was found to be present at high signal intensity at station Y3 independent of the season, but was not detected or was detected at low signal intensity at the other stations. *alkB* is responsible for degradation of relatively shorter *n*-alkanes, whereas *alkM* and *alkB* & *alkB1* are for longer *n*-alkanes (Belhaj et al., 2002; Kohno et al., 2002; Sei et al., 2003). Thus it can be inferred that natural river water has shorter *n*-alkane degradation ability as a general function.

3.3.2 Functions related to carbon cycle

Amongst the methane oxidizing genes, the *pmoA* was ubiquitous in all samples. In contrast, the *mmoX* was not detected in most samples. Almost all methane oxidizing bacteria possess *pmoA*, whereas *mmoX* gene encodes a special methane oxidizing enzyme which is activated only under the copper limitation conditions. Methane oxidizing bacteria possessing *mmoX* are limited to 5 genera such as *Methylococcus* (Cheng et al., 1999; Nakamura et al., 2007). Thus, it is suggested that *mmoX* gene is minor in the riverine

Table 3-1 Distribution of functional bacterial genes in Yodo and Kita Rivers

Environmental function	Target gene	October, 2005				August, 2006				January, 2007				May, 2007			
		Yodo River		Kita River		Yodo River		Kita River		Yodo River		Kita River		Yodo River		Kita River	
		1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2
Chemical degradation	C12O DNA	+	-	++	+	-	-	++	+	++	+	-	+	+	-	+	+
	C23O DNA	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	<i>alkB</i>	++	++	++	++	+	+	++	++	++	++	++	++	++	++	++	++
	<i>alkM</i>	+	-	++	-	+	++	+	+	+	+	+	+	+	+	+	+
	<i>alkB</i> & <i>B1</i>	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Carbon cycle (methane cycle)	<i>mmoX</i>	-	+	+	-	-	-	+	-	+	+	-	+	-	-	-	-
	<i>pmoA</i>	++	++	++	++	-	+	++	++	++	++	++	++	++	++	++	++
	<i>mcrA</i>	-	-	+	-	-	-	+	+	+	-	-	+	-	-	+	+
Carbon cycle (energy flow)	<i>phaZ</i>	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	<i>apr</i>	+	-	++	+	-	+	+	+	++	+	-	+	++	+	++	++
	<i>npr</i>	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-
	<i>sub</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>sub</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>chiA</i>	++	++	++	+	-	+	++	++	++	++	++	+	++	++	++	++
Nitrogen cycle	<i>amoA</i>	+	-	++	-	-	-	+	+	+	-	-	+	-	+	+	+
	<i>napA</i>	++	+	++	+	-	-	+	+	++	+	-	+	+	+	+	++
	<i>narG</i>	+	+	++	+	-	-	++	++	++	+	+	+	++	+	++	++
	<i>nirK</i>	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	<i>nirS</i>	++	+	++	+	-	-	++	++	++	++	+	+	++	+	++	++
	<i>qnorB</i>	+	-	+	-	-	-	+	-	+	-	-	-	+	+	-	+
	<i>cnorB</i>	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
	<i>nosZ</i>	++	-	++	+	-	-	++	+	++	-	-	+	++	+	++	++
	<i>nifH</i>	++	++	++	+	-	++	++	++	++	++	++	++	++	++	++	++
	<i>nrfA</i>	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	16S rDNA (anammox)	-	-	+	-	-	-	+	+	+	-	-	+	+	+	+	+
Sulfur cycle	<i>dsrAB</i>	+	-	+	-	-	-	+	-	+	-	-	+	+	-	++	++
	<i>soxB</i>	-	-	+	-	-	-	+	+	+	-	-	+	-	-	+	+
Metal metabolism	<i>ferA</i>	++	+	++	+	-	-	++	++	++	+	++	++	++	++	++	++
	<i>mofA</i>	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
	<i>merA</i>	+	-	+	+	-	-	++	++	++	+	-	+	++	+	++	++
	<i>tpm</i>	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	<i>cadA</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	<i>pcoR</i>	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-

++, Signal intensity (SI) ≥ 15000; +, 2500 < SI < 15000; -, SI < 2500

waters. The *mcrA* gene that encodes the enzyme for methane generation during anaerobic conditions was rarely detected probably because the samples were collected from the sub-surface aerobic zone.

The genes relevant to energy flow: *phaZ* *chiA*, and genes related to poly(3-hydroxybutyrate) (PHB) degradation and chitin degradation, were dominantly detected in almost all the samples. In recent years, the production and consumption of PHB have been increasing and its environmental release should increase (Sei et al., 2001). In addition, chitin is a biological macromolecule that is universally present on earth (LeClerc et al., 2004). The evidence suggests that chitin degradation and PHB degradation genes should be widely present in river water systems. On the other hand, amongst the genes related to peptide hydrolysis, neither *npr* nor *sub* were detected in the river samples although *apr* genes were mainly found in many samples. Although peptide compounds are also thought to be ubiquitous in the environment, hydrolytic functions were rarely detected. The primer used in this study was originally developed for evaluating the peptide degradation genes in soil environments (Bach et al., 2001), and may be inappropriate for those in aquatic environments. Thus, more specific primers and probes are needed for better evaluating the peptide hydrolytic functions in the aquatic environment.

3.3.3 Functions related to nitrogen cycle

amoA gene responsible for the ammonia oxidation was always detected at station Y3 although it was rarely detected in upstream stations of both monitoring rivers. Because station Y3 is located downstream of WWTPs, ammonium nitrogen released from the WWTPs increased the concentration of ammonium nitrogen in river water and consequently ammonia oxidizing bacteria grew (Cébron et al., 2004).

Among two genes related to nitrite reduction, *nirK* gene was detected in all samples, whereas *nirS* gene was not always detected. The abundance and diversity of *nirK* and *nirS* have been reported to be different depending on the target environment (Priemé et al. 2002; Braker et al., 2006). Yan et al. (2003) have reported that although *nirK* gene is generally dominated in groundwater contaminated with nitrate and uranium, *nirS* gene is activated at low pH and high nitrate concentration and it is suppressed under high DO condition. pH of river water samples analyzed in this study was around neutral and their DO was generally high. Therefore, *nirK* gene was generally dominated in the samples. On the other hand, *qnorB* and *cnorB* genes responsible for nitric oxide reduction were rarely detected. Nitric oxide is produced under the anaerobic condition, and thus *qnorB* and *cnorB*

genes were not present in surface waters under the aerobic condition.

nifH gene responsible for nitrogen fixation was detected in almost all the samples, excepting station K1 where the gene was detected only in summer.

nrfA responsible for reduction of nitrite to ammonium and 16S rDNA of anammox were hardly detected. Results of this study suggest that these special functions are rarely present in river water environments.

From the results, each gene included in the nitrogen cycle showed different spatial variation. As a whole, the nitrogen cycling potential in the river environment seems to vary depending on the environmental conditions along the flow. In particular, most of the target genes were detected in station Y3. Although previous study has revealed the elevation of *amoA* gene by the discharge of WWTP effluent (Cébron et al., 2004), our results suggest that WWTP effluent influences entire genes related to the nitrogen cycle.

3.3.4 Functions related to sulfur cycle

dsrAB responsible for the dissimilatory sulfate reduction was detected in the downstream station in Kita River and upstream stations in Yodo River, however the signal intensity was always low. Sulfate reduction occurs under the anaerobic condition in soil and sediment (Scholten et al., 2005; Leloup et al., 2006). Thus, the presence of sulfate reducing genes in sub-surface waters would be resulted from the transport of river sediments or soils of river bank. On the other hand, thiosulfate oxidizing gene *soxB* was hardly detected in the samples, suggesting that this function is unusual in the river waters.

In this study only two genes were targeted for detecting bacterial functions related to the sulfur cycle. Since these two genes are rarely present in the samples analyzed, further investigation targeting different functional bacterial genes would be needed to evaluate the sulfur recycling function in the river environment.

3.3.5 Functions related to metal metabolism

The selenium methylating gene *tpm* was commonly detected in river water samples. The iron reduction gene *ferA* was detected in all but two samples from Kita River in summer. The mercury reduction gene *merA* was also detected in most samples although it was rarely detected in autumn. By contrast, manganese oxidation gene *mofA*, cadmium resistance gene *cadA* and copper resistant gene *pcoR* were undetected in almost all the samples.

3.3.6 Overall evaluation of bacterial functions

Based on the detection frequencies in the river water samples, the genes related to bacterial functions were classified into three major groups as shown in Table 3-2. Group (A) consisted of universally present genes; group (B) consisted of genes which are specifically present under certain geographical and seasonal condition; and group (C) consisted of non existent genes. Group A included the chemical degradation genes of C23O DNA, *alkB*, *alkB & B1*, carbon cycle genes of *pmoA*, *phoZ*, *chiA*, nitrogen cycle gene *nirK* and metal metabolism gene *tpm*. Except for *nirK*, the genes classified in group A are responsible for aerobic functions. Group B consisted of the chemical degradation genes of C12O DNA, *alkM*, nitrogen cycle genes of *amoA*, *napA*, *narG*, *nirS*, *nosZ*, *nifH*, sulfur cycle gene *dsrAB*, and metal metabolism genes of *ferA* and *merA*. The genes related to the nitrogen cycle were abundant in this group, and thus it was suggested that they may largely reflect the specific condition of river environment. Genes categorized in group C were detected in very small number or not at all detected. It was concluded that group C genes did not have any significant contribution towards the functions of river water environment.

Table 3-2 Classification of functional bacterial genes based on detection frequency

(A)	(B)	(C)
C23O DNA	C12O DNA	<i>mmoX</i>
<i>alkB</i>	<i>alkM</i>	<i>mcrA</i>
<i>alkB&B1</i>	<i>amoA</i>	<i>qnorB</i>
<i>pmoA</i>	<i>napA</i>	<i>cnorB</i>
<i>nirK</i>	<i>narG</i>	16S rDNA (anammox)
<i>tpm</i>	<i>nirS</i>	<i>soxB</i>
<i>phaZ</i>	<i>nosZ</i>	<i>npr</i>
<i>chiA</i>	<i>nifH</i>	<i>nrfA</i>
	<i>dsrAB</i>	<i>mofA</i>
	<i>ferA</i>	<i>cadA</i>
	<i>merA</i>	<i>pcoR</i>
	<i>apr</i>	<i>sub</i>

(A); Detected in most samples (++ ≥ 20), (B); Detected in several samples,
(C); Hardly or not detected (++ = 0)

3.4 Conclusion

In this chapter, spatial and seasonal variations of the occurrence of 33 functional bacterial genes related to carbon, nitrogen and sulfur cycles, chemical degradation, metal metabolisms, and energy flows were studied using DNA microarray. The presence of functional bacterial genes in river water was greatly influenced by the seasonal variations. Especially, a wide variety of bacterial functions seems to be present in summer as compared to the other seasons. The number of genes seemed to be reduced by water retention in a dam lake. Conversely, discharge of WWTP effluent increase the diversity of bacterial functions.

The 33 kinds of functions analyzed were classified into three groups based on the detection frequency in the seasonal samples: (A) universally present genes, (B) genes specifically present under certain geographical and seasonal conditions, and (C) non-existent genes. Group A included the chemical degradation genes of C23O DNA, *alkB*, *alk B & B1*, carbon cycle genes of *pmoA*, *phoZ*, *chiA*, nitrogen cycle gene *nirK* and metal metabolism gene *tpm*. Group B consisted of the chemical degradation genes of C12O DNA, *alkM*, nitrogen cycle genes of *amoA*, *napA*, *narG*, *nirS*, *nosZ*, *nifH*, sulfur cycle gene *dsrAB*, and metal metabolism genes of *ferA* and *merA*. From the results, functions included in the group B may be used as an indicator for evaluating the quality of river environment as these functions are sensitive towards varying environmental conditions than other studied genes, as they have special impact on river water environment depending on seasonal or geographical variations.

CHAPTER 4

Spatial and Temporal Variations of Eubacterial Community in Rivers

4.1 Background

Microorganisms play pivotal roles in breaking down organic matter and remineralizing nutrients; these actions strongly influence energy flux and elemental and material circulation in aquatic ecosystems. Microbial populations in the natural environment fluctuate spatiotemporally with changes in the chemical and physical conditions of the surrounding environment (Branco et al., 2005; Chénier et al., 2003). In addition, inflow of wastewaters containing xenobiotics and toxic chemicals causes a drastic shift in microbial community structure; this shift may influence the ecological functions of the environment (Brummer et al., 2000; Feris et al., 2004; Fossi et al., 1995; Rodriguez et al., 2007). Thus, microbial flora can serve as a bioindicator for estimating water quality (Brummer et al., 2003; Douterelo et al., 2004) and environmental soundness.

In this chapter, total eubacterial community in the monitoring rivers was analyzed using a eubacterial microarray and assessed the correlation with various physicochemical parameters.

4.2 River water quality

Table 4-1 shows the physicochemical and biological quality of river water samples collected over a span of 20 months. Water temperature varied by the seasonal pattern was 21.6°C to 30.2°C in summer, 11.8°C to 22°C in spring and autumn, and 6.5°C to 13.1°C in winter. The pH values of river samples were around neutral; however summer samples exhibited a tendency to be mildly alkaline. Electrical conductivity was low (0 to 0.3 mS/cm) in almost all of the samples, with an exception of samples from downstream in the Kita River (station K2), which had values of 50.1, 13.9 and 7 (mS/cm) for summer, autumn and spring respectively. This occurs due to a brackish water environment that was created owing to the backflow of seawater. DO levels vary widely among the samples ranging from 3.3 to 9.5 mg/l. Concentrations of DOC, CHB and eubacterial 16S rDNA copies were lowest in the upstream of Kita River (station K1) in most seasons, where the influx of anthropogenic disturbances was presumed lowest amongst all the sampling stations. DOC and CHB increased by 1.7 to 5 times and more than 10 times during the flow from station K1 to station K2 in Kita River, respectively, possibly due to the inflow of effluents from surrounding areas and the backflow of seawater containing contaminants accumulated in the Wakasa Bay. In Yodo River, concentrations of CHB, DOC, and T-N usually increased between sampling stations Y2 and Y3, indicating that effluents from WWTPs

Table 4-1 Physicochemical and biological water quality parameters in river water^a

Sampling date	River	Station	Temp (°C)	pH	DO (mg/l)	Conductivity (mS/cm)	DOC (mg/l)	T-N (mg/l)	Heterotrophic bacteria (CFU/ml)	Eubacterial 16S rDNA (MPN-copies/ml)	Coliform count (MPN-copies/100ml)
Spring (May 2007)	Yodo	Y1	19.8	7.7	9.1	0.1	2.4	0.34	4.6×10^4	2.4×10^6	2.3×10^2
		Y2	19.4	7.4	7.6	0.1	2.7	0.69	7.9×10^3	2.4×10^4	2.9×10^2
		Y3	21.0	7.1	7.0	0.2	8.2	1.9	4.7×10^3	9.3×10^6	1.5×10^3
		Y4	20.5	7.4	7.9	0.1	4.5	1.5	1.8×10^4	9.3×10^4	2.1×10^2
	Kita	K1	12.8	7.2	8.8	0.1	8.2	0.88	4.5×10^3	2.4×10^4	NA
		K2	16.8	6.6	6.2	7.0	3.3	0.90	5.7×10^4	9.3×10^4	9.3×10^3
Summer (August 2006)	Yodo	Y1	30.0	9.0	6.9	1.4	NA ^a	NA	1.1×10^4	2.3×10^4	4.3×10^2
		Y2	28.6	7.3	4.8	0.1	NA	NA	1.4×10^4	2.3×10^4	9.0×10^1
		Y3	29.8	7.5	6.1	0.2	NA	NA	4.0×10^4	9.3×10^4	9.3×10^2
		Y4	30.2	8.5	6.3	0.1	NA	NA	4.0×10^4	9.3×10^4	9.3×10^2
	Kita	K1	21.6	8.2	9.3	ND	NA	NA	6.3×10^3	2.4×10^4	1.4×10^2
		K2	30.0	7.8	3.3	50.1	NA	NA	1.1×10^5	1.5×10^4	4.3×10^2
Autumn (October 2005)	Yodo	Y1	21.2	8.1	7.9	0.1	1.5	1.1	2.7×10^4	2.4×10^5	NA
		Y2	21.5	7.2	6.8	0.1	1.1	1.4	7.1×10^3	1.5×10^5	NA
		Y3	22.0	7.3	7.4	0.2	1.9	2.0	1.3×10^4	2.1×10^5	NA
		Y4	21.8	6.6	6.4	0.2	2.0	1.6	3.3×10^4	2.4×10^4	NA
	Kita	K1	13.4	6.7	9.5	0.1	0.36	2.4	5.7×10^3	2.3×10^3	NA
		K2	11.8	6.5	6.5	13.9	1.8	2.6	1.9×10^5	7.0×10^3	NA
Winter (January 2007)	Yodo	Y1	7.2	7.5	7.8	0.1	6.3	0.87	4.6×10^4	9.3×10^3	3.6×10^1
		Y2	6.5	7.6	8.1	0.1	5.5	0.80	7.8×10^3	4.3×10^3	ND
		Y3	13.1	7.1	5.9	0.3	11.3	4.6	1.7×10^4	2.4×10^4	7.4×10^2
		Y4	8.4	7.5	4.2	0.1	6.1	1.7	4.4×10^4	9.3×10^3	2.1×10^2
	Kita	K1	6.6	7.2	6.3	ND	2.8	0.67	4.1×10^3	1.5×10^3	3.6×10^1
		K2	7.1	6.9	6.8	0.1	4.8	0.50	4.4×10^4	2.1×10^3	2.4×10^3

^a NA, not analyzed ; ND, not detected

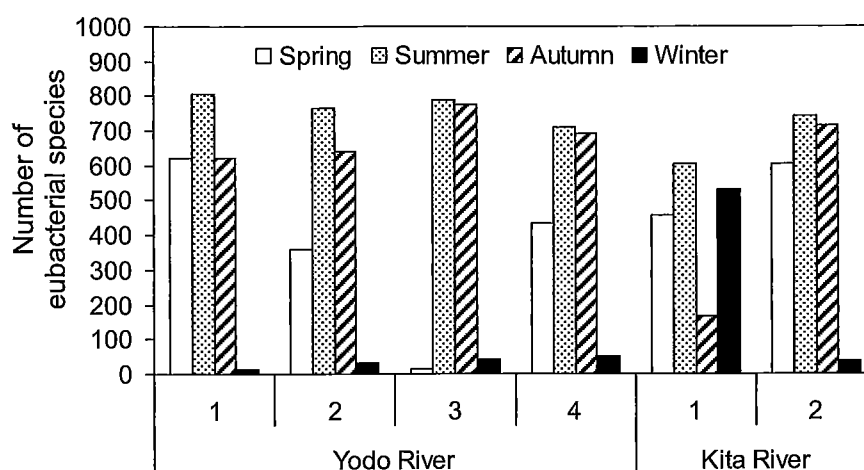


Fig. 4-1 Number of bacterial species detected in river water samples collected from Yodo and Kita Rivers. Probes with relative signal intensity > 0.1 of Cy3 control spot were judged as positive.

located between these sampling stations largely influenced the water quality at station Y3.

4.3 Diversity of bacterial community

The number of bacterial species detected in river water samples varied ranging from 15 to 807 (Fig. 4-1). Of the 1016 bacterial species targeted, a total of 854 (84%) were detected in one or more samples. A heat map (Fig. 4-2) shows the presence of bacterial species to better understand the results visually.

Higher numbers of bacterial species (610 to 807 species) were detected in summer and autumn, with the exception of an autumn sample from station K1 that had only 159 detectable bacterial species. Winter samples had the lowest numbers of bacterial species, ranging from 15 to 50, although one sample from station K1 contained as many as 524 different species. In spring, the numbers of bacterial species detected at stations K1, K2, Y1, Y2 and Y4 ranged from 362 to 620 – higher than in winter and lower than in autumn and summer – whereas a spring sample from station Y3 contained an exceptionally low number (17 species).

In the Kita River, fewer bacterial species were found at upstream station K1 than at downstream station K2 in spring, summer, and autumn (Fig. 4-1 and Table 4-2), and a very sharp increase in the numbers of almost all phyla was observed between K1 and K2 in autumn. In contrast, in winter the community at station K1 had over 10 times more species than at station K2, as indicated above. In the Yodo River, although the number of bacterial species detected did not drastically change along the flow in summer, autumn, and winter, it

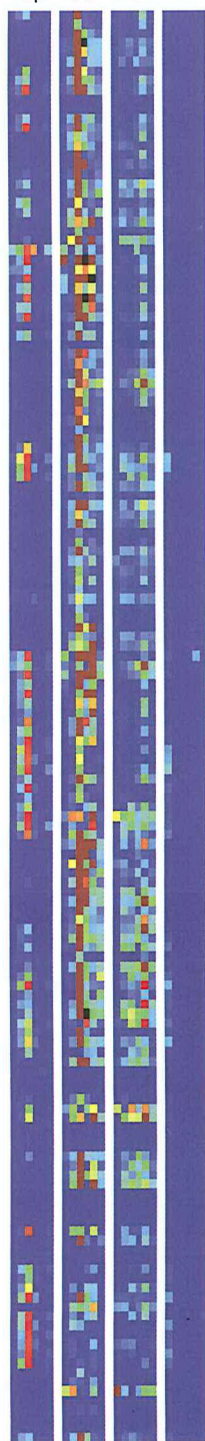
Table 4-2 Detailed distribution of bacterial species in each sample

Phylum (Total probes) ^a	Spring						Summer						Autumn						Winter					
	Y1	Y2	Y3	Y4	K1	K2	Y1	Y2	Y3	Y4	K1	K2	Y1	Y2	Y3	Y4	K1	K2	Y1	Y2	Y3	Y4	K1	K2
<i>Actinobacteria</i> (152)	77 [51] ^b	63 [41]	6 [4]	55 [36]	58 [38]	77 [51]	131 [86]	130 [86]	137 [90]	125 [82]	101 [66]	129 [85]	113 [74]	118 [78]	126 [83]	117 [77]	85 [56]	119 [78]	1 [1]	9 [6]	6 [4]	12 [8]	71 [47]	1 [1]
<i>Bacteroidetes</i> (48)	36 [75]	21 [44]	1 [2]	22 [46]	28 [58]	34 [71]	45 [94]	38 [79]	46 [96]	44 [92]	36 [75]	43 [90]	37 [77]	28 [58]	43 [90]	40 [83]	5 [10]	40 [83]	1 [2]	2 [4]	5 [10]	3 [6]	27 [56]	2 [4]
<i>Cyanobacteria</i> (39)	35 [90]	23 [59]	1 [3]	21 [54]	25 [64]	34 [87]	33 [85]	37 [95]	31 [79]	31 [79]	36 [92]	34 [87]	29 [74]	26 [67]	36 [92]	29 [74]	1 [3]	29 [74]	0 [0]	0 [0]	0 [0]	0 [0]	33 [85]	1 [3]
<i>Firmicutes</i> (226)	174 [77]	86 [38]	1 [0]	113 [50]	114 [50]	165 [73]	180 [80]	167 [74]	166 [73]	141 [62]	114 [50]	143 [63]	126 [56]	134 [59]	173 [77]	149 [66]	14 [6]	145 [64]	6 [3]	7 [3]	8 [4]	9 [4]	144 [64]	11 [5]
<i>Proteobacteria</i> (455)	264 [58]	155 [34]	6 [1]	203 [45]	212 [47]	261 [57]	338 [74]	336 [74]	336 [74]	306 [67]	271 [60]	334 [73]	290 [64]	307 [67]	342 [75]	327 [72]	50 [11]	341 [75]	6 [1]	11 [2]	15 [3]	20 [4]	232 [51]	24 [5]
- <i>Alpha</i> (123)	105 [85]	65 [53]	1 [1]	89 [72]	81 [66]	103 [84]	119 [97]	119 [97]	118 [96]	108 [88]	101 [82]	117 [95]	102 [83]	110 [89]	119 [97]	114 [93]	11 [9]	117 [95]	1 [1]	4 [3]	5 [4]	6 [5]	98 [80]	12 [10]
- <i>Beta</i> (78)	37 [47]	23 [29]	0 [0]	31 [40]	30 [38]	36 [46]	59 [76]	53 [68]	62 [79]	53 [68]	55 [71]	56 [72]	51 [65]	54 [69]	58 [74]	58 [74]	15 [19]	56 [72]	4 [5]	1 [1]	3 [4]	2 [3]	32 [41]	7 [9]
- <i>Gamma</i> (133)	80 [60]	46 [35]	1 [1]	60 [45]	67 [50]	77 [58]	101 [76]	112 [84]	99 [74]	93 [70]	77 [58]	107 [80]	85 [64]	94 [71]	103 [77]	102 [77]	8 [6]	109 [82]	1 [1]	5 [4]	7 [5]	10 [8]	64 [78]	5 [4]
- <i>Delta</i> (108)	37 [34]	19 [18]	3 [3]	21 [19]	31 [29]	39 [36]	47 [44]	44 [41]	45 [42]	45 [42]	33 [31]	42 [39]	44 [41]	45 [42]	50 [46]	45 [42]	15 [14]	47 [44]	0 [0]	1 [1]	0 [0]	2 [2]	33 [31]	0 [0]
- <i>Epsilon</i> (13)	5 [38]	2 [15]	1 [8]	2 [15]	3 [23]	6 [46]	12 [92]	8 [62]	12 [92]	7 [54]	5 [38]	12 [92]	8 [62]	4 [31]	12 [92]	8 [62]	1 [8]	12 [92]	0 [0]	0 [0]	0 [0]	0 [0]	5 [38]	0 [0]
Others (96)	34 [35]	14 [14]	2 [2]	19 [19]	19 [19]	30 [31]	80 [82]	74 [76]	78 [80]	67 [68]	52 [53]	63 [64]	29 [30]	27 [28]	55 [56]	29 [30]	4 [4]	41 [42]	1 [1]	2 [2]	6 [6]	6 [6]	17 [17]	0 [0]
Total (1,016)	620 [61]	362 [36]	17 [2]	433 [43]	456 [45]	601 [59]	807 [79]	782 [77]	794 [78]	714 [70]	610 [60]	746 [73]	624 [61]	640 [63]	775 [76]	691 [68]	159 [16]	715 [70]	15 [1]	31 [3]	40 [4]	50 [5]	524 [51]	39 [4]

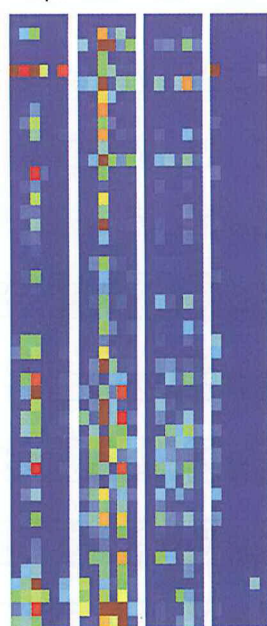
^a Numbers in round parentheses indicate total targeted species.

^b Numbers in boxed parentheses indicate percentage of detected probes. Note the numbers are rounded-off to eliminate decimal values.

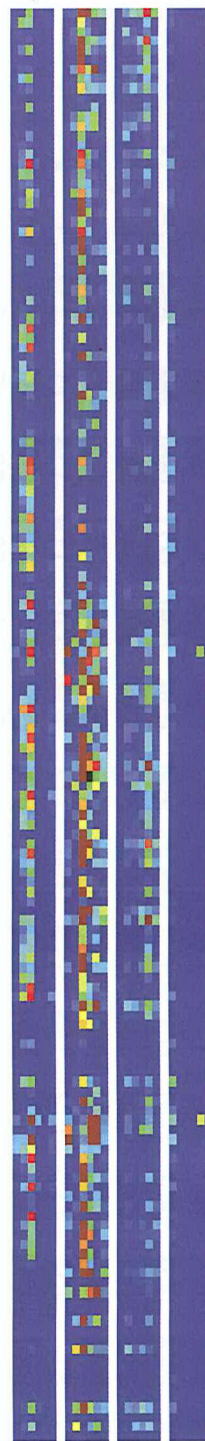
Actinobacteria
Sp Su Au Wi



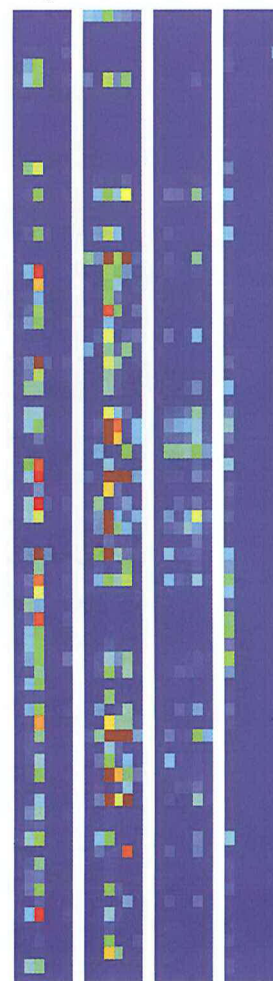
Bacteroidetes
Sp Su Au Wi



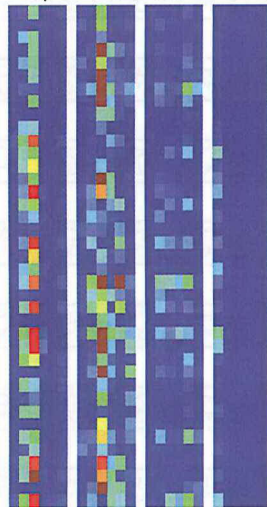
Firmicutes
Sp Su Au Wi



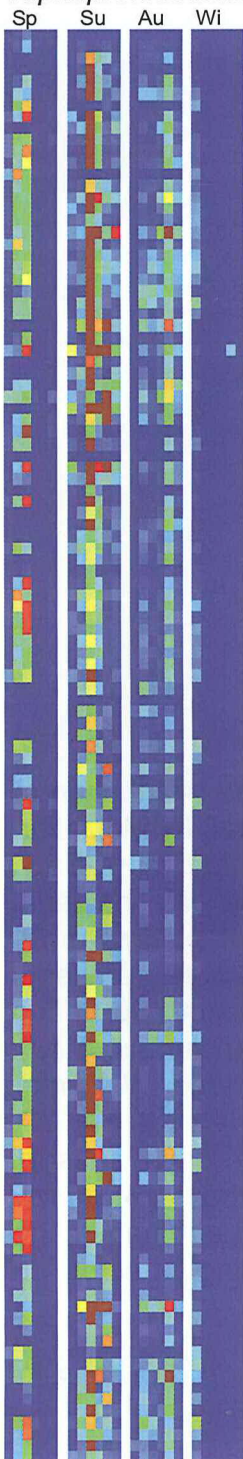
Firmicutes (contd.)
Sp Su Au Wi



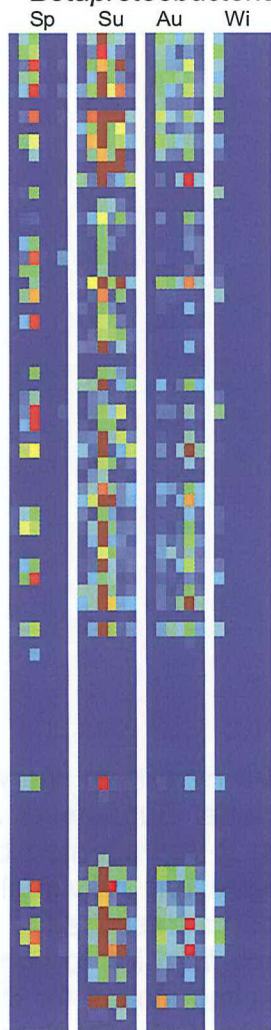
Cyanobacteria
Sp Su Au Wi



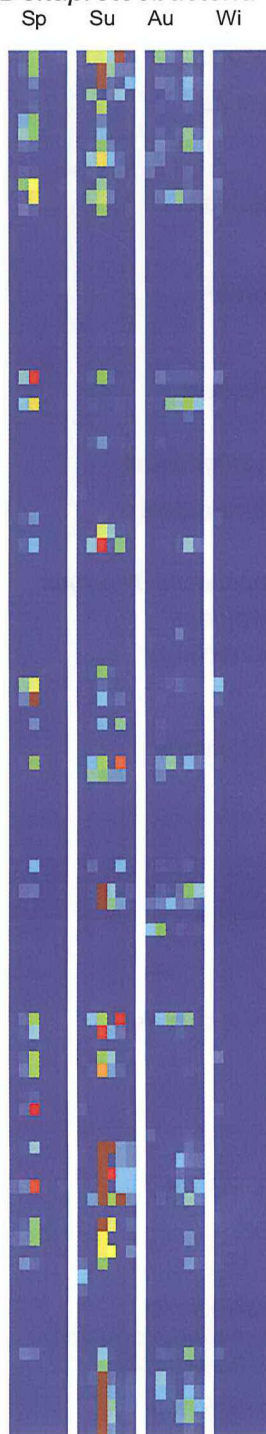
Alphaproteobacteria



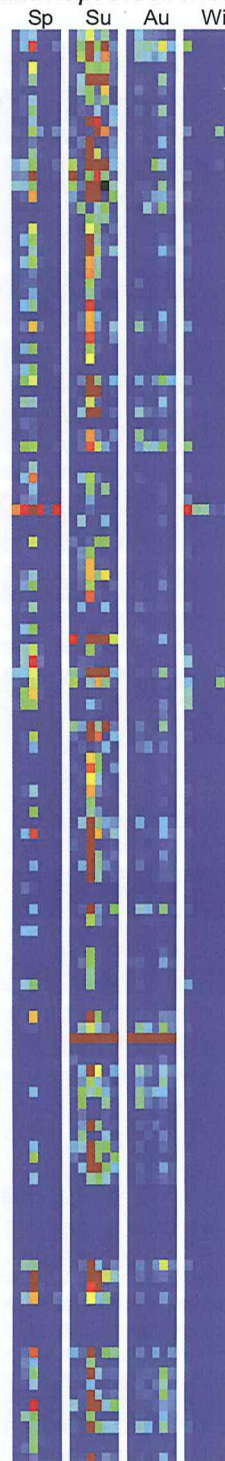
Betaproteobacteria



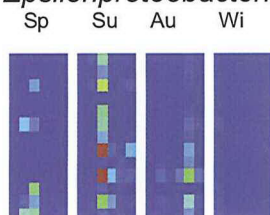
Deltaproteobacteria



Gammaproteobacteria



Epsilonproteobacteria



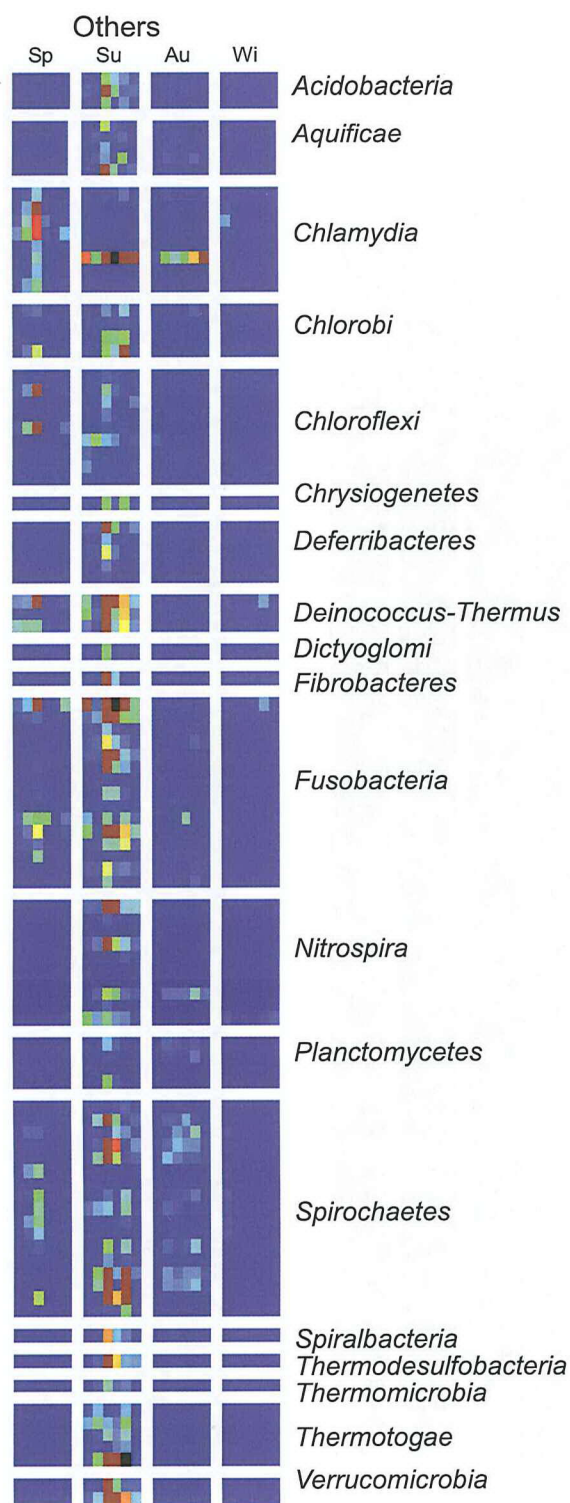


Fig. 4-2 Heat map showing the distribution of eubacteria. Eubacterial species' hybridization pattern is represented by a heat map. Each row represent individual probe of a bacterial species as described in table 4-2, each of 24 columns from left to right direction represents a sampling station for the seasons Sp-Spring (columns 1-6), Su-Summer (columns 7-12), Au-Autumn (columns 13-18) and Wi-Winter (columns 19-24) in the order: K1, K2, Y1, Y2, Y3 and Y4 (left to right) for each season. Relative signal intensity data from microarray analysis was converted into a heat map image which is generated by using macros in Microsoft Excel® (Reference website: <http://homepage.mac.com/yabyab/program/heatmap.html> accessed: February 2011).

Color scheme for relative signal intensity is explained below:



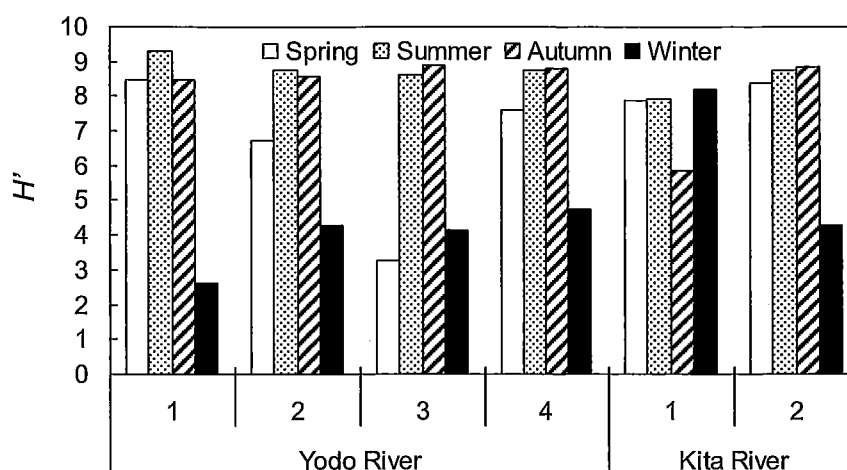


Fig. 4-3 Shannon-Weaver's diversity index (H') calculated from the data of eubacterial microarray analyses. Calculations were carried out for spots whose relative signal intensity was > 0.1 of control Cy3 spot.

increased slightly between stations Y2 and Y3 (Fig. 4-1 and Table 4-2). Exceptionally in spring the number of bacterial species largely decreased from station Y1 (620 species) to station Y3 (17 species) but recovered in Y4 (433 species).

The Shannon-Weaver's diversity index H' was calculated from the RSIs of the detected bacterial species. Figure 4-3 shows the spatiotemporal variation similar to that of the detected number of bacterial species described above. H' accounts for both abundance and evenness of species detected. H' value increases if all species are equally abundant or additional new species are present. Lower H' values means that both abundance and evenness of detected species is low.

4.4 Composition of bacterial community

Bacterial species detected in the analysis are summarized at the phylum level in Table 4-2. A total of 854 bacterial species detected were classified into six groups according to their seasonal occurrence patterns (Table 4-3): Group A consists of 505 species commonly present in spring, summer and autumn seasons; group B consists of 220 species specific to the season of summer and autumn; groups C, D and E consisted of 44, 6 and 60 species specific to summer, autumn and spring, respectively; and group F consisted of 19 species randomly present in winter and more samples.

Five phyla, *Cyanobacteria*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* and *Firmicutes* were dominant in the Yodo and Kita Rivers in spring, summer, and autumn with

Table 4-3 Classification of detected bacterial species at the phylum level according to seasonal occurrence pattern

Phylum ^a	Pattern						Undetected in any of four seasons
	A Ubiquitous in spring, summer, and autumn	B Specific to summer and autumn	C Specific to summer	D Specific to autumn	E Specific to spring	F Random presence in winter and one or more seasons	
<i>Actinobacteria</i> (152)	70 [46] ^b	51 [34]	5 [3]	0 [0]	3 [2]	2 [1]	21 [14]
<i>Bacteroidetes</i> (48)	28 [58]	15 [31]	1 [2]	0 [0]	2 [4]	1 [2]	1 [2]
<i>Cyanobacteria</i> (39)	33 [85]	4 [10]	1 [3]	0 [0]	1 [3]	0 [0]	0 [0]
<i>Firmicutes</i> (226)	129 [57]	31 [14]	3 [1]	1 [0]	31 [14]	5 [2]	26 [12]
<i>Proteobacteria</i> (455)	230 [51]	93 [20]	10 [2]	5 [1]	14 [3]	10 [2]	93 [20]
– <i>Alpha</i> (123)	98 [80]	17 [14]	0 [0]	0 [0]	2 [2]	4 [3]	2 [2]
– <i>Beta</i> (78)	31 [40]	20 [26]	2 [3]	2 [3]	1 [1]	4 [5]	18 [23]
– <i>Gamma</i> (133)	63 [47]	36 [27]	6 [5]	0 [0]	8 [6]	1 [1]	19 [14]
– <i>Delta</i> (108)	33 [31]	13 [12]	2 [2]	3 [3]	3 [3]	1 [1]	53 [49]
– <i>Epsilon</i> (13)	5 [38]	7 [54]	0 [0]	0 [0]	0 [0]	0 [0]	1 [8]
Others (96)	15 [15]	26 [27]	24 [24]	0 [0]	9 [9]	1 [1]	23 [23]
Total (1,016)	505 [50]	220 [22]	44 [4]	6 [0]	60 [6]	19 [2]	164 [16]

^a Numbers in round parentheses indicate total targeted species.

^b Numbers in boxed parentheses indicate percentage of detected probes. Note the numbers are rounded-off to eliminate decimal values.

70%, 68%, 65%, 57% and 57% of the average detection ratio to total targeted species, respectively, although the most dominant phylum varied depending on the sample (Table 4-3). In the *Proteobacteria*, the alpha subclass (average detection ratio in spring, summer and autumn: 77%) was the most frequently detected, followed by gamma (average detection ratio: 59%) and beta (average detection ratio: 56%) subclasses. The numbers of species detected among the dominant phyla clearly decreased in the following order: summer > autumn > spring > winter; this was in accordance with the seasonal variation in the total number of bacterial species detected (Fig. 4-1). Thus, fluctuations in the abundance of the dominant bacterial phyla strongly influenced those of the entire bacterial community.

4.5 Principal component analysis

In PCA based on the data of microarray analysis, almost half (49.6%) of the total variation was explained by extracting the first (PC1) and second (PC2) principal components (Fig. 4-4). Scatter plot based on the PC1 and PC2 revealed that the 24 samples analyzed could be divided into three core groups, A, B and C. Core group A consisted of summer and autumn samples; core group B consisted of winter samples (as well as an anomalous sample from K1 and a spring sample from Y3); and core group C consisted of spring samples (as well as an anomalous sample from Y3 and a winter sample from K1). Further PCA performed on the samples classified into core group A clearly separated summer samples from autumn samples (Fig. 4-5). These results suggest that the bacterial species composition of the river water changed seasonally. The classification of a winter sample from K1 and a spring sample from Y3 into core groups C and B, respectively, was resulted from the tendency for the PCA score which depended on the total number of bacterial species detected in a sample. Namely, a spring sample from Y3 that had a lower number of bacterial species than the other spring samples was located in core group B, which usually had lower numbers of species; conversely, a winter sample from K1 that had a higher number of bacterial species than the other winter samples was grouped into core group C. The PC1 values obtained in the PCA (Fig. 4-4) showed a strong positive

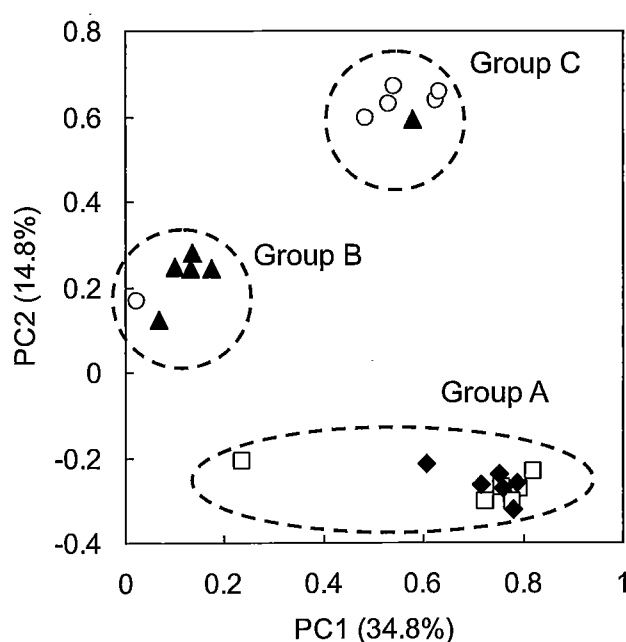


Fig. 4-4 Ordination produced from PCA based on microarray profiles in river water eubacterial community obtained from Yodo and Kita Rivers in spring (open circle), summer (closed diamond), autumn (open square) and winter (closed triangle).

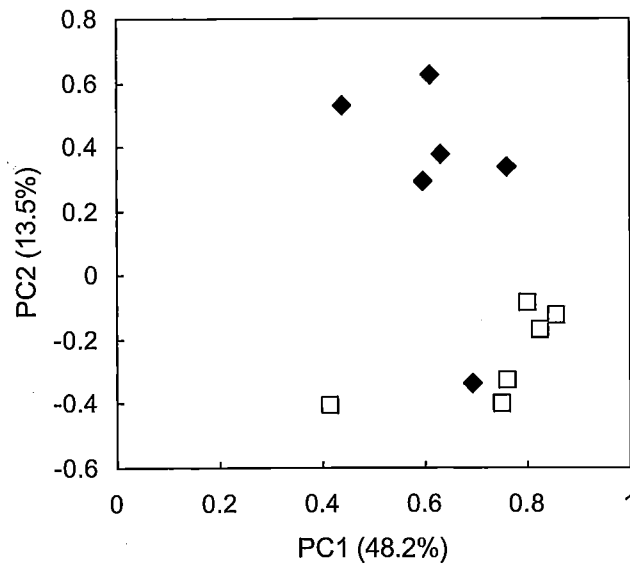


Fig. 4-5 PCA Ordination produced for group A in Fig. 4-4, with river samples obtained from Yodo and Kita Rivers in summer (closed diamond) and autumn (open square).

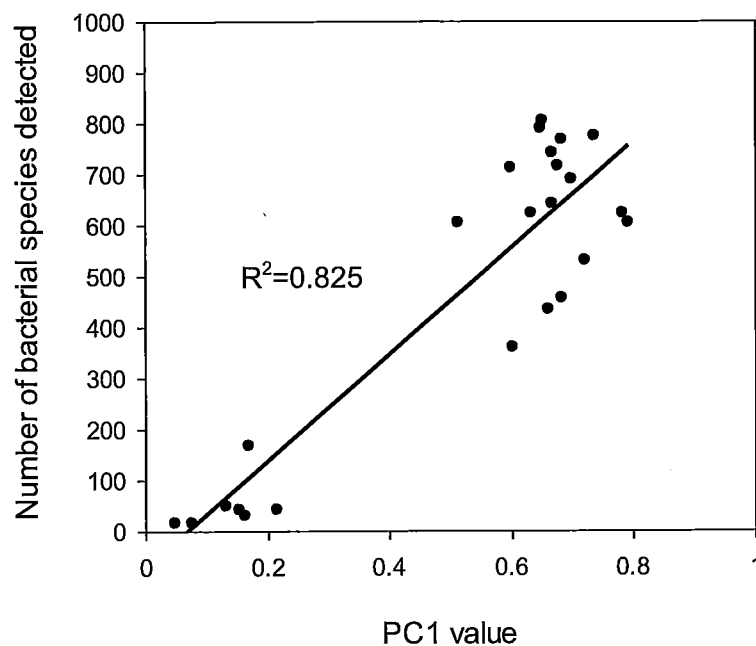


Fig. 4-6 Correlation between the value of principal component factor 1 (PC1) and the number of detected EU bacteria in river water samples.

correlation with the number of bacterial species detected as shown in Fig. 4-6. In contrast, no clear relationship was observed between the PC2 values and any of the physicochemical and biological parameters measured.

4.6 Discussion

DNA microarray analysis revealed that the bacterial community in river water environments varied primarily according to the seasons. Although, previous studies have also reported a strong influence of seasonal variation in environmental conditions on the bacterial composition of river water environment (Bell et al., 1982; Rubin et al., 2007), however in these studies the targeted bacterial species were limited to a few numbers and a comprehensive monitoring of broad variety of bacteria at *phylum* level was not carried out. Fewer bacterial species were detected in winter than in the other seasons, probably because most of the dominant bacteria in river water are mesophilic and the cold winter temperatures are unsuitable for their growth. In particular, although the numbers of culturable bacteria were similar in spring and winter (Table 4-1), the species diversity observed in the microarray analysis was much lower in winter than in spring (Table 4-2). The differences in 16S rDNA copy number between winter and the other seasons (Table 4-1) suggest that most of the unculturable bacteria, which may account for >99% of all bacteria in fresh water (Amann et al., 1995), do not persist under cold conditions. The recording of an exceptionally large number of bacterial species at K1 in winter suggests the presence of largely diverse bacteria at low concentrations and without highly dominant species. At Y3 in spring, despite the large 16S rDNA copy number, the number of detected species was quite low (Table 4-1 and Fig. 4-1), and their RSI was only marginal. This implies that those bacterial species, which are not targeted by the microarray, were abundantly present in this sample.

In each sampling season, the bacterial species composition at K1, the upstream station on the Kita River, was highly different from that at the downstream station (K2) on the same river and at any stations on the Yodo River. The physicochemical water quality parameters suggested that the upstream part of the Kita River generally had lower pollution levels, although the spring sample had relatively high organic and nitrogen concentrations. A number of previous studies have reported that the input of anthropogenic wastewaters containing various pollutants, including easily degradable organics, nutrients, and xenobiotic compounds, can affect the composition of riverine microbial communities (Fossi et al., 1995; Feris et al., 2004; Rodriguez et al., 2007; Rubin et al., 2007; Pesce et al., 2008). Thus, the difference in community composition between station K1 and the other stations may be due to dissimilarities in pollution levels. The slight increase in the number of bacterial species between Y2 and Y3 in the Yodo River (except spring) may have occurred partly because of the growth of some species that were present at undetectable levels

upstream and preferred the polluted conditions formed by the discharge of effluent containing various pollutants from the WWTPs located between Y2 and Y3. The elucidation on positive correlation between concentration of some bacterial species and pollution level was previously reported (Feris et al., 2004). The inflow of bacterial species in the WWTP effluent itself may also have contributed to the increase in the bacterial diversity between Y2 and Y3 (Iwane et al., 2001; Cébron et al., 2004).

Samples from station K2 showed exceptionally higher electrical conductivity in spring, summer, and autumn (7.0, 50.1 and 13.9 mS/cm, respectively) than the other samples (0.1 to 1.4 mS/cm; Table 4-1). Correspondingly, the bacterial communities in these K2 samples exhibited high species diversity. In contrast, the bacterial diversity at the same station in winter, when the electrical conductivity was surprisingly low (0.1 mS/cm), was the lowest among the samples from the Kita River. In the brackish environment near the mouth of a river, freshwater from the river and backflow from the sea intermix, creating unique conditions with characteristics intermediate between those of freshwater and seawater and with an increased hydraulic retention time. Consequently, a highly divergent microbial community can be established in the brackish environment (Crump et al., 1999). Therefore, the high bacterial diversity observed at station K2 in spring, summer, and autumn can be attributed to the formation of such brackish conditions.

Previous studies have reported that bacterial community composition gradually changes in large rivers along the course of flow (Sekiguchi et al., 2002; Winter et al., 2007). Especially Winter et al. (2007) suggested that such gradual shifts result from the adaptation of a riverine community to changing environmental conditions over the course of the river. In contrast, in the two rivers investigated here, the bacterial community appeared to respond sharply to specific geographic features and facilities which affect the river water quality rather successively adapt to changing conditions in the course of the water flow. Such differential spatial variation was likely to have been caused by the short retention times of the rivers we monitored. Moreover we targeted free-living bacteria in order to investigate the spatiotemporal changes in bacterial communities in the river environment. However, it has been pointed out that particle-attached biofilm bacteria are also an important part of the microbial ecosystem in riverine environments (Meyer 1994; Crump et al., 1999; Brummer et al., 2003; Olapade et al., 2005). Thus, further studies focused on biofilm bacteria, including clarification of the relationship between biofilm bacteria and the free-living bacteria analyzed here, are needed if we are to thoroughly understand the spatiotemporal variations in riverine bacterial communities.

The phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Cyanobacteria*, and

Bacteroidetes were found to be the dominant bacterial groups in the two rivers. *Proteobacteria*, *Firmicutes*, and *Cytophaga–Flavobacterium–Bacteroidetes* have been commonly detected as dominant bacterial groups in riverine environments (Brummer et al., 2000; Feris et al., 2004; Crum et al., 1999; Sekiguchi et al., 2002). In the phylum *Proteobacteria*, the *beta* subclass has been observed as dominant in freshwater and the *alpha* subclass as dominant in seawater (Crum et al., 1999). In contrast, we found that the *Alphaproteobacteria* were dominant in the two rivers in our study. Although the reason for the discrepancy has not been completely elucidated, this may be a local characteristic of the rivers we monitored. The presence of such indicator bacterial species helps in comparing the characteristics feature of the aquatic environment and thereby aid in predicting the environmental soundness.

4.7 Conclusion

The results from bacterial community analysis revealed the spatiotemporal variation in various species occurring in two small and steep rivers typical of those present in Japan. Seasonal variables most strongly affected the bacterial community, although geographical characteristics, including pollution level and specific sources (effluent from WWTPs and backflow of seawater), were also significant influences. We targeted free-living bacteria in order to investigate the spatiotemporal changes in bacterial communities in the river environment. However, it has been pointed out that particle-attached biofilm bacteria are also an important part of the microbial ecosystem in riverine environments (Meyer1994; Crump et al., 1999; Brummer et al., 2003; Olapade et al., 2005) and further studies are needed to investigate them.

CHAPTER 5

Occurrence of Bacterial Pathogens in Rivers

5.1 Background

Since surface freshwater is a primary source of drinking water for the majority of the world's human population, appropriate assessment and management of surface water quality is of great importance to protect against potential health risks associated with unintentional ingestion of microbiologically contaminated surface water. Nevertheless, during the last decade, 21 health hazard cases in Japan were caused by pathogenic microorganisms, including *Escherichia coli*, *Campylobacter jejuni*, *Shigella sonnei*, *Plesiomonas shigelloides*, *Yersinia enterocolitica*, *Leptospira* spp., *Clostridium botulinum*, norovirus, and rotavirus, in drinking water supplied from public or private water supply systems (Yamada et al., 2007). Plant and fish and shellfish pathogens have also caused serious damage to agriculture and fisheries and have disrupted natural ecosystems. To reduce the incidence of disease and problems caused by pathogenic microorganisms, more adequate prediction of pathogenic contamination of water sources is required, in addition to improvement of water resource management and disinfection systems.

In this chapter, bacterial pathogens were investigated using a DNA microarray targeting 1012 species/groups of bacterial pathogens infectious to humans, animals, plants, fish, and shellfish to comprehensively understand the occurrence and behavior of multiple bacterial pathogens in rivers. Based on the results, correlation of pathogens detected in multiple samples with total coliforms, a conventional hygienic water quality indicator, was assessed.

5.2 Pathogen profile in river waters

As shown in Table 4-1 (section 4.2), heterotrophic bacteria occurred in river water samples in quantities on the order of 10^3 to 10^5 CFU/ml. Total coliform counts in spring, summer, and winter samples varied from $<3.6 \times 10^1$ to 9.3×10^3 MPN/100 ml. Spring and summer samples tended to have higher total coliform counts than winter samples.

A total of 87 bacterial pathogen species/group, including 21 biosafety level 2 (BSL2) pathogens, were detected by the DNA microarray of 24 river water samples (Table 5-1). Forty-nine of the 87 pathogens (listed in Table 5-2) were present in two or more samples, and 45 of these pathogens were found in both rivers. Furthermore, 27 of the 49 pathogens were present in two distinct seasons, whereas the other 22 were detected in only one sampling period. The distribution of the remaining 38 pathogens, which each occurred in only one sample, was as follows: 3 at Y2, 6 each at Y1, Y3, and Y4, and 17 at K2. Although most of the detected pathogens were human or animal pathogens, two

fish/shellfish-infectious pathogens, *Pseudoalteromonas atlantica* (Costa-Ramos et al., 2004) and *Vibrio cholerae/mimicus* (Kiiyukia et al., 1992), and two plant pathogens, *Agrobacterium tumefaciens* (Escobar et al., 2003) and *Clavibacter michiganensis* (Jahr et al., 1999), were also detected, each in only one of the 4 sampling periods.

Table 5-1 Relative signal intensity of positive pathogen probes in 24 river water samples^a

Pathogen species/group	October, 2005						August, 2006						January, 2007						May, 2007					
	Y1	Y2	Y3	Y4	K1	K2	Y1	Y2	Y3	Y4	K1	K2	Y1	Y2	Y3	Y4	K1	K2	Y1	Y2	Y3	Y4	K1	K2
<i>Acetivibrio cellulosolvens</i>	0.62	0.25	0.51	0.30		0.60					0.31	1.42												
<i>Acinetobacter aceti</i>							0.30																	
<i>Acinetobacter anitratum</i>						0.56																		
<i>Acinetobacter baumannii</i>						0.56																		
<i>Acinetobacter haemolyticus</i>						0.54																		
<i>Acinetobacter johnsoni</i>						0.61																		
<i>Acinetobacter junii</i>						0.57																		
<i>Acinetobacter lwoffii</i>						0.41																		
<i>Acinetobacter radioresistens</i>						0.25																		
<i>Actinobacillus muris</i>													0.61	0.43	0.64	0.62	0.87	0.65	0.57	0.56	0.63	0.81	0.57	
<i>Actinobacillus pleuropneumoniae</i>													1.06	0.76	0.95	0.82	0.82	0.79	1.05	0.58	0.65	0.93	0.63	0.57
<i>Actinomadura</i> spp.	0.71					0.57																		0.83
<i>Aegyptianella pullorum</i>	0.29					0.49																		
<i>Agrobacterium tumefaciens</i> group						0.43																		
<i>Anaplasma marginale/centrale</i>	0.35					0.29																		
<i>Anaplasma phagocytophila</i>	0.30					0.37																		
<i>Arcobacter</i> genus	0.68	0.26	0.60	0.61		0.59																		
<i>Arcobacter</i> sp.	0.28																							
<i>Bacteroides distasonis</i>																						0.47	0.30	0.62
<i>Bacteroides fragilis</i>																								0.97
<i>Bacteroides urealyticus</i>	0.66	0.54	0.58	0.69		0.64					0.54													
<i>Balneatrix alpica</i>													0.57	0.49	0.90	0.62	0.79	0.63	0.54	0.55	0.64	0.58	0.59	0.97
<i>Bordetella avium</i> group	0.26																							
<i>Borrelia burgdorferi/valaisiana</i>									0.44															
<i>Brevundimonas diminuta</i>	0.75	0.37	0.63	0.56		0.66					0.72													
<i>Brevundimonas</i> group	0.68	0.43	0.58	0.62		0.63					0.49													
<i>Campylobacter concisus</i>	0.71	0.52	0.63	0.30		0.54																		
<i>Campylobacter fetus</i> group	0.33		0.26			0.34																		
<i>Campylobacter jejuni</i> group	0.69		0.61	0.27		0.58																		
<i>Campylobacter rectus</i>									0.57			0.50												
<i>Campylobacter sputorum</i>	0.59					0.40																		
<i>Centipeda periodontii</i>																					0.39			
<i>Chromobacterium violaceum</i>									0.42															
<i>Chryseobacterium meningosepticum</i> group (1) ^b	0.59	0.33	0.52	0.30		0.56					0.29													
<i>Chryseobacterium meningosepticum</i> group (2)								0.27	0.95															
<i>Chryseobacterium proteolyticum</i>						0.36																		
<i>Chryseobacterium scophthalmum</i>						0.30																		
<i>Clavibacter michiganensis</i>							1.19	1.19	0.91	1.42		1.35												
<i>Corynebacterium mycetoides</i>						0.31																		
<i>Eggerthella lenta</i> group										0.33														
<i>Eperythrozoon</i> spp.							1.15	1.13	1.30	0.61		1.04												
<i>Erysipelothrix</i> spp.						0.31																		
<i>Erysipelothrix rhusiopathiae</i>	1.29	0.38	0.63	0.81		0.67					0.65													
<i>Erysipelothrix tonsillarum</i>	0.65	0.29	0.37	0.66		0.57					0.56													

[illegible]

^b The same species with different numbers in parentheses indicate the probes designed based on different sequences from the same species.

Table 5-2 Biosafety level of pathogens detected in two or more samples^a

Pathogen species/group	BSL ^b	Pathogen species/group	BSL	Pathogen species/group	BSL
Acetivibrio cellulosolvens	–	<i>Campylobacter sputorum</i>	1	Leptospira noguchii	1
Actinobacillus muris	–	<i>Chryseobacterium meningosepticum</i> group (1) ^c	2	Leptospira parva	–
Actinobacillus pleuropneumoniae	1	C. meningosepticum group (2)	2	Leptospira santarosai	1
<i>Actinomadura</i> spp.	2	Clavibacter michiganensis	–	Mannheimia granulomatis	1
<i>Aegyptianella pullorum</i>	1	Eperythrozoon spp.	1	Mannheimia haemolytica	1
<i>Anaplasma marginale/centrale</i>	1-2	<i>Erysipelothrix rhusiopathiae</i>	2	Marinospirillum megaterium	–
<i>Anaplasma phagocytophila</i>	2	<i>Erysipelothrix tonsillarum</i>	–	Moraxella caviae	–
<i>Arcobacter</i> genus	1	Ewingella americana	1	Moraxella lacunata group	1
Bacteroides distasonis	1	Haemophilus influenzae (1)	2	Mycobacterium mucogenicum	2
<i>Bacteroides urealyticus</i>	1	H. influenzae (2)	2	<i>Olsenella uli</i>	1
Balneatrix alpica	1	Haemophilus parasuis (1)	2	Pasteurella bettyae	1
<i>Brevundimonas diminuta</i>	1	H. parasuis (2)	2	Pasteurella caballi	–
<i>Brevundimonas</i> group	1	Hafnia alvei	1	Pasteurella pneumotropica (1)	2
<i>Campylobacter concisus</i>	1	Klebsiella oxytoca group	2	P. pneumotropica (2)	2
<i>Campylobacter fetus</i> group	2	<i>Kluyvera ascorbata</i>	1	Treponema denticola	1
<i>Campylobacter jejuni</i> group	1-2	<i>Kluyvera cryocrescens</i>	1		
Campylobacter rectus	1	<i>Lactobacillus</i> spp.	1		

^a Pathogen species/groups shown in boldface were assessed for their correlation with total coliforms.

^b Biosafety level (BSL) according to the Japanese Society for Bacteriology (Japanese Society for Bacteriology, 2007).

^c Numbers in parentheses following the species name indicate that probes were designed based on different sequences of the same species.

The number of pathogen species/groups found varied among samples (Fig. 5-1). Similar numbers of pathogens (16 to 20 species) were detected at all six sampling stations in spring and winter. Even the pathogen profile was almost identical in the 12 samples collected in these seasons; the following species were present in all 12 samples: *Actinobacillus pleuropneumoniae*, *Balneatrix alpica*, *Haemophilus influenzae* and *H. parasuis*, *Mannheimia granulomatis*, *M. haemolytica*, *Marinospirillum megaterium*, and *Moraxella caviae* and *M. lacunata*. Summer samples contained the lowest numbers of bacterial pathogen species (9 to 16 species) among the four seasons. In autumn, 10, 15, and 13 pathogen species occurred at stations Y2, Y3, and Y4, respectively. In contrast, in the same season, 24 and 32 different pathogens occurred at stations Y1 and K2, respectively. Exceptionally, all 1012 bacterial pathogens targeted were below the detection limit in autumn at station K1.

In the Yodo River, 16, 5, 9, and 15 species/groups were detected at all four stations in spring, summer, autumn, and winter, respectively (Table 5-1; Fig. 5-1). The number of pathogen species increased from Y2 to Y3 and slightly decreased from Y3 to Y4 in summer and autumn. In summer, 7 and 5 species/groups that were never detected at the upstream stations Y1 and Y2, respectively, were found at Y3 and Y4, respectively. In the Kita River, the pathogens detected at stations K1 and K2 were completely different between stations in summer and autumn although almost identical in spring and winter, as mentioned above.

In the PCA against the occurrence pattern of bacterial pathogens in river water samples, excluding the autumn sample from K1, in which no pathogen was detected, 73.0% of the total variation was explained by the first (PC1) and second (PC2) principal components.

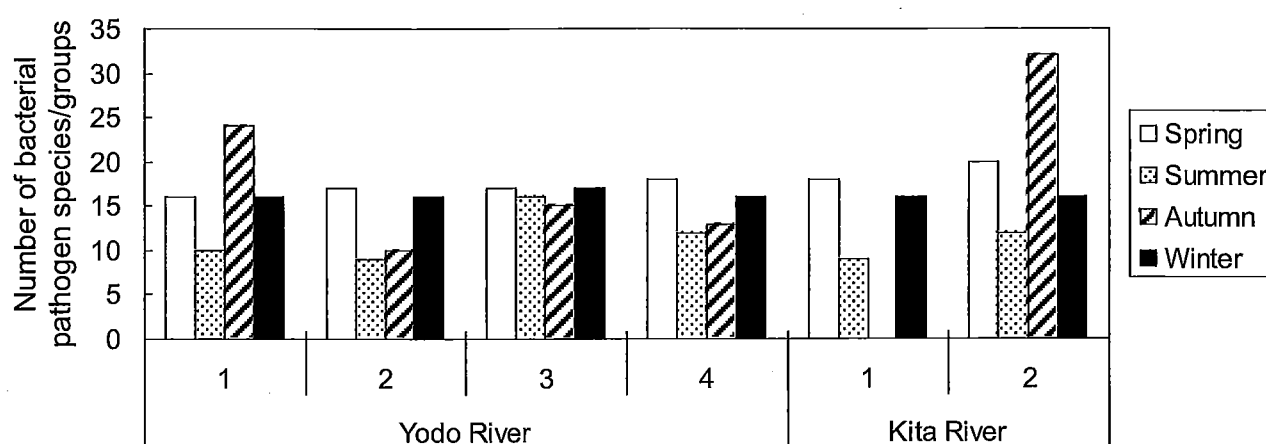


Fig. 5-1 Spatial and temporal variations in the number of pathogenic bacterial species in the Yodo and Kita rivers. Probes with relative intensity > 0.25 in the microarray analysis were judged as positive.

Scatter plot based on PC1 and PC2 revealed that the 23 analyzed samples fell into three distinct groups A, B, and C, depending basically on the season of sample collection (Fig. 5-2); group A consisted of all of the spring and winter samples, group B consisted of the autumn samples from all stations on the Yodo River and station K2 on the Kita River and a summer sample from K1, and group C consisted of the summer samples from all stations on the Yodo River and from K2 on the Kita River.

5.3 Correlation between pathogens detected by microarray and total coliforms

Traditionally, water quality is measured in terms of fecal bacterial count. In this study we wanted to establish a correlation between coliform count and the detected pathogens from microarray experiments. The correlation between the RSIs of the 30 probes detected in two or more samples in spring, summer, or winter (shown in boldface in Table 5-2) and the relative total coliform count was assessed. The RSI of 18 probes increased with the relative total coliform count (e.g., Fig. 5-3 A–C). In contrast, the remaining 12 probes corresponding to 11 pathogenic bacteria did not show a positive correlation with the relative total coliform count (e.g., Fig. 5-3 D–F). The latter group included 4 fecal bacteria (*Campylobacter rectus*,

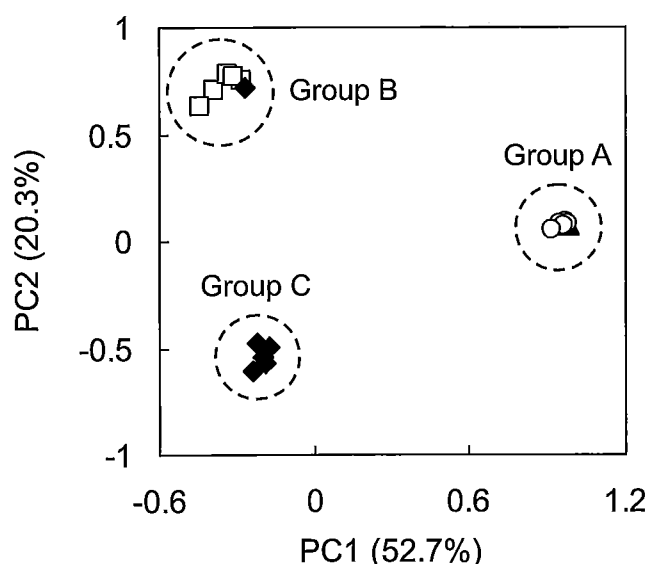


Fig. 5-2 Ordination produced from a principal component analysis based on pathogen profiles of river water samples collected from the Yodo and Kita rivers in spring (open circles), summer (closed diamonds), autumn (open squares), and winter (closed triangles).

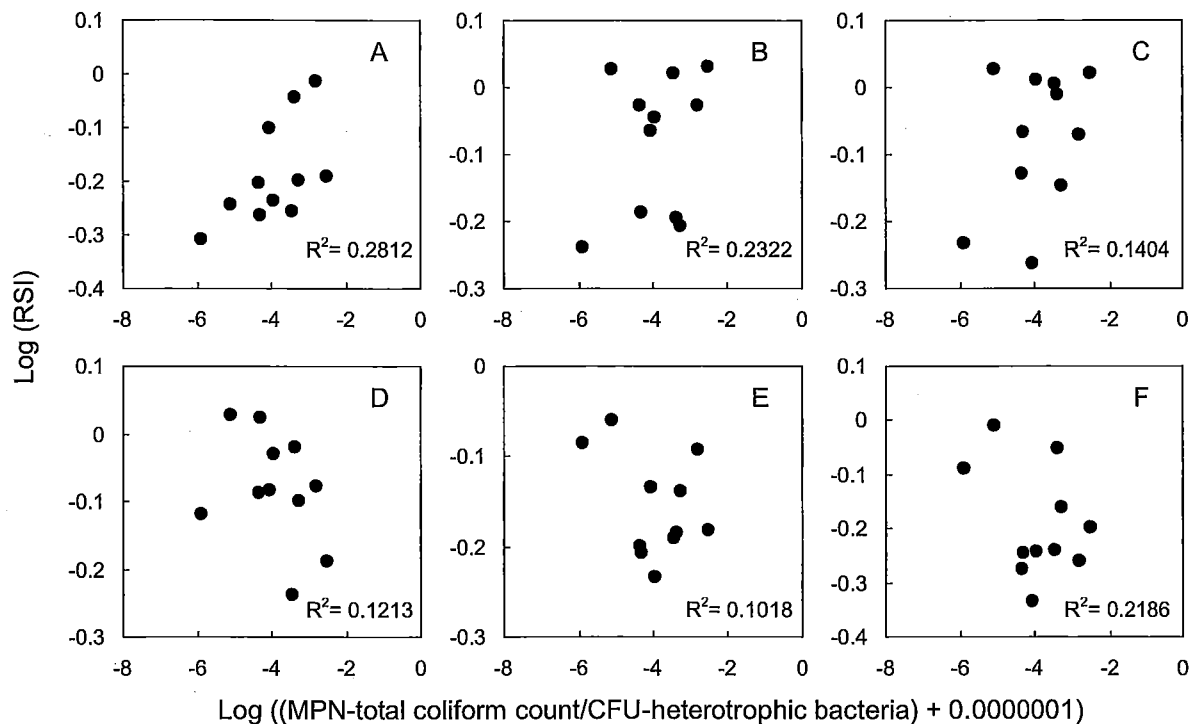


Fig. 5-3 Examples of correlations between the coliform count and the relative signal intensity of pathogen probe detected. The total coliform count relative to the total number of heterotrophic bacteria was used. A, *Balneatrix alpica*; B, *Moraxella caviae*; C, *Pasteurella bettyae*; D, *Actinobacillus pleuropneumoniae*; E, *Haemophilus influenzae* (1); F, *Mannheimia granulomatis*. A, B, and C are examples of a positive correlation, and D, E, and F are examples without any positive correlation.

Leptospira noguchii, *Leptospira parva*, *Leptospira santarosai*) and 7 non-fecal bacteria (*A. pleuropneumoniae*, *Eperythrozoon* spp., *H. influenzae*, *H. parasuis*, *Klebsiella oxytoca* group, *M. granulomatis*, and *Pasteurella pneumotropica* [2 targets]).

5.4 Discussion

In this chapter, the simultaneous detection of multiple pathogens in surface waters from the Yodo and Kita Rivers in the Kinki district of Japan was carried out. Of the two monitored rivers, the Yodo River is a relatively polluted urban river, whereas the Kita River is a clean, rural river. The geographical features of the two river basins suggest that neither has any potential fecal sources other than WWTPs. No unusual abundance of fecal indicator bacteria in the WWTP effluents or in the surface waters of either river has been reported in recent years. Therefore, the health risk associated with waterborne pathogens does not appear to be easily predictable by use of the conventional fecal indicators in these basins. Nevertheless, there were a total of 87 pathogen species/groups in the survey. In addition,

more than half were present in both rivers, and one-third occurred in two seasons. These results suggest that specific groups of bacterial pathogens may be commonly present in surface waters in the monitoring region. Furthermore, the detection of fish/shellfish and plant pathogens, in addition to human and animal pathogens, indicates that the surface waters may pose health hazards to various organisms, with consequent economic and ecological damage.

The pathogen profile in the surface waters of the monitored rivers varied primarily according to the season. As described in chapter 3, total bacterial community in surface waters in Yodo River and Kita River varied seasonally. Thus, it was expected that the pathogen profile in the rivers changes in accordance with the natural, season-dependent appearance/disappearance of microorganisms. It has been also reported that the incidence of some kinds of pathogens in river environments is influenced by seasonal variables, particularly water temperature (Eyles et al., 2003; Pfeffer et al., 2003; Caruso et al., 2005; Hsieh et al., 2008).

Bacterial pathogens enter surface waters from both point and non-point sources, including in raw sewage, effluent from WWTPs, and run-off from agriculture and livestock farming (Lemarchand et al., 2003; Exall et al., 2004; Kim et al., 2005; Savichtcheva et al., 2006). Among these potential sources, effluent from WWTPs is recognized as an important pathogen source that can alter the pathogen profile along a river's course. In our monitoring area, several WWTPs are located between stations Y2 and Y4 on the Yodo River. The number of pathogen species/groups became marginally elevated between Y2 and Y3 in summer and autumn, and several pathogens that were absent at the upstream stations emerged at Y3 and Y4 in summer. From these results, we inferred that effluent from WWTPs slightly impacted the pathogen profile of the Yodo River in summer and autumn. However, no noticeable impact was observed in spring or winter, suggesting that the influence of WWTPs on the pathogen profile in the Yodo River is marginal, despite the input of a large amount of WWTP effluent by repeated use of river water (by the time it reaches the river's mouth the downstream, the water has been used five times) (Sumitomo et al., 1998), compared with the predominant impact by seasonal factors. In the Yodo River basin, coverage of the sewer system was nearly 90% in FY2004. In addition, WWTPs within the basin are equipped with satisfactory disinfection units. These facts suggest that WWTPs are not a significant pathogen source in the basin because the public sewer system has been sufficiently improved, even though the Yodo River basin is relatively polluted according to the BOD level.

The bacterial species diversity in river water increases at the mouth, where a brackish

Table 5-3 Pathogenic bacteria uncorrelated with coliform count

Fecal/Non-fecal Pathogen species		Biosafety level ^a
Fecal	<i>Campylobacter rectus</i>	2
	<i>Klebsiella oxytoca</i> group	2
	<i>Leptospira noguchii</i>	2
	<i>Leptospira parva</i>	—
	<i>Leptospira santarosai</i>	—
Non-fecal	<i>Actinobacillus pleuropneumoniae</i>	—
	<i>Eperythrozoon</i> spp.	—
	<i>Haemophilus influenzae</i> (serotype A)	2
	<i>Haemophilus parasuis</i> (serotype B)	2
	<i>Mannheimia granulomatis</i>	2
	<i>Pasteurella pneumotropica</i> (serotypes B, C)	2

a: Biosafety level to human determined by World Health Organization.

water environment results from the mixing of river water and seawater (Crump et al., 1999; Hewson et al., 2004). Thus, we speculated that the pathogen diversity would also be higher at the mouth of a river than further upstream where no mixing occurred. As expected, our results showed that the pathogen species richness in the Kita River was greater at K2, near the mouth of the river (nearly 500 m upstream from the mouth), than at K1. The occurrence of the highest number of distinct pathogens at K2 presumably also resulted from the unusual condition of a brackish water environment, namely, characteristics intermediate between those of freshwater and seawater. Therefore, waters at the river mouth, under brackish water conditions, are likely to serve as a reservoir of diverse bacterial pathogens even in the case of a less-polluted river like the Kita River.

Recent studies have shown that conventional hygienic water quality indicators are not well correlated with feces-related bacterial pathogens such as the *Bacteroides-Prevotella* group (Okabe et al., 2007), *Campylobacter* spp. (Hörman et al., 2004), *Salmonella* spp. (Lemarchand et al., 2003), and *Yersinia* spp. (Lund, 1996) or eukaryotic pathogens such as *Cryptosporidium* spp. (Lund, 1996; Lemarchand et al., 2003; Hörman et al., 2004). In this study, it was observed that 11 of the 30 pathogen species/groups assessed did not show a significant positive correlation with total coliforms (Table 5-3). These species/groups included not only opportunistic but also BSL2 pathogens (*Haemophilus influenzae*, *Haemophilus parasuis*, *Klebsiella oxytoca*, and *Pasteurella pneumotropica*), and more than half were non-fecal. To our knowledge, this is the first report suggesting the possibility that multiple pathogens, including both fecal and non-fecal ones, show a low correlation with the conventional hygienic indicator. The lack of a positive correlation between these pathogens

and the conventional fecal indicator may reflect dissimilarity in the environmental behavior between some bacterial pathogens and the indicator bacteria (Lemarchand et al., 2003; Hörman et al., 2004). Moreover, with respect to non-fecal pathogens, the source and route of their discharge into surface waters differ from those of the fecal indicator bacteria, which may be another important reason for the low correlation. Because species of *Leptospira*, which have in fact caused a human health hazard via the drinking water supply in Japan (Yamada et al., 2007), were among the pathogens that did not correlate with total coliforms, it is clear that the conventional hygienic indicators cannot necessarily predict the occurrence of significant bacterial pathogens, as observed from our results. Therefore, systematization of a new set of indicators that can comprehensively predict the occurrence of various pathogens is strongly required for assessment of the health risks associated with waterborne pathogens. Among the pathogens that did not positively correlate with the total coliforms in this study, high-risk (BSL2) and non-fecal pathogens may be candidates for new indicators. From the viewpoint of preventing damage to agriculture and fisheries as well as ecological damage, pathogens infectious to fish/shellfish and plants should also be considered as candidate indicators for advanced management of the aquatic environment. Further study on the simultaneous determination of the occurrence of the candidate indicator pathogens suggested here and in earlier studies (Savichtcheva et al., 2007) should be performed by cost-effective and reliable molecular tools such as multiplex real-time PCR (Ibekwe et al., 2002; Elizaquível et al., 2008) to establish an ideal set of hygienic indicators for advanced management of the aquatic environment.

5.5 Conclusion

The distribution and frequent occurrence of multiple pathogens from Yodo River and Kita River have been studied in this chapter. A broad range of pathogens infecting humans, animals, plants, fish, and shellfish were targeted. A total of 87 pathogens were detected in 24 river water samples, and more than half of them were present in both the rivers. There was a strong influence of seasonal variation in the pathogen occurrence. Several non-fecal pathogens showed a negative or no correlation with the total coliforms. Thus the conventional hygienic indicator for fecal contamination is inadequate for comprehensive determination of the health risks associated with contamination of river water by bacterial pathogens, and systematization of a new set of indicators that can comprehensively predict the occurrence of various pathogens is strongly required for advanced management of the aquatic environment.

CHAPTER 6

General Conclusions

Biological indicators have received much attention for the assessment of soundness of aquatic environment under the goal of creating and conserving healthy aquatic ecosystem and healthy water circulation system. In particular, microorganisms play pivotal roles in breaking down organic matter and remineralizing nutrients and thus largely influence the energy flux and elemental and material cycles in aquatic ecosystem, which suggests that microbial indicators would be greatly helpful to understand and assess the soundness of whole ecosystem. However, aquatic microbial community largely fluctuates depending on the natural variables (e.g. seasonal variables) and anthropogenic impacts. To establish practical microbial indicators, it is of great importance to profoundly understand the shift of microbial community (populations) in response to the change of environmental condition.

Therefore, in this study, total bacterial community, specific functional bacterial genes and pathogenic bacteria were selected as candidate microbial indicators, and their fates in river water were analyzed using DNA microarray technique. A total of 24 surface water samples collected seasonally from 4 stations of Yodo River and 2 stations of Kita River were subjected to the analyses.

In chapter 3, the occurrence and distribution of a total of 33 bacterial functions using 85 gene probes related to organic pollutant degradation, carbon, nitrogen and sulfur cycles, metal metabolisms, and energy flow in river water were investigated. It was observed that the presence of functional bacterial genes was greatly influenced by the seasonal variations. Especially, in summer, the detected genes were in larger numbers than in other seasons. Further it was revealed that the number of detected functions decreased by water retention time in a dam or lake, whereas they increased by receiving WWTP effluent. Results obtained here suggested that 33 microbial functions were classified into three groups: (A) universally present genes, (B) genes specifically present under specific geographical and seasonal conditions and (C) non-existent genes. Functional bacterial genes classified in the group (B) may be useful as indicators for the assessment of environmental soundness because their presence (or absence) is influenced by the physiochemical parameters and the water quality conditions than group A or group C genes.

In chapter 4, total bacterial community in river water samples was analyzed using a DNA microarray targeting 1016 eubacterial species (*Actinobacteria*, 152; *Bacteroidetes*, 48; *Cyanobacteria*, 39; *Firmicutes*, 226; *Proteobacteria*, 455 (*alpha* subclass, 123; *beta* subclass, 78; *gamma* subclass, 133; *delta* subclass, 108; *epsilon* subclass, 13); others, 96). It was revealed that seasonal variables most strongly affected the diversity and composition of bacterial community. In particular, the number of bacterial species in winter was quite low

as compared with that in the other seasons. In addition, the diversity of bacterial community in river water was influenced by the inflow of WWTP effluent and backflow of seawater. These results suggested that the overall bacterial diversity can be expressed in terms of seasonal influencing factors, geographical features and river management factors for the use in assessment of soundness of aquatic environment.

In chapter 5, the occurrence of 1012 species/groups of bacterial pathogens infectious to humans, animals, plants, fish, and shellfish in river waters was investigated. A total of 87 pathogens including 21 BSL2 pathogens were detected in 24 river water samples, and more than half of them were present commonly in Yodo River and Kita River. There was a strong influence of seasonal variation in the pathogen occurrence. In addition, the number of pathogenic bacteria in river water appeared to be increased by the WWTP effluent load and backflow of seawater (Crump et al., 1999; Hewson et al., 2004). Several fecal and non-fecal pathogens did not show a positive correlation with the total coliforms, suggesting that the conventional hygienic indicator for fecal contamination is inadequate for comprehensive determination of the health risks associated with contamination of river water by bacterial pathogens.

According to the results in this study, a hypothetical scheme for evaluating the soundness of aquatic environment is proposed. In case of functional bacterial genes, those functions belonging to group (B) (genes specifically present under certain geographical and seasonal conditions), namely organic pollutant degradation, elemental and material cycles (carbon, nitrogen and sulfur cycles and energy flow) and metal metabolism, can be applied in the environmental assessment scheme. As for total bacterial community, the total bacterial diversity, which is expressed by the diversity index and certain coefficients based on seasonal factors and geographical and river management factors, can be an indicator. In case of pathogens, hygienic safety can be evaluated based on the population and BSL of each detected pathogen although certain weighting coefficients for specific geographical feature and seasonal variations were not considered. In addition, the physicochemical parameters used in current environmental assessment should be included because they greatly influence the soundness of the environment.

Therefore, by calculating the scores for total bacterial diversity, organic pollutant degradation function, elemental and material cycling function, metal metabolizing function, hygienic safety and physicochemical status and expressing in radar chart, the soundness of aquatic environment can be visually understood. Fig. 6-1 shows an example of the application of evaluating the soundness of aquatic environment proposed here. In the radar chart, high scores of total bacterial diversity and organic pollutant degradation, elemental

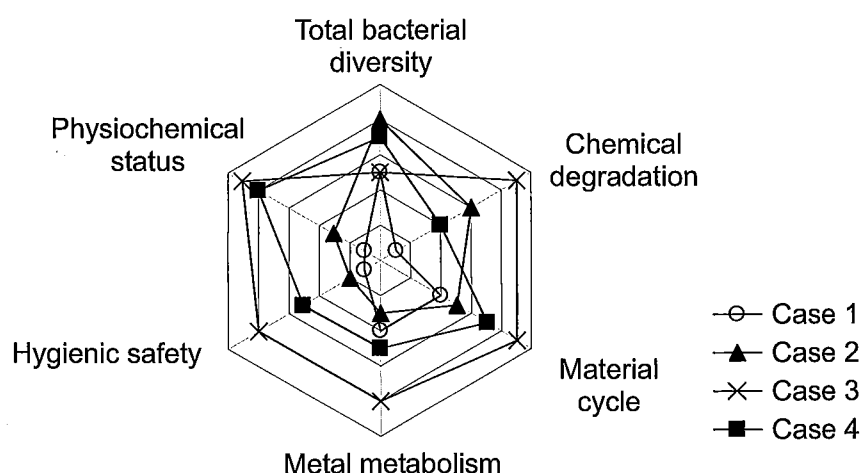


Fig. 6-1 Proposed scheme for evaluation of the soundness of riverine environment

and material cycling and metal metabolizing functions mean high diversity and high potentials for the functions, respectively. Conversely, high scores of hygienic safety and physicochemical status mean high pollutant level. Based on the score distribution pattern, environmental status can be classified into a variety of types. For example, organic pollutant degradation function can be roughly classified into four grades, coupled with the pollution level. That is, a riverine environment in which the pollutant load is very low and the organic carbon degradation potential is also low as shown by a hypothetical case 1 in Fig. 6-1 is a virgin area without impact from anthropological activities for a long period. In an environment with low pollutant load and high organic pollutant degradation potential shown as case 2 in Fig. 6-1, high water quality has been kept by sufficient natural purification. Station K1 in Kita River investigated in this study may be under this condition. By contrast, in an environment when both the pollutant load and organic pollutant degradation potential are high as case 3 in Fig. 6-1, although the natural purification potential has been enhanced due to long-term pollution, water quality is not maintained. An environment where the pollutant load is very high but organic pollutant degradation potential is low, as case 4 in Fig. 6-1, indicates that natural purification does not work well due to abrupt increase of pollutant load. This scheme can be used to assess the overall condition of a given environment by identifying the above input parameters and evaluating the microbial effectiveness in reconditioning the aquatic environment.

By the use of the scheme as proposed here, evaluation of the environmental soundness from various viewpoints can be accomplished. However, there exist significant issues to be addressed so as to accomplish adequate assessment of the environmental soundness. Firstly, the investigation was performed once for each season and the data from DNA

microarray analyses were not quantitative. To accurately understand influential factors, investigation should be continued for long period. Because it is difficult to get quantitative data in DNA microarray analysis, quantitative methods such as real-time PCR should be applied for target bacteria or genes selected in DNA microarray analysis. It is necessary to accumulate quantitative data for target bacteria or genes so as to quantitatively evaluate individual indicator. Secondly, although this study targeted free-living bacteria in surface water, bacteria attached to particles and present in sediment also make a great contribution to the pollutant degradation and material/elemental cycles in the river environment. Thus, bacterial community in whole river environment should be included in the investigation. Thirdly, the target in the investigation of bacterial functions and pathogens was insufficient and should be expanded for overall understanding of bacterial community. As for bacterial functions, those functions related to the degradation of various xenobiotic compounds, phosphorus cycle and so on should be included in the analysis of environmental functions. In addition, virus and bacteriophage should be included in the analysis of pathogens. Fourthly, the scoring system in the environmental soundness assessment scheme proposed above should be scientifically established. Further studies to overcome these issues can provide a practically usable environmental soundness assessment system for creation and conservation of healthy aquatic environment.

CHAPTER 7

References

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APPENDIX A

Microarray Analysis of Eubacterial Community and Bacterial Pathogens in Landfill Leachate from Three Different Landfills in Japan

A.1 Introduction

Leachates are generated when rainwater enters a landfill site and in the absence of a proper landfill lining this leachate may percolates easily in the environment contaminating the groundwater sources. Landfills are deposited with hundreds of thousands of heterogeneous waste materials, thus leachates can have a complex characteristic often containing various toxic and life threatening compounds (Ömana et al., 2008). Leachate characteristics vary considerably according to the type and composition of wastes, the age, status and design of landfill, and so on (Kumar et al., 2005). Microbiological studies in landfills are of great importance for the following two reasons. Firstly, as microorganisms are vital in decomposing waste compounds in landfills, in particular, members of the domain Bacteria undertake most of the biochemical reactions toward the waste decomposition. Thus, their fates are closely linked with the stabilization of landfills like the quality of leachates generated, release of harmful gases, and so on. Secondly, landfills are a potential source of pathogens because wastes disposed in landfills include sewage sludge, garbage and animal feces. Thus, leachates running from landfills can pose a health-risk if they get released without proper treatment of the pathogens (Sobsey, 1978; Fedorak et al., 1991; Straub et al., 1993).

In recent years, several researches have analyzed the entire bacterial communities (Huang et al., 2004; Huang et al., 2005; Sawamura et al., 2007) and also reported the occurrence of frank and opportunistic pathogens in bulk soils as well as leachates (Cooper et al., 1975; Sobsey, 1978; Hale Boothe et al., 2001; Graczyk et al., 2008). However, detection of bacterial pathogens was limited to culturable populations, and the occurrence of pathogens in a viable but non-culturable state was not reported yet. For exactly assessing a possible microbial health risk derived from landfilling and establishing a preventative strategy, what kinds of and how many pathogens emerge in landfill leachates should be elucidated by the application of molecular methods.

To give some insights into the microbiology in landfill ecosystems and the potential microbial contamination in leachates, the overall eubacterial community and the bacterial pathogens occurring in leachates collected from three landfills that were different each other in the disposed wastes and the landfill age were analyzed using DNA microarray technique.

A.2 Materials and methods

A.2.1 Leachate samples

Leachate samples used in this study were collected once during FY2005 from three different landfill sites in Japan. Characteristics of three landfills and their leachates are summarized in Table A-1. Three landfills received different type of solid wastes, and landfill B was still active while landfills A and C had been closed. Leachate in landfill C contained much higher concentration of organic compound and nitrogen than in the other landfills.

A.2.2 DNA microarray analysis

Microarray analysis was carried out using two types of DNA microarrays, eubacterial microarray targeting 1016 eubacterial species and pathogen microarray targeting 1012 bacterial pathogens. Microarray analysis and thereafter statistical analysis was performed in accordance with the methods described in chapter 2, with the exception that test spots whose RSI exceeded 0.01 were assigned as positive signal intensities in both microarrays.

Table A-1 Characteristics of landfills and their leachate samples analyzed

Item	Landfill		
	A	B	C
Major constituents	Raw garbage, sewage sludge, and construction debris without pretreatment	Plastics, shredded unburnable residue and incineration ash	Shredder residue, sludge, incineration ash and unburnable wastes
Landfilling status (Disposition period)	Closed (1979-2003)	Active (since 1992)	Closed (1992-1999)
Precipitation (mm) ^a			
- in FY 2005	990	1148	2425
- from opening to 2005	728-1538 (1059) ^b	1535-2642 (2093)	938-1870 (1297)
Leachate quality			
- pH	7.7	6.8	na
- COD (mg/L)	60	10-20	600
- T-N (mg/L)	45	na	400

^a Precipitation data was obtained from records for a point which was geographically nearest to each of the sampling station.

^b Numbers indicate minimum - maximum (mean) precipitation values.
COD, Chemical oxygen demand; T-N, total nitrogen; na, not analyzed.

A.3 Results and discussion

A.3.1 Eubacterial community diversity

Eubacterial population profiles obtained in DNA microarray analysis are shown in Fig. A-1 and summarized at the phylum level in Table A-2. More than 70% of the targeted

species in phyla *Actinobacteria*, *Bacteroides*, *Cyanobacteria*, *Firmicutes* and *Proteobacteria* were detected in all three samples. Samples A and B exhibited marginally more abundant species in the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, especially *alpha*-, *beta*- and *gamma*-subclasses, than sample C. Several species in *Deferribacteres*, *Deinococcus-Thermus*, *Fusobacteria* and *Thermomicrobia* classified in “Others” in Table A-2 were specifically detected in sample B. *Proteobacteria*, *Firmicutes* and *Cytophaga-Flexibacter-Bacteroides* group have been predominantly and commonly detected in leachates from landfills in Japan (Okinawa), Vietnam, Thailand (Sawamura et al., 2007) and Korea (Huang et al., 2004; Huang et al., 2005). It was thus suggested that predominant eubacterial populations in landfill leachate may be common in broad parts of East and Southeast Asia, irrespective of the climate zone: Tropical and subtropical climate in Okinawa, Vietnam and Thailand or mesothermal climate in Korea and our monitoring area of Japan.

In contrast, as expected, each of three leachate samples analyzed here showed slightly different eubacterial community structure (Fig. A-1, Table A-2). Previous study has reported that eubacterial community composition in landfill leachate can alter significantly according to various factors such as waste type and volume, landfill age and hydrogeologic positioning (Lee et al., 2006). Interestingly, PCA based on RSI of detected species showed

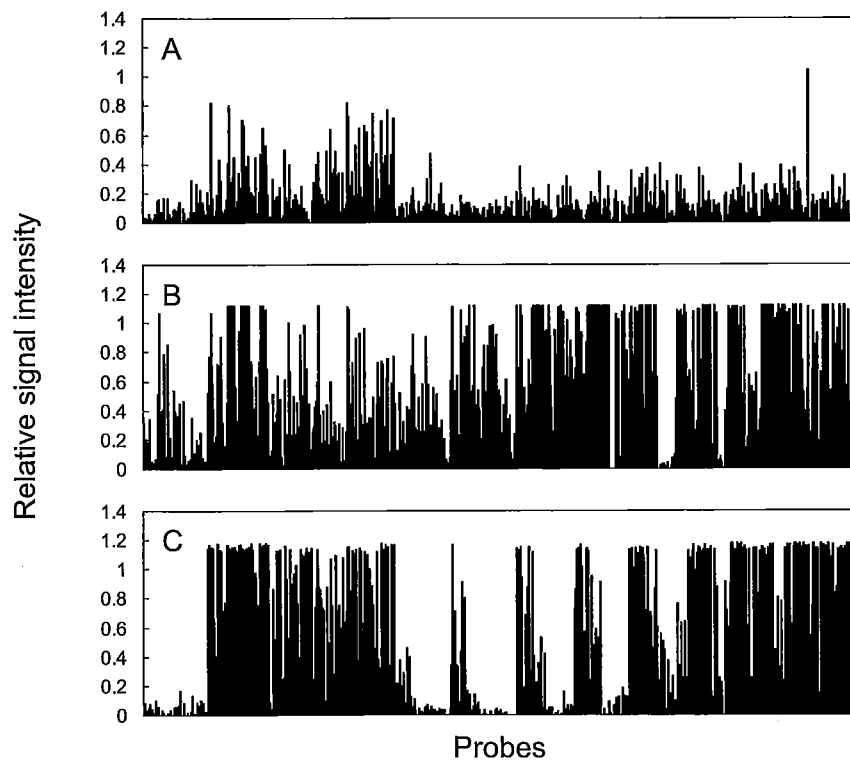


Fig. A-1 Relative signal intensities of eubacterial populations in leachate samples A, B and C obtained from DNA microarray analysis

Table A-2 Distribution of eubacterial species in leachate samples

Phylum ^a	Sample ^b		
	A	B	C
<i>Actinobacteria</i> (152)	126 [82]	123 [81]	106 [70]
<i>Bacteroidetes</i> (48)	45 [94]	41 [85]	37 [77]
<i>Cyanobacteria</i> (39)	35 [90]	36 [92]	36 [92]
<i>Firmicutes</i> (226)	169 [75]	174 [77]	151 [67]
<i>Proteobacteria</i> (455)	362 [80]	363 [80]	335 [74]
- <i>Alpha</i> (123)	119 [97]	121 [98]	108 [88]
- <i>Beta</i> (78)	62 [79]	64 [82]	59 [76]
- <i>Gamma</i> (133)	111 [83]	116 [87]	105 [79]
- <i>Delta</i> (108)	58 [54]	51 [47]	51 [47]
- <i>Epsilon</i> (13)	12 [92]	11 [85]	12 [92]
Others (96)	49 [51]	63 [66]	40 [42]
Total (1,016)	786 [77]	800 [79]	705 [69]

^a Numbers in round parentheses indicate total targeted species.

^b Numbers in box parentheses indicate percentage of detected species to the total targeted species.

that eubacterial community compositions in samples B and C are relatively similar, but different from that in sample A (Fig. A-2). The dissimilarity of the opening period, namely in 1979 for landfill A whereas in 1992 for landfills B and C, may relate to such difference of eubacterial community structure between landfill A and landfills B and C. However, further studies should be conducted to completely elucidate the influential factor(s) on the entire eubacterial community structure in landfill environment. The diversity of the eubacterial populations in sample B was considerably higher than those in samples A and C (Fig. A-3).

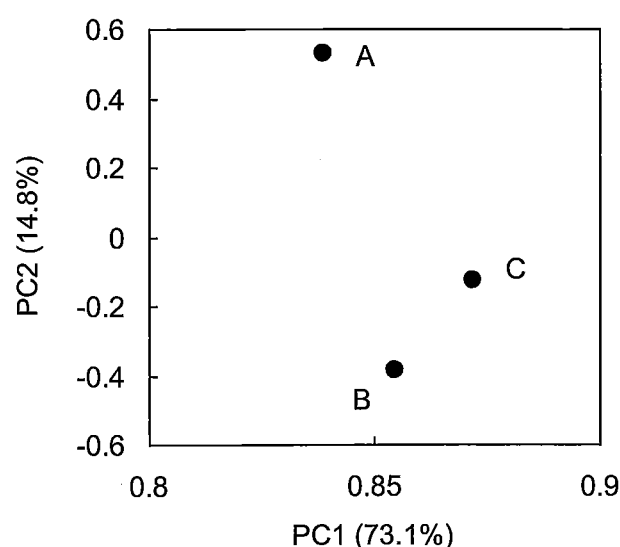


Fig. A-2 Ordination produced from principal component analysis for eubacterial community in three leachate samples.

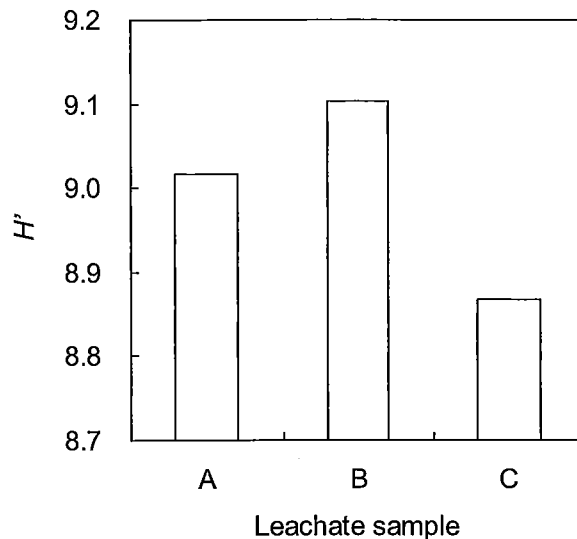


Fig. A-3 Shannon-Weaver diversity index (H') calculated from DNA microarray analyses of eubacterial community in three leachate samples.

It was thus suggested that the landfill status would be one of the critical factors determining the eubacterial diversity in landfills: eubacterial community would be more divergent in active landfills than in closed landfills. Minor species specifically detected in sample B mentioned above may be specific populations in active landfill where the wastes, in which a certain energy and carbon sources are contained, are still constantly supplied and active metabolic reactions occur. In contrast, the lowest eubacterial diversity in landfill C (Fig. A-3) may be caused by a deleterious effect of xenobiotic compounds as implied by a high concentration of chemical oxygen demand (Table A-1). Samples B and C were located closely in PCA (Fig. A-2), but their diversities were quite different due to the presence of several minor species in sample B. Such minor species carry less weightage during the PCA calculation and we get an overall similarity between samples B and C, however in case of diversity calculations, the presence of minor species contributed to the overall diversity index and consequently sample B has higher species diversity as compared to sample C.

A.3.2 Pathogenic bacterial distribution

Pathogen profiles in 3 leachate samples are shown in Fig. A-4, and a total of 44 bacterial pathogens detected are listed in Table A-3. Samples A, B and C contained 43, 27 and 42 species/groups of bacterial pathogens, respectively. Twenty-six of the 44 detected pathogens were commonly present in all three samples. Samples A and C contained a quite greater diversity of pathogens than sample B, and 41 species/groups out of a total of

Table A-3 Distribution of pathogenic bacteria in leachate samples

Pathogen species/group	Sample			Biosafety Level ^a
	A	B	C	
<i>Acetivibrio cellulosolvens</i>	0.545 ^b	0.679	0.695	— ^c
<i>Actinomadura</i> spp.	0.211	0.020	0.705	1–2 ^d
<i>Aegyptianella pullorum</i>	0.014	ND ^e	0.127	1
<i>Agrobacterium tumofaciens</i> group	0.312	0.016	0.669	—
<i>Anaerococcus hydrogenalis</i>	0.185	ND	0.082	1
<i>Anaplasma marginale/centrale</i>	0.015	ND	0.168	—
<i>Arcobacter butzleri</i>	0.116	ND	0.026	1
<i>Arcobacter</i> genus	0.675	0.171	0.291	1
<i>Bacteroides urealyticus</i>	0.726	0.070	0.629	1
<i>Bartonella</i> group	0.192	ND	0.690	1–2
<i>Bordetella avium</i> group	0.090	ND	0.221	1
<i>Bordetella pertussis</i> group	0.047	0.031	0.175	2
<i>Brevundimonas diminuta</i>	0.759	0.220	0.701	1
<i>Brevundimonas</i> group	0.727	0.350	0.755	1
<i>Campylobacter concisus</i>	0.769	0.706	0.457	1
<i>Campylobacter fetus</i> group	0.382	0.034	0.123	2
<i>Campylobacter jejuni</i> group	0.065	0.014	0.270	1–2
<i>Campylobacter sputorum</i>	0.245	0.033	0.075	1
<i>Caulobacter fusiformis</i>	0.281	0.022	0.336	—
<i>Caulobacter halobacterioide</i>	0.235	0.019	0.334	—
<i>Caulobacter henricii</i>	0.213	ND	0.168	—
<i>Caulobacter intermedius</i>	0.378	0.051	0.634	—
<i>Chryseobacterium meningosepticum</i> group	0.571	0.477	0.628	2
<i>Chryseobacterium proteolyticum</i>	0.190	ND	0.074	—
<i>Chryseobacterium scophthalmum</i>	0.040	ND	0.106	—
<i>Chryseomonas luteola</i>	0.029	ND	0.161	1
<i>Corynebacterium auris</i>	0.157	ND	0.028	1
<i>Corynebacterium mycetoides</i>	0.635	ND	0.079	—
<i>Eperythrozoon</i> spp.	0.268	0.035	0.146	1
<i>Erysipelothrix rhusiopathiae</i>	0.781	0.123	0.680	2
<i>Erysipelothrix</i> spp.	0.120	ND	0.054	—
<i>Erysipelothrix tonsillarum</i>	0.658	0.139	0.686	—
<i>Flavimonas oryzihabitans</i>	0.055	0.015	0.145	1
<i>Francisella</i> group	0.092	0.024	0.166	2–3
<i>Kluyvera ascorbata</i>	0.075	0.648	0.705	1
<i>Kluyvera cryocrescens</i>	0.650	0.407	0.649	1
<i>Kluyvera georgiana</i>	0.010	0.025	0.154	—
<i>Legionella feeleii</i>	0.054	0.304	ND	2
<i>Megasphaera elsdenii</i>	0.113	ND	ND	1
<i>Moraxella caviae</i>	0.125	0.023	0.066	—
<i>Mycoplasma kahnei</i>	0.070	0.054	0.192	—
<i>Sphingomonas parapaucimobilis</i>	0.018	ND	0.279	1
<i>Sphingomonas paucimobilis</i>	ND	ND	0.188	1
<i>Yokenella regensburgei</i> group	0.124	ND	0.083	1

^aBiosafety level classified by Japanese Society for Bacteriology in 2007.

^bNumbers for each pathogen/sample indicate relative signal intensities in microarray analysis.

^c—, Biosafety level is not identified.

^dDifferent biosafety levels are set for pathogens within the same group.

^eND, not detected.

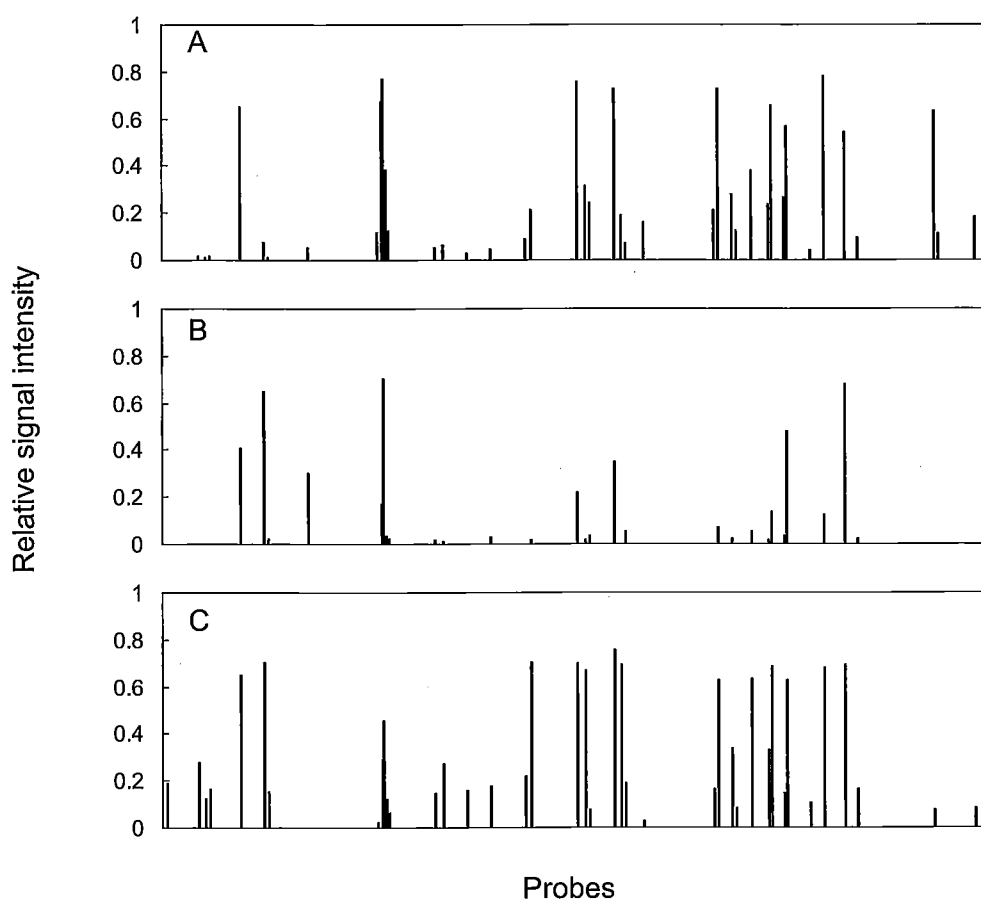


Fig. A-4 Relative signal intensities of bacterial pathogens in leachate samples A, B and C obtained from DNA microarray analysis

44 detected pathogens were commonly found in them. These results suggest that pathogens existing in landfill leachates are limited to certain common taxonomies. Twenty-six pathogens were commonly present in landfill leachates irrespective of the waste type and landfill age. Hale Boothe et al. (Hale Boothe et al., 2001) have reported that certain pathogens persist in the landfill site even after the landfill is closed. Commonly detected pathogens in our monitoring landfills included *Francisella*, although the abundance seems low. Genus *Francisella* is a small gram-negative bacteria which includes *Francisella tularensis* that is able to infect a wide range of animals including rodents and even humans and causes the highly debilitating or fatal disease tularemia (Oyston et al., 2005). Common pathogens also included several pathogens classified into the biosafety level 2 under the Japanese classification (Japanese Society of Bacteriology, 2007) (Table A-3). Ubiquitous presence of such high-risk pathogens may be a great concern, although the possibility of microbial risks derived from our monitoring sites is low because possible environmental impacts are eliminated by some leachate treatment before discharging.

Landfills A and C, where diverse pathogens were detected, commonly contained a large

amount of organic wastes such as sludge and raw garbage. Sludge contains a great number of pathogens, but conventional sludge treatment is not always effective in reducing numbers of pathogen (Straub et al., 1993). Therefore, sludge would be a major source of pathogens that specifically occur in these landfills. Abundant organics and nutrients provided from organic wastes may also support the growth of specific pathogens. However, since the pathogen profile in landfill would vary along with the waste decomposition stage as with the entire eubacterial community, abundant species of pathogens present in landfills A and C may decrease with time. Particularly, this would be possible by depletion of organics and nutrients resulted from further progression of the waste decomposition. Furthermore, fewer pathogen species in landfill B, where pretreated wastes were mainly disposed (Table A-1), may suggest that advanced waste management with appropriate waste pretreatment can lower the potential health-risks associated with the landfill and ensure the public health.

Recently, strict standards were implicated to evaluate a landfill's performance based on pretreatment of waste, biological stabilization by aeration, emissions of leachates and gases from the landfill into the environment, and after-use of its land (Tanaka et al., 2005). Nevertheless, a criterion to evaluate pathogenicity has never indicated. This would be obviously because of the lack of field data on the type of existing pathogens in landfills and their leachates. In that context, results of this study would be valuable for establishing a set of indicators and a criterion for preventing possible microbial risks derived from landfill sites.

A.4 Conclusion

Eubacterial communities and bacterial pathogens occurring in the leachates collected from three full-scale landfills in mesothermal area of Japan were analyzed using DNA microarrays targeting approximately a thousand eubacterial and pathogen species/groups. The leachate samples showed almost similar eubacterial compositions with marginal variations. Comparison of the results with precedents suggested that the predominant eubacterial populations in the landfills may be generally similar in East and Southeast Asia, irrespective of the climate zone. Analysis of the bacterial pathogens showed that the three leachates included a total of 44 species/groups of bacterial pathogens and 26 species/groups including *Francisella*, a frank zoonotic pathogens causing a serious health risk, and other high-risk pathogens were commonly found. It is suggested that sludge can be a source of specific pathogens. In addition, the 2 leachate samples from the landfills, where organic wastes such as sludge and raw garbage were mainly disposed, had 15

specific pathogens. It was suggested that sludge can increase the variation of the pathogens occurring in landfill leachates. Overall, results of this study posed some insights into the underlying microbial ecology in the landfill and the possible microbiological health risks associated with the landfill leachate.

APPENDIX B

Probes used in making DNA Microarrays

B.1 Eubacterial probes

Phylum	Class	Order	Family	Genus	Species
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Acidimicrobium	acidobacterium
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Geothrix	geothrix
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Holophaga	holophaga
Actinobacteria	Acidimicrobiae	Acidimicrobiales	Acidimicrobineae	Acidimicrobium	acidimicrobium
Actinobacteria	Actinobacteridae	Actinobacteriales	Corynebacteriaceae	Corynebacterium	corynebacterium
Actinobacteria	Actinobacteridae	Actinobacteriales	Corynebacteriaceae	Dietzia	dietzia
Actinobacteria	Actinobacteridae	Actinobacteriales	Actinosynnemataceae	Actinokineospora	actinokineospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Actinosynnemataceae	Actinosynnema	actinosynnema
Actinobacteria	Actinobacteridae	Actinobacteriales	Actinosynnemataceae	Lentzea	lentzea
Actinobacteria	Actinobacteridae	Actinobacteriales	Actinosynnemataceae	Saccharothrix	saccharothrix
Actinobacteria	Actinobacteridae	Actinobacteriales	Frankiaceae	Frankia	frankia
Actinobacteria	Actinobacteridae	Actinobacteriales	Gordoniaceae	Gordonia	gordonia
Actinobacteria	Actinobacteridae	Actinobacteriales	Gordoniaceae	Skermania	skermania
Actinobacteria	Actinobacteridae	Actinobacteriales	Geodermatophilaceae	Blastococcus	blastococcus
Actinobacteria	Actinobacteridae	Actinobacteriales	Geodermatophilaceae	Geodermatophilus	geodermatophilus
Actinobacteria	Actinobacteridae	Actinobacteriales	Geodermatophilaceae	Modestobacter	modestobacter
Actinobacteria	Actinobacteridae	Actinobacteriales	Glycomycetaceae	Glycomyces	glycomyces
Actinobacteria	Actinobacteridae	Actinobacteriales	Kineosporiaceae	Cryptosporangium	cryptosporangium
Actinobacteria	Actinobacteridae	Actinobacteriales	Kineosporiaceae	Kineococcus	kineococcus
Actinobacteria	Actinobacteridae	Actinobacteriales	Kineosporiaceae	Kineosporia	kineosporia
Actinobacteria	Actinobacteridae	Actinobacteriales	Mycobacteriaceae	Mycobacterium	mycobacterium
Actinobacteria	Actinobacteridae	Actinobacteriales	Microsphaeraceae	Microsphaera	microsphaera
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Actinoplanes	actinoplanes
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Catellatospora	catellatospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Catenuloplanes	catenuloplanes
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Couchioplanes	couchioplanes
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Dactylosporangium	dactylosporangium
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Micromonospora	micromonospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Pilimelia	pilimelia
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Spirilliplanes	spirilliplanes
Actinobacteria	Actinobacteridae	Actinobacteriales	Micromonosporaceae	Verrucosispora	verrucosispora
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardiaceae	Nocardia	nocardia
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardiaceae	Rhodococcus	rhodococcus
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardiaceae	Rhodococcus	rhodochronus2
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardiaceae	Rhodococcus	rhodochrous1
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardioidaceae	Actinopolymorpha	actinopolyspora
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardioidaceae	Aeromicrobium	aeromicrobium
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardioidaceae	Friedmanniella	friedmanniella
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardioidaceae	Hongia	hongia
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardioidaceae	Kribbella	kribbella
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardioidaceae	Marmoricola	marmoricola
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardioidaceae	Micropruina	micropruina
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardioidaceae	Nocardioides	nocardioides
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardiopsaceae	Nocardiosis	nocardiosis

Phylum	Class	Order	Family	Genus	Species
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardiopsaceae	Thermobifida	thermobifida
Actinobacteria	Actinobacteridae	Actinobacteriales	Propionibacteriaceae	Luteococcus	luteococcus
Actinobacteria	Actinobacteridae	Actinobacteriales	Propionibacteriaceae	Microlunatus	microlunatus
Actinobacteria	Actinobacteridae	Actinobacteriales	Propionibacteriaceae	Propionibacterium	propionibacterium
Actinobacteria	Actinobacteridae	Actinobacteriales	Propionibacteriaceae	Propioniferax	propioniferax
Actinobacteria	Actinobacteridae	Actinobacteriales	Propionibacteriaceae	Tessaracoccus	tessaracoccus
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Actinoalloteichus	actinoalloteichus
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Amycolata	amycolata
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Amycolatopsis	amycolatopsis
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Kibdelosporangium	kibdelosporangium
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Kutzneria	kutzneria
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Prauserella	prauserella
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Pseudonocardia	pseudonocardia
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Saccharomonospora	saccharomonospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Saccharopolyspora	saccharopolyspora
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Streptoalloteichus	streptoalloteichus
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Thermobispora	thermobispora
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Thermocrisum	thermocrisum
Actinobacteria	Actinobacteridae	Actinobacteriales	Promicrosporaceae	Promicromonospora	promicromonospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptomycetaceae	Streptomyces	streptomyces
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Acrocarpospora	acrocarpospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Microbispora	microbispora
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Microtetraspora	microtetraspora
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Nonomuraea	nonomuraea
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Planobispora	planobispora
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Planomonospora	planomonospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Planopolyspora	planopolyspora
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Planotetraspora	planotetraspora
Actinobacteria	Actinobacteridae	Actinobacteriales	Streptosporangiaceae	Streptosporangium	streptosporangium
Actinobacteria	Actinobacteridae	Actinobacteriales	Symbiobacterium	Symbiobacterium	symbiobacterium
Actinobacteria	Actinobacteridae	Actinobacteriales	Sporichthyaceae	Acidotherrus	acidotherrus
Actinobacteria	Actinobacteridae	Actinobacteriales	Sporichthyaceae	Sporichthya	sporichthya
Actinobacteria	Actinobacteridae	Actinobacteriales	Thermomonosporaceae	Actinomadura	actinomadura
Actinobacteria	Actinobacteridae	Actinobacteriales	Thermomonosporaceae	Spirillospora	spirillospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Thermomonosporaceae	Thermomonospora	thermomonospora
Actinobacteria	Actinobacteridae	Actinobacteriales	Tsukamurellaceae	Tsukamurella	tsukamurella
Actinobacteria	Actinobacteridae	Actinobacteriales	Williamsiaceae	Williamsia	williamsia
Actinobacteria	Actinobacteridae	Actinomycetales	Actinomycetaceae	Actinobaculum	actinobaculum
Actinobacteria	Actinobacteridae	Actinomycetales	Actinomycetaceae	Actinomyces	actinomyces
Actinobacteria	Actinobacteridae	Actinomycetales	Actinomycetaceae	Arcanobacterium	arcanobacterium
Actinobacteria	Actinobacteridae	Actinomycetales	Actinomycetaceae	Mobiluncus	mobiluncus
Actinobacteria	Actinobacteridae	Actinomycetales	Actinomycetaceae	Mobiluncus	mobiluncus
Actinobacteria	Actinobacteridae	Actinomycetales	Beutenbergiaceae	Beutenbergia	beutenbergia
Actinobacteria	Actinobacteridae	Actinomycetales	Bogoriellaceae	Bogoriella	bogoriella
Actinobacteria	Actinobacteridae	Actinomycetales	Brevibacteriaceae	Brevibacterium	brevibacterium
Actinobacteria	Actinobacteridae	Actinomycetales	Cellulomonadaceae	Cellulomonas	cellulomonas

Phylum	Class	Order	Family	Genus	Species
Actinobacteria	Actinobacteridae	Actinomycetales	Cellulomonadaceae	Oerskovia	oerskovia
Actinobacteria	Actinobacteridae	Actinomycetales	Dermabacteraceae	Brachybacterium	brachybacterium
Actinobacteria	Actinobacteridae	Actinomycetales	Dermabacteraceae	Dermabacter	dermabacter
Actinobacteria	Actinobacteridae	Actinomycetales	Dermacoccaceae	Dermacoccus	dermacoccus
Actinobacteria	Actinobacteridae	Actinomycetales	Dermacoccaceae	Kytococcus	kytococcus
Actinobacteria	Actinobacteridae	Actinomycetales	Dermatophilaceae	Dermatophilus	dermatophilus
Actinobacteria	Actinobacteridae	Actinomycetales	Intrasporangiaceae	Intrasporangium	intrasporangium
Actinobacteria	Actinobacteridae	Actinomycetales	Intrasporangiaceae	Janibacter	janobacter
Actinobacteria	Actinobacteridae	Actinomycetales	Intrasporangiaceae	Ornithinicoccus	ornithinicoccus
Actinobacteria	Actinobacteridae	Actinomycetales	Intrasporangiaceae	Ornithinimicrobium	ornithinimicrobium
Actinobacteria	Actinobacteridae	Actinomycetales	Intrasporangiaceae	Terrabacter	terrabacter
Actinobacteria	Actinobacteridae	Actinomycetales	Intrasporangiaceae	Terracoccus	terracoccus
Actinobacteria	Actinobacteridae	Actinomycetales	Intrasporangiaceae	Tetrasphaera	tetrasphaera
Actinobacteria	Actinobacteridae	Actinomycetales	Jonesiaceae	Jonesia	jonesia
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Agrococcus	agrococcus
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Agromyces	agromyces
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Aureobacterium	aureobacterium
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Clavibacter	clavibacter
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Cryobacterium	cryobacterium
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Curtobacterium	curtobacterium
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Frigoribacterium	frigoribacterium
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Leifsonia	leifsonia
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Leucobacter	leucobacter
Actinobacteria	Actinobacteridae	Actinomycetales	Microbacteriaceae	Subtercola	subtercola
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Arthrobacter	arthrobacter
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Arthrobacter	1
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Arthrobacter	2
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Arthrobacter	3
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Demetria	demetria
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Kocuria	kocuria
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Micrococcus	micrococcus
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Micrococcus	1
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Micrococcus	2
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Micrococcus	3
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Nesterenkonia	nesterenkonia
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Renibacterium	renibacterium
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Rothia	rothia
Actinobacteria	Actinobacteridae	Actinomycetales	Micrococcaceae	Stomatococcus	stomatococcus
Actinobacteria	Actinobacteridae	Actinomycetales	Nocardiaceae	Nocardia	1
Actinobacteria	Actinobacteridae	Actinomycetales	Nocardiaceae	Nocardia	2
Actinobacteria	Actinobacteridae	Actinomycetales	Nocardiaceae	Nocardia	3
Actinobacteria	Actinobacteridae	Actinomycetales	Nocardiaceae	Nocardia	4
Actinobacteria	Actinobacteridae	Actinomycetales	Rarobacteraceae	Rarobacter	rarobacter
Actinobacteria	Actinobacteridae	Actinomycetales	Sanguibacteraceae	Sanguibacter	sanguibacter
Actinobacteria	Actinobacteridae	Actinomycetales	Nocardioidaceae	Rhodococcus	1
Actinobacteria	Actinobacteridae	Actinomycetales	Nocardioidaceae	Rhodococcus	2

Phylum	Class	Order	Family	Genus	Species
Actinobacteria	Actinobacteridae	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	bifidobacterium
Actinobacteria	Actinobacteridae	Bifidobacteriales	Bifidobacteriaceae	Gardnerella	gardnerella
Actinobacteria	Actinobacteridae	Bifidobacteriales	Unknown affiliation	Actinobispora	actinobispora
Actinobacteria	Actinobacteridae	Bifidobacteriales	Unknown affiliation	Actinocorallia	actinocorallia
Actinobacteria	Actinobacteridae	Bifidobacteriales	Unknown affiliation	Excellospora	excellospora
Actinobacteria	Actinobacteridae	Bifidobacteriales	Unknown affiliation	Turicella	turicella
Actinobacteria	Actinobacteridae	Coriobacteriales	Coriobacteriaceae	Atopobium	atopobium
Actinobacteria	Actinobacteridae	Coriobacteriales	Coriobacteriaceae	Collinsella	collinsella
Actinobacteria	Actinobacteridae	Coriobacteriales	Coriobacteriaceae	Coriobacterium	coriobacterium
Actinobacteria	Actinobacteridae	Coriobacteriales	Coriobacteriaceae	Cryptobacterium	cryptobacterium
Actinobacteria	Actinobacteridae	Coriobacteriales	Coriobacteriaceae	Denitrobacterium	denitrobacterium
Actinobacteria	Actinobacteridae	Coriobacteriales	Coriobacteriaceae	Eggerthella	eggerthella
Actinobacteria	Actinobacteridae	Coriobacteriales	Coriobacteriaceae	Slackia	slackia
Actinobacteria	Actinobacteridae	Micrococcineae	Microbacteriaceae	Microbacterium	1
Actinobacteria	Actinobacteridae	Micrococcineae	Microbacteriaceae	Microbacterium	2(barkeri)
Actinobacteria	Actinobacteridae	Micrococcineae	Microbacteriaceae	Microbacterium	3
Actinobacteria	Actinobacteridae	Rubrobacteriales	Rubrobacteriaceae	Rubrobacter	rubrobacter
Actinobacteria	Actinobacteridae	Sphaerobacterales	Sphaerobacteriaceae	Sphaerobacter	sphaerobacter
Actinobacteria	Rubrobacteridae	Rubrobacterales	Rubrobacterineae	Thermoleophilum	thermoleophilum
Aquificae	Aquificae	Aquificales	Aquificaceae	Aquifex	aquifex
Aquificae	Aquificae	Aquificales	Aquificaceae	Calderobacterium	calderobacterium
Aquificae	Aquificae	Aquificales	Aquificaceae	Hydrogenobacter	hyddrogenobacter
Aquificae	Aquificae	Aquificales	Aquificaceae	Thermocrinis	thermocrinis
Aquificae	Aquificae	Aquificales	Genera icertae sedis	Desulfurobacterium	desulfurobacterium
Bacteroidetes	Bacteroidetes	Bacteroidales	Bacteroidaceae	Anaerorhabdus	anaerorhabdus
Bacteroidetes	Bacteroidetes	Bacteroidales	Bacteroidaceae	Bacteroides	bacteroides
Bacteroidetes	Bacteroidetes	Bacteroidales	Bacteroidaceae	Megamonas	megamonas
Bacteroidetes	Bacteroidetes	Bacteroidales	Porphyomonadaceae	Dysgonomonas	dysgonomonas
Bacteroidetes	Bacteroidetes	Bacteroidales	Porphyomonadaceae	Porphyromonas	porphyromonas
Bacteroidetes	Bacteroidetes	Bacteroidales	Porphyromonadaceae	Oribaculum	oribaculum
Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Hallella	hallella
Bacteroidetes	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	prevotella
Bacteroidetes	Bacteroidetes	Bacteroidales	Rikenellaceae	Marinilabilia	marinilabilia
Bacteroidetes	Bacteroidetes	Bacteroidales	Rikenellaceae	Rikenella	rikenella
Bacteroidetes	Flavobacteria	Flavobacteriales	Blattbacteriaceae	Blattabacterium	blattabacterium
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Bergeyella	bergeyella
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	capnocytophaga
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Cellulophaga	cellulophaga
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	chryseobacterium
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Coenonia	coenonia
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Empedobacter	empedobacter
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium	flavobacterium

Phylum	Class	Order	Family	Genus	Species
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Gelidibacter</i>	<i>gelidibacter</i>
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Myroides</i>	<i>myroides</i>
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Ornithobacterium</i>	<i>ornithobacterium</i>
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Polaribacter</i>	<i>polaribacter</i>
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Psychroflexus</i>	<i>psychroflexus</i>
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Psychroserpens</i>	<i>psychroserpens</i>
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Riemerella</i>	<i>riemerella</i>
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Salegentibacter</i>	<i>salegentibacter</i>
<i>Bacteroidetes</i>	<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Weeksella</i>	<i>weeksella</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Crenotrichaceae</i>	<i>Chitinophaga</i>	<i>chitinophaga</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Crenotrichaceae</i>	<i>Rhodothermus</i>	<i>rhodothermus</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flammeovirgaceae</i>	<i>Flammeovirga</i>	<i>flammeovirga</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flammeovirgaceae</i>	<i>Flexithrix</i>	<i>flexithrix</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flammeovirgaceae</i>	<i>Persicobacter</i>	<i>persicobacter</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flammeovirgaceae</i>	<i>Thermonema</i>	<i>thermonema</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Cyclobacterium</i>	<i>cyclobacterium</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Cytophaga</i>	<i>cytophaga</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Dyadobacter</i>	<i>dyadobacter</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Flectobacillus</i>	<i>flectobacillus</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Flexibacter</i>	<i>flexibacter</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Hymenobacter</i>	<i>hymenobacter</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Microscilla</i>	<i>microscilla</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Runella</i>	<i>runella</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Spirosoma</i>	<i>spirosoma</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Flexibacterraceae</i>	<i>Sporocytophaga</i>	<i>sporocytophaga</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Sapraspiraceae</i>	<i>Haliscomenobacter</i>	<i>haliscomenobacter</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Sapraspiraceae</i>	<i>Lewinella</i>	<i>lewinella</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Sapraspiraceae</i>	<i>Saprospira</i>	<i>saprospira</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Sphingobacteriaceae</i>	<i>Pedobacter</i>	<i>pedobacter</i>
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Sphingobacteriales</i>	<i>Sphingobacteriaceae</i>	<i>Sphingobacterium</i>	<i>sphingobacterium</i>
<i>Chlamydia</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Chlamydiaceae</i>	<i>Chlamydia</i>	<i>chlamydia</i>
<i>Chlamydia</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Chlamydiaceae</i>	<i>Chlamydophila</i>	<i>chlamydophila</i>
<i>Chlamydia</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Chlamydiaceae</i>	<i>Chlamydophila</i>	<i>chlamydophila</i>
<i>Chlamydia</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Chlamydiaceae</i>	<i>Chlamydophila</i>	<i>chlamydophila</i>
<i>Chlamydia</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Chlamydiaceae</i>	<i>Chlamydophila</i>	<i>chlamydophila</i>
<i>Chlamydia</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Parachlamydiaceae</i>	<i>Parachlamydia</i>	<i>parachlamydia</i>
<i>Chlamydia</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>Simkaniaceae</i>	<i>simkaniaceae</i>
<i>Chlamydia</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Waddliaceae</i>	<i>Waddlia</i>	<i>waddlia</i>
<i>Chlorobi</i>	<i>Chlorobia</i>	<i>Chlorobiales</i>	<i>Chlorobiaceae</i>	<i>Chlorobium</i>	<i>chlorobium</i>
<i>Chlorobi</i>	<i>Chlorobia</i>	<i>Chlorobiales</i>	<i>Chlorobiaceae</i>	<i>Chloroherpeton</i>	<i>chloroherpeton</i>

Phylum	Class	Order	Family	Genus	Species
<i>Chlorobi</i>	<i>Chlorobia</i>	<i>Chlorobiales</i>	<i>Chlorobiaceae</i>	<i>Pelodictyon</i>	<i>pelodictyon</i>
<i>Chlorobi</i>	<i>Chlorobia</i>	<i>Chlorobiales</i>	<i>Chlorobiaceae</i>	<i>Prosthecochloris</i>	<i>prosthecochloris</i>
<i>Chloroflexi</i>	<i>Chloroflexi</i>	<i>Chloroflexales</i>	<i>Chloroflexaceae</i>	<i>Chloroflexus</i>	<i>chloroflexus</i>
<i>Chloroflexi</i>	<i>Chloroflexi</i>	<i>Chloroflexales</i>	<i>Chloroflexaceae</i>	<i>Chloronema</i>	<i>chloronema</i>
<i>Chloroflexi</i>	<i>Chloroflexi</i>	<i>Chloroflexales</i>	<i>Chloroflexaceae</i>	<i>Heliothrix</i>	<i>heliothrix</i>
<i>Chloroflexi</i>	<i>Chloroflexi</i>	<i>Chloroflexales</i>	<i>Oscillochloridaceae</i>	<i>Oscillochloris</i>	<i>oscillochloris</i>
<i>Chloroflexi</i>	<i>Chloroflexi</i>	<i>Herpetosiphonales</i>	<i>Herpetosiphonaceae</i>	<i>Herpetosiphon</i>	<i>herpetosiphon</i>
<i>Chloroflexi</i>	<i>Dehalococcoidetes</i>	<i>Dehalococcoides</i>	<i>Dehalococcoidaceae</i>	<i>Dehalococcoides</i>	<i>ethenogenes</i> 1
<i>Chloroflexi</i>	<i>Dehalococcoidetes</i>		<i>Dehalococcoidaceae</i>	<i>Dehalococcoides</i>	2
<i>Chloroflexi</i>	<i>Dehalococcoidetes</i>		<i>Dehalococcoidaceae</i>	<i>Dehalococcoides</i>	3
<i>Chloroflexi</i>	<i>Dehalococcoidetes</i>		<i>Dehalococcoidaceae</i>	<i>Dehalococcoides</i>	4
<i>Chrysiogenetes</i>	<i>Chrysiogenetes</i>	<i>Chrysiogenales</i>	<i>Chrysiogenaceae</i>	<i>Chrysiogenes</i>	<i>chrysiogenes</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Cyanobacterium</i>	<i>cyanobacterium</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Cyanothece</i>	<i>cyanothece</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Dactylococcopsis</i>	<i>dactylococcopsis</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Gloeobacter</i>	<i>gloeobacter</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Gloeocapsa</i>	<i>gloeocapsa</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Gloeotheca</i>	<i>gloeotheca</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Microcystis</i>	<i>microcystis</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Prochlorococcus</i>	<i>prochlorococcus</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Prochloron</i>	<i>prochloron</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O1	<i>Cyanobacteria</i> F1.1	<i>Synechocystis</i>	<i>synechocystis</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O2	<i>Cyanobacteria</i> F2.1	<i>Dermocapella</i>	<i>dermocapella</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O2	<i>Cyanobacteria</i> F2.1	<i>Stanieria</i>	<i>stanieria</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O2	<i>Cyanobacteria</i> F2.1	<i>Xenococcus</i>	<i>xenococcus</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O2	<i>Cyanobacteria</i> F2.2	<i>Pleurocapsa</i>	<i>pleurocapsa</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Geitlerinema</i>	<i>geitlerinema</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Halospirulina</i>	<i>halospirulina</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Limnothrix</i>	<i>limnothrix</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Lyngbya</i>	<i>lyngbya</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Planktothrix</i>	<i>planktothrix</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Prochlorothrix</i>	<i>prochlorothrix</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Pseudanabaena</i>	<i>pseudanabaena</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Spirulina</i>	<i>spirulina</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Starria</i>	<i>starria</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Symploca</i>	<i>symploca</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O3	<i>Cyanobacteria</i> F3.1	<i>Trichodesmium</i>	<i>trichodesmium</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O4	<i>Cyanobacteria</i> F3.2	<i>Oscillatoria</i>	<i>oscillatoria</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O4	<i>Cyanobacteria</i> F4.1	<i>Anabaena</i> 4A	<i>anabaena</i>
<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>Cyanobacteria</i> O4	<i>Cyanobacteria</i> F4.1	<i>Anabaena</i> 4A	<i>anabaena</i>

Phylum	Class	Order	Family	Genus	Species
Cyanobacteria	Cyanobacteria	Cyanobacteria O4	Cyanobacteria F4.1	Anabaena 4A	anabaena
Cyanobacteria	Cyanobacteria	Cyanobacteria O4	Cyanobacteria F4.1	Anabaenopsis	anabaenopsis
Cyanobacteria	Cyanobacteria	Cyanobacteria O4	Cyanobacteria F4.1	Aphanizomenon	aphanizomenon
Cyanobacteria	Cyanobacteria	Cyanobacteria O4	Cyanobacteria F4.1	Cyanospira	cyanospira
Cyanobacteria	Cyanobacteria	Cyanobacteria O4	Cyanobacteria F4.1	Cylindrospermopsis	cylindrospermopsis
Cyanobacteria	Cyanobacteria	Cyanobacteria O4	Cyanobacteria F4.1	Cylindrospermum	cylindrospermum
Cyanobacteria	Cyanobacteria	Cyanobacteria O4	Cyanobacteria F4.1	Nodularia	nodularia
Cyanobacteria	Cyanobacteria	Cyanobacteria O5	Cyanobacteria F5.1	Chlorogloeopsis	chlorogloeopsis
Cyanobacteria	Cyanobacteria	Cyanobacteria O5	Cyanobacteria F5.1	Fischerella	fischerella
Cyanobacteria	Cyanobacteria	Cyanobacteria O5	Cyanobacteria F5.1	Umezakia	umezakia
Cyanobacteria	Cyanobacteria	Oscillatoriales	Arthrospiraceae	Arthrospira	arthrospira
Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae	Deferribacter	deferribacter
Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae	Denitrovibrio	denitrovibrio
Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae	Flexistipes	flexistipes
Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae	Geovobrio	geovibrio
Deferribacteres	Deferribacteres	Inscertae sedis	Inscertae sedis	Synergistes	synergistes
Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	Deinococcus	deinococcus
Deinococcus-Thermus	Deinococci	Thermales	Thermaceae	Meiothermus	meiothermus
Deinococcus-Thermus	Deinococci	Thermales	Thermaceae	Thermus	thermus
Dictyoglomi	Dictyoglomi	Dictyoglomales	Dictyoglomaceae	Dictyoglomus	dictyoglomus
Fibrobacteres	Fibrobacteres	Fibrobacterales	Fibrobacteraceae	Fibrobacter	fibrobacter
Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	Alicyclobacillus	alicyclobacillus
Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	Sulfobacillus	sulfobacillus
Firmicutes	Bacilli	Bacillales	Bacillaceae	Amphibacillus	amphibacillus
Firmicutes	Bacilli	Bacillales	Bacillaceae	Anoxybacillus	anoxybacillus
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	bacillus
Firmicutes	Bacilli	Bacillales	Bacillaceae	Exiguobacterium	exiguobacterium
Firmicutes	Bacilli	Bacillales	Bacillaceae	Gracilibacillus	gracilibacillus
Firmicutes	Bacilli	Bacillales	Bacillaceae	Halobacillus	halobacillus
Firmicutes	Bacilli	Bacillales	Bacillaceae	Saccharococcus	saccharococcus
Firmicutes	Bacilli	Bacillales	Bacillaceae	Salibacillus	salibacillus
Firmicutes	Bacilli	Bacillales	Bacillaceae	Virgibacillus	virgibacillus
Firmicutes	Bacilli	Bacillales	Carnobacteriaceae	Agitococcus	agitococcus
Firmicutes	Bacilli	Bacillales	Cartophanaceae	Caryophanon	caryophanon
Firmicutes	Bacilli	Bacillales	Listeriaceae	Brochothrix	brochothrix
Firmicutes	Bacilli	Bacillales	Listeriaceae	Listeria	listeria
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Ammoniphilus	ammoniphilus
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Aneurinibacillus	aneurinibacillus
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Brevibacillus	brevibacillus
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Oxalophagus	oxalophagus

Phylum	Class	Order	Family	Genus	Species
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Paenibacillaceae</i>	<i>Paenibacillus</i>	<i>paenibacillus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Paenibacillaceae</i>	<i>Thermicanus</i>	<i>thermicanus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Paenibacillaceae</i>	<i>Thermobacillus</i>	<i>thermobacillus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Planococcaceae</i>	<i>Filibacter</i>	<i>filibacter</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Planococcaceae</i>	<i>Planococcus</i>	<i>planococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Planococcaceae</i>	<i>Sporosarcina</i>	<i>sporosarcina</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Sporolactobacillaceae</i>	<i>Sporolactobacillus</i>	<i>sporolactobacillus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Staphylococcaceae</i>	<i>Macrococcus</i>	<i>macrococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Sporolactobacillaceae</i>	<i>Marinococcus</i>	<i>marinococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Staphylococcaceae</i>	<i>Gemella</i>	<i>gemella</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Staphylococcaceae</i>	<i>Salinicoccus</i>	<i>salinicoccus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Staphylococcaceae</i>	<i>Staphylococcus</i>	<i>staphylococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Thermoactinomycetaceae</i>	<i>Thermoactinomyces</i>	<i>thermoactinomyces</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Aerococcaceae</i>	<i>Abiotrophia</i>	<i>abiotrophia</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Aerococcaceae</i>	<i>Aerococcus</i>	<i>aerococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Aerococcaceae</i>	<i>Dolosicoccus</i>	<i>dolosicoccus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Aerococcaceae</i>	<i>Eremococcus</i>	<i>eremococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Aerococcaceae</i>	<i>Facklamia</i>	<i>facklamia</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Aerococcaceae</i>	<i>Globicatella</i>	<i>globicatella</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Aerococcaceae</i>	<i>Ignavigranum</i>	<i>ignavigranum</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Carnobacteriaceae</i>	<i>Alloiococcus</i>	<i>alloiococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Carnobacteriaceae</i>	<i>Carnobacterium</i>	<i>carnobacterium</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Carnobacteriaceae</i>	<i>Desemzia</i>	<i>desemzia</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Carnobacteriaceae</i>	<i>Dolosigranulum</i>	<i>dolosigranulum</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Carnobacteriaceae</i>	<i>Granulicatella</i>	<i>granulicatella</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Carnobacteriaceae</i>	<i>Lactosphaera</i>	<i>lactosphaera</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Carnobacteriaceae</i>	<i>Trichococcus</i>	<i>trichococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Atopobacter</i>	<i>atopobacter</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Enterococcus</i>	<i>enterococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Leuconostoc</i>	<i>leuconostoc</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Melissococcus</i>	<i>melissococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Oenococcus</i>	<i>oenococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Tetragenococcus</i>	<i>tetragenococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Vagococcus</i>	<i>vagococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Weissella</i>	<i>weissella</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Incertae sedis</i>	<i>Oscillospira</i>	<i>osillospira</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Incertae sedis</i>	<i>Syntrophococcus</i>	<i>syntrophococcus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Lactobacillaceae</i>	<i>Lactobacillus</i>	<i>lactobacillus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Lactobacillaceae</i>	<i>Paralactobacillus</i>	<i>paralactobacillus</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Lactobacillales</i>	<i>Lactobacillaceae</i>	<i>Pediococcus</i>	<i>pediococcus</i>

Phylum	Class	Order	Family	Genus	Species
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	lactococcus
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	streptococcus
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Acetonema	acetonema
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Acidaminococcus	acidaminococcus
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Anaeroarcus	anaeroarcus
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Anaeromusa	anaeromusa
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Anaerosinus	anaerosinus
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Anaerovibrio	anaerovibrio
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Dialister	dialister
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Megasphaera	megasphaera
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Mitsuokella	mitsuokella
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Papillibacter	papillibacter
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Papillibacter	papillibacter
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Pectinatus	pectinatus
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Phascolarctobacterium	phascolarctobacterium
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Propionispora	propionispira
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Quinella	quinella
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Schwartzia	schwartzia
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Selenomonas	selenomonas
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Sporomusa	sporomusa
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Succiniclasticum	succiniclasticum
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Succinispira	succinispira
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Veillonella	veillonella
Firmicutes	Clostridia	Clostridiales	Acidaminococcaceae	Zymophilus	zymophilus
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Acetivibrio	acetivibrio
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Acidaminobacter	acidaminobacter
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Anaerobacter	anaerobacter
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Caloramator	caloramator
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	clostridium
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Coprobacillus	coprobacillus
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Natronincola	natronincola
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Oxobacter	oxobacter
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Sarcina	sarcina
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Sporobacter	sporobacter
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Thermobrachium	thermobrachium
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Thermohalobacter	thermohalobacter
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Tindallia	tindallia
Firmicutes	Clostridia	Clostridiales	Clostridiaceae I	Clostridium I	clostridium
Firmicutes	Clostridia	Clostridiales	Clostridiaceae II	Clostridium II	clostridium
Firmicutes	Clostridia	Clostridiales	Clostridiaceae III	Clostridium III	clostridium

Phylum	Class	Order	Family	Genus	Species
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Clostridiaceae</i> IX	<i>Clostridium</i> <i>Ix</i>	<i>clostridium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Clostridiaceae</i> XII	<i>Clostridium</i> <i>Xii</i>	<i>clostridium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Clostridiaceae</i> XIVa	<i>Clostridium</i> <i>Xiva</i>	<i>clostridium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Clostridiaceae</i> XVI	<i>Clostridium</i> <i>Xvi</i>	<i>clostridium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Clostridiaceae</i> XVIII	<i>Clostridium</i> <i>Xviii</i>	<i>clostridium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Clostridiaceae</i> XIX	<i>Clostridium</i> <i>Xix</i>	<i>clostridium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Dehalobacterium</i>	<i>Dehalobacterium</i>	<i>formicoaceticum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Eubacteriaceae</i>	<i>Acetobacterium</i>	<i>acetobacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Eubacteriaceae</i>	<i>Acetobacterium</i>	<i>carbinolicum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Eubacteriaceae</i>	<i>Acetobacterium</i>	<i>halotolerans</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Eubacteriaceae</i>	<i>Acetobacterium</i>	<i>woodii</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Eubacteriaceae</i>	<i>Anaerovorax</i>	<i>anaerovorax</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Eubacteriaceae</i>	<i>Eubacterium</i>	<i>eubacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Eubacteriaceae</i>	<i>Mogibacterium</i>	<i>mogibacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Eubacteriaceae</i>	<i>Pseudoramibacter</i>	<i>pseudoramibacter</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Heliobacteriaceae</i>	<i>Heliobacillus</i>	<i>heliobacillus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Heliobacteriaceae</i>	<i>Heliobacterium</i>	<i>heliobacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Heliobacteriaceae</i>	<i>Heliophilum</i>	<i>heliophilum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Heliobacteriaceae</i>	<i>Heliorestis</i>	<i>heliorestis</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Acetitomaculum</i>	<i>acetitomaculum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Anaerofilum</i>	<i>anaerofilum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Butyrivibrio</i>	<i>butyrivibrio</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Catenibacterium</i>	<i>catenibacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Catonella</i>	<i>catonella</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Coprococcus</i>	<i>coprococcus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Johnsonella</i>	<i>johnsonella</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Lachnospira</i>	<i>lachnospira</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Pseudobutyrvibrio</i>	<i>pseudobutyrvibrio</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Roseburia</i>	<i>roseburia</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Ruminococcus</i>	<i>ruminococcus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Lachnospiraceae</i>	<i>Sporobacterium</i>	<i>sporobacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Centipeda</i>	<i>centipeda</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Dehalobacter</i>	<i>1</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Dehalobacter</i>	<i>dehalobacter</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Dehalobacter</i>	<i>restrictus2</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Dehalobacter</i>	<i>restrictus3</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Dendrosporobacter</i>	<i>dendrosporobacter</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfitobacterium</i>	<i>dehalogenans</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfitobacterium</i>	<i>desulfitobacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfitobacterium</i>	<i>dichloroeliminans</i>

Phylum	Class	Order	Family	Genus	Species
<i>Firmicutes</i>	<i>Clostridia</i>		<i>Peptococcaceae</i>	<i>Desulfitobacterium</i>	<i>frappieri1</i>
<i>Firmicutes</i>	<i>Clostridia</i>		<i>Peptococcaceae</i>	<i>Desulfitobacterium</i>	<i>frappieri2</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfitobacterium</i>	<i>hafniense</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfitobacterium</i>	<i>metalireducens</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfonispota</i>	<i>desulfonispota</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfonosporus</i>	<i>thiosulfogenes</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfosporosinus</i>	<i>desulfosporosinus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfosporosinus</i>	<i>orientis</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>nigrificans2</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>acetoxidans</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>aeronauticum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>alkaliphilum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>auripigmentum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>desulfotomaculum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>guttoideum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>halophilum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>kuznetsovii</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>nigrificans1</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>reducens</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>ruminis</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Desulfotomaculum</i>	<i>thermobenzoicum</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Peptococcus</i>	<i>niger</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Peptococcus</i>	<i>peptinophilus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Syntrophobotulus</i>	<i>glycolicus1</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Syntrophobotulus</i>	<i>glycolicus2</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Syntrophobotulus</i>	<i>syntrophobotulus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Thermoterrabacterium</i>	<i>ferrireducens</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptococcaceae</i>	<i>Thermoterrabacterium</i>	<i>thermoterrabacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptopstreptococcaceae</i>	<i>Anaerococcus</i>	<i>anaerococcus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptopstreptococcaceae</i>	<i>Filifactor</i>	<i>filifactor</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptopstreptococcaceae</i>	<i>Finegoldia</i>	<i>finegoldia</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptopstreptococcaceae</i>	<i>Fusibacter</i>	<i>fusibacter</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptopstreptococcaceae</i>	<i>Helcococcus</i>	<i>helcococcus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptopstreptococcaceae</i>	<i>Micromonas</i>	<i>micromonas</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptopstreptococcaceae</i>	<i>Peptoniphilus</i>	<i>peptococcus</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Peptopstreptococcaceae</i>	<i>Tissierella</i>	<i>tissierella</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Syntrophomonadaceae</i>	<i>Acetogenium</i>	<i>acetogenium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Syntrophomonadaceae</i>	<i>Aminobacterium</i>	<i>aminobacterium</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Syntrophomonadaceae</i>	<i>Aminomonas</i>	<i>aminomonas</i>
<i>Firmicutes</i>	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Syntrophomonadaceae</i>	<i>Anaerobaculum</i>	<i>anaerobaculum</i>

Phylum	Class	Order	Family	Genus	Species
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Anaerobranca	anaerobranca
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Caldicellulosiruptor	caldicellulosiruptor
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Dethiosulfovibrio	dethiosulfovibrio
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Pelospora	pelospora
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Syntrophomonas	syntrophomonas
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Syntrophospora	syntrophospora
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Syntrophothermus	syntrophothermus
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Thermaerobacter	thermaerobacter
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Thermanaerovibrio	thermanaerovibrio
Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae	Thermosyntropha	thermosyntropha
Firmicutes	Clostridia	Haloanaerobiales	Haloanaerobiaceae	Halocella	halocella
Firmicutes	Clostridia	Haloanaerobiales	Haloanaerobiaceae	Halothermothrix	halothermothrix
Firmicutes	Clostridia	Haloanaerobiales	Haloanaerobiaceae	Natroniella	natroniella
Firmicutes	Clostridia	Haloanaerobiales	Haloanaerobiaceae	Spolohalobacter	spolohalobacter
Firmicutes	Clostridia	Haloanaerobiales	Halobacteroidaceae	Acetohalobium	acetohalobium
Firmicutes	Clostridia	Haloanaerobiales	Halobacteroidaceae	Haloanaerobacter	haloanaerobacter
Firmicutes	Clostridia	Haloanaerobiales	Halobacteroidaceae	Haloanaerobium	haloanaerobium
Firmicutes	Clostridia	Haloanaerobiales	Halobacteroidaceae	Halobacteroides	halobacteroides
Firmicutes	Clostridia	Haloanaerobiales	Halobacteroidaceae	Orenia	orenia
Firmicutes	Clostridia	Haloanaerobiales	Halobacteroidaceae	Sporohalobacter	sporohalobacter
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacter	Thermoanaerobacteroides	thermoanaerobacteroides
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacteriaceae	Ammonifex	ammonifex
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacteriaceae	Coprothermobacter	coprothermobacter
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacteriaceae	Moorella	moorella
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacteriaceae	Sporotomaculum	sporotomaculum
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacteriaceae	Thermacetogenium	thermacetogenium
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacteriaceae	Thermoanaerobacter	thermoanaerobacter
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacteriaceae	Thermoanaerobacterium	thermoanaerobacterium
Firmicutes	Clostridia	Thermoanaerobacteriales	Thermoanaerobacteriaceae	Thermoanaerobium	thermoanaerobium
Firmicutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma	acholeplasma
Firmicutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	anaeroplasma
Firmicutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Asteroleplasma	asteroleplasma
Firmicutes	Mollicutes	Anaeroplasmatales	Erysipelotrichaceae	Bulleidia	bulleidia
Firmicutes	Mollicutes	Entomoplasmatales	Entomoplasmataceae	Mesoplasma	mesoplasma
Firmicutes	Mollicutes	Entomoplasmatales	Spiroplasmataceae	Spiroplasma	spiroplasma
Firmicutes	Mollicutes	Incertae sedis	Erysipelothrichaceae	Erysipelothrix	erysipelothrix
Firmicutes	Mollicutes	Incertae sedis	Erysipelothrichaceae	Solobacterium	solobacterium
Firmicutes	Mollicutes	Incertae sedis	Erysipelothrichaceae	Holdemania	holdemania
Firmicutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Eperythrozoon	eperythrozoon
Firmicutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Ureaplasma	ureaplasma

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bartonellaceae</i>	<i>Rochalimaea</i>	<i>rochalimaea</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Beijerinckiaceae</i>	<i>Beijerinckia</i>	<i>beijerinckia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Beijerinckiaceae</i>	<i>Chelatococcus</i>	<i>chelatococcus</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Beijerinckiaceae</i>	<i>Methylocella</i>	<i>methylocella</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Afipia</i>	<i>afipia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Agromonas</i>	<i>agromonas</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Blastobacter</i>	<i>blastobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Bosea</i>	<i>bosea</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Bradyrhizobium</i>	<i>bradyrhizobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Nitrobacter</i>	<i>nitrobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Oligotropha</i>	<i>oligotropha</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Rhodopseudomonas</i>	<i>rhodopseudomonas</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Bradyrhizobiaceae</i>	<i>Rhodopseudomonas</i>	<i>rhodopseudomonas</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Brucellaceae</i>	<i>Brucella</i>	<i>brucella</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Brucellaceae</i>	<i>Mycoplana</i>	<i>mycoplana</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Brucellaceae</i>	<i>Ochrobactrum</i>	<i>ochrobacterium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Ancylobacter</i>	<i>ancylobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Azorhizobium</i>	<i>azorhizobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Blastochloris</i>	<i>blastochloris</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Devosia</i>	<i>devosia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Hyphomicrobium</i>	<i>hyphomicrobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Methylorhabdus</i>	<i>methylorhabdus</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Pedomicrobium</i>	<i>pedomicrobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Prosthecomicrobium</i>	<i>prosthecomicrobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Rhodomicrobium</i>	<i>rhodomicrobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Rhodoplanes</i>	<i>rhodoplanes</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Xanthobacter</i>	<i>flavus</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Xanthobacter</i>	<i>xanthobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Methylobacteriaceae</i>	<i>Methylobacterium</i>	<i>methylobacterium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Methylocystaceae</i>	<i>Methylocystis</i>	<i>methylocystis</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Methylocystaceae</i>	<i>Methylopila</i>	<i>methylopila</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Methylocystaceae</i>	<i>Methylosinus</i>	<i>methylosinus</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Phyllobacteriaceae</i>	<i>Aminobacter</i>	<i>aminobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Phyllobacteriaceae</i>	<i>Aquamicrobium</i>	<i>aquamicrobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Phyllobacteriaceae</i>	<i>Mesorhizobium</i>	<i>mesorhizobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Phyllobacteriaceae</i>	<i>Phyllobacterium</i>	<i>phenylobacterium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Phyllobacteriaceae</i>	<i>Phyllobacterium</i>	<i>phyllobacterium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Phyllobacteriaceae</i>	<i>Pseudaminobacter</i>	<i>pseudaminobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Rhizobiaceae</i>	<i>Agrobacterium</i>	<i>agrobacterium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Rhizobiaceae</i>	<i>Allorhizobium</i>	<i>allorhizobium</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Rhizobiaceae</i>	<i>Chelatobacter</i>	<i>chelatobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Rhizobiaceae</i>	<i>Ensifer</i>	<i>ensifer</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Rhizobiaceae</i>	<i>Rhizobium</i>	<i>rhizobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Rhizobiaceae</i>	<i>Sinorhizobium</i>	<i>sinorhizobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Rhodobiaceae</i>	<i>Rhodobium</i>	<i>rhodobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Ahrensia</i>	<i>ahrensia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Ahrensia</i>	<i>ahrensia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Amaricoccus</i>	<i>amaricoccus</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Antarctobacter</i>	<i>antarctobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Hirschia</i>	<i>hirschia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Hyphomonas</i>	<i>hyphomonas</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Maricaulis</i>	<i>maricaulis</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Methylarcula</i>	<i>methylarcula</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Octadecabacter</i>	<i>octadecabacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Paracoccus</i>	<i>paracoccus</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Rhodobacter</i>	<i>rhodobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Rhodothalassium</i>	<i>rhodothalassium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Rhodovulum</i>	<i>rhodovulum</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Roseibium</i>	<i>roseibium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Roseinatronobacter</i>	<i>roseinatronobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Roseivivax</i>	<i>roseivivax</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Roseobacter</i>	<i>roseobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Roseovarius</i>	<i>roseovarius</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Rubrimonas</i>	<i>rubrimonas</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Ruegeria</i>	<i>ruegeria</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Sagittula</i>	<i>sagittula</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Silicibacter</i>	<i>silicibacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Stappia</i>	<i>stappia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Sulfitobacter</i>	<i>sulfitobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodobacterales</i>	<i>Rhodobacteraceae</i>	<i>Sulfitobacter</i>	<i>sulfitobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Acidiphilium</i>	<i>acidiphilium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Acidisphaera</i>	<i>acidisphaera</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Acidocella</i>	<i>acidocella</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Asaia</i>	<i>asaia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Gluconacetobacter</i>	<i>erythromonas</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Gluconobacter</i>	<i>gluconobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Gluconobacter</i>	<i>gluconacetobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Paracraurococcus</i>	<i>paracraurococcus</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Rhodopila</i>	<i>rhodopila</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Roseococcus</i>	<i>roseococcus</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteriaceae</i>	<i>Acetobacter</i>	<i>acetobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteriaceae</i>	<i>Craurococcus</i>	<i>craurococcus</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Azospirillum</i>	<i>azospirillum</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Azospirillum</i>	<i>azospirillum</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Magnetospirillum</i>	<i>magnetospirillum</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Magnetospirillum</i>	<i>magnetospirillum</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Phaeospirillum</i>	<i>pedomicrobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Rhodocista</i>	<i>rhodocista</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Rhodospira</i>	<i>rhodospira</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Rhodospirillum</i>	<i>rhodospirillum</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Rhodovibrio</i>	<i>rhodovibrio</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Roseospira</i>	<i>roseospira</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Skermanella</i>	<i>skermanella</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Anaplasmataceae</i>	<i>Aegyptianella</i>	<i>aegyptianella</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Anaplasmataceae</i>	<i>Anaplasma</i>	<i>anaplasma</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Anaplasmataceae</i>	<i>Cowdria</i>	<i>cowdria</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Anaplasmataceae</i>	<i>Ehrlichia</i>	<i>ehrlichia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Anaplasmataceae</i>	<i>Neorickettsia</i>	<i>neorickettsia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Anaplasmataceae</i>	<i>Wolbachia</i>	<i>wolbachia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Genera incertae sedis</i>	<i>Caedibacter</i>	<i>caedibacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Holosporaceae</i>	<i>Holospira</i>	<i>holospira</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Rickettsiaceae</i>	<i>Orientia</i>	<i>orientia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>Rickettsiaceae</i>	<i>Rickettsia</i>	<i>rickettsia</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Blastomonas</i>	<i>blastomonas</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Erythrobacter</i>	<i>enthydrobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Erythromonas</i>	<i>erythromicrobium</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Porphyrobacter</i>	<i>porphyrobacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Sandaracinobacter</i>	<i>sandaracinnbacter</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Sphingomonas</i>	<i>sphingomonas</i>
<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>	<i>Sphingomonadaceae</i>	<i>Zymomonas</i>	<i>zymomonas</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Alcaligenaceae</i>	<i>Achromobacter</i>	<i>achromobacter</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Alcaligenaceae</i>	<i>Alcaligenes</i>	<i>alcaligenes</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Alcaligenaceae</i>	<i>Bordetella</i>	<i>bordetella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Alcaligenaceae</i>	<i>Sutterella</i>	<i>sutterella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Alcaligenaceae</i>	<i>Taylorella</i>	<i>taylorella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Burkholderia</i>	
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Burkholderia</i>	<i>burkholderia</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Cupriavidus</i>	<i>cupriavidus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Lautropia</i>	<i>lautropia</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Pandoraea</i>	<i>pandoraea</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Polynucleobacter</i>	<i>polynucleobacter</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Ralstonia</i>	<i>ralstonia</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Thermothrix</i>	<i>thermothrix</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Acidovorax</i>	<i>acidovorax</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Brachymonas</i>	<i>brachymonas</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Commamonadaceae</i>	<i>Commamonas</i>	<i>comamonas</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Hydrogenophaga</i>	<i>hydrogenophaga</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Macromonas</i>	<i>macromonas</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Polaromonas</i>	<i>polaromonas</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Rhodoferrax</i>	<i>rhodoferrax</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Variovorax</i>	<i>variovorax</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Aquabacterium</i>	<i>aquabacterium</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Leptothrix</i>	<i>leptothrix</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Roseateles</i>	<i>roseateles</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Rubrivivax</i>	<i>rubrivivax</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Sphaerotilus</i>	
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Sphaerotilus</i>	<i>sphaerotilus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Tepidimonas</i>	<i>tepidimonas</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Thiomonas</i>	<i>thiomonas</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Genera incertae sedis</i>	<i>Xylophilus</i>	<i>xylophilus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Oxalobacteraceae</i>	<i>Duganella</i>	<i>duganella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Oxalobacteraceae</i>	<i>Herbaspirillum</i>	<i>herbaspirillum</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Oxalobacteraceae</i>	<i>Massilia</i>	<i>massilia</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Oxalobacteraceae</i>	<i>Oxalobacter</i>	<i>oxalobacter</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Piscirickettsiaceae</i>	<i>Oligella</i>	<i>oligella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Hydrogenophilaes</i>	<i>Hydrogenophilaceae</i>	<i>Hydrogenophilus</i>	<i>hydrogenophilus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Hydrogenophilaes</i>	<i>Hydrogenophilaceae</i>	<i>Thiobacillus</i>	<i>thiobacillus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Methylophilales</i>	<i>Methylophilaceae</i>	<i>Methylobacillus</i>	<i>methylobacillus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Methylophilales</i>	<i>Methylophilaceae</i>	<i>Methylophilus</i>	<i>methylophilus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Neisseriales</i>	<i>Neisseriaceae</i>	<i>Alysiella</i>	<i>alysiella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Neisseriales</i>	<i>Neisseriaceae</i>	<i>Formivibrio</i>	<i>formivibrio</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Neisseriales</i>	<i>Neisseriaceae</i>	<i>Neisseria</i>	<i>neisseria</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Neisseriales</i>	<i>Neisseriaceae</i>	<i>Simonsiella</i>	<i>simonsiella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Neisseriales</i>	<i>Neisseriaceae</i>	<i>Vitreoscilla</i>	<i>vitreoscilla</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Neisseriales</i>	<i>Neisseriaceae</i>	<i>Vogesella</i>	<i>vogesella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Gallionellaceae</i>	<i>Gallionella</i>	1
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Gallionellaceae</i>	<i>Gallionella</i>	<i>gallionella</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosolobus</i>	1
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>communis</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>aestuarii</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>europaea</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>oligotroph</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>halophila</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>nitrosa</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>cryotolerans</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>ureae</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>sp</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrosomonas</i>	<i>nitrosomonas</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrospira</i>	<i>marina</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrospira</i>	<i>sp</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Nitrospira</i>	<i>moscoviensis</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>Spirillum</i>	
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrospiraceae</i>	<i>Nitrosomonas</i>	<i>sp.</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Spirillaceae</i>	<i>Spirillum</i>	<i>spirillum</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Azoarcus</i>	<i>azoarcus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Azonexus</i>	<i>azonexus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Azospira</i>	<i>azospira</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Azovibrio</i>	<i>azovibrio</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Ferribacterium</i>	<i>ferribacterium</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Propionibacter</i>	<i>propionibacter</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Rhodocyclus</i>	<i>rhodocyclus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Rhodocyclus</i>	<i>rhodocyclus</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Thauera</i>	<i>thauera</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Zoogloea</i>	<i>zoogloea</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Zoogloea</i>	<i>ramigera1</i>
<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Zoogloea</i>	<i>ramigera2</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Bdellovibrionales</i>	<i>Bdellovibrionaceae</i>	<i>Bacteriovorax</i>	<i>bacteriovorax</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Bdellovibrionales</i>	<i>Bdellovibrionaceae</i>	<i>Bdellovibrio</i>	<i>bdellovibrio</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfarcales</i>	<i>Desulfarculaceae</i>	<i>Desulfarculus</i>	<i>desulfoarculus</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacter</i>	<i>Desulfofaba</i>	<i>desulfofaba</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfobacter</i>	<i>desulfobacter</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfobacterium</i>	<i>desulfobacterium</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfobacula</i>	<i>desulfobacula</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfocella</i>	<i>desulfocella</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfococcus</i>	<i>desulfococcus</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfofrigus</i>	<i>desulfofrigus</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfonema</i>	<i>desulfonema</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfosarcina</i>	<i>desulfosarcina</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteraceae</i>	<i>Desulfospira</i>	<i>desulfospira</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteriaceae</i>	<i>Desulfobacter</i>	4
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteriaceae</i>	<i>Desulfobacter</i>	3
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteriaceae</i>	<i>Desulfobacter</i>	1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteriaceae</i>	<i>Desulfobacter</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteriaceae</i>	<i>Desulfobotulus</i>	
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteriaceae</i>	<i>Desulfococcus</i>	1(biacutus)
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteriaceae</i>	<i>Desulfococcus</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobacteriaceae</i>	<i>Desulfosarcina</i>	
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfobulbus</i>	1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfobulbus</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfobulbus</i>	4
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfobulbus</i>	3
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfobulbus</i>	desulfobulbus
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfocapsa</i>	3
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfocapsa</i>	desulfocapsa
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfofustis</i>	desulfofustis
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfofustis</i>	
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulforhopalus</i>	desulforhopalus
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulforhopalus</i>	3
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulforhopalus</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfotalea</i>	1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Desulfobulbaceae</i>	<i>Desulfotalea</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfobacterales</i>	<i>Nitrospinaceae</i>	<i>Nitrospina</i>	nitrospina
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfomonaceae</i>	<i>Desulfuromonas</i>	desulfuromonas
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfomonaceae</i>	<i>Pelobacter</i>	pelobacter
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromonas</i>	1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromusa</i>	1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromonas</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromusa</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromonas</i>	3
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromusa</i>	3
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromonas</i>	4
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromonas</i>	5
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Desulfuromonadaceae</i>	<i>Desulfuromonas</i>	6
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Geobacteriaceae</i>	<i>Geobacter</i>	geobacter
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Geobacteriaceae</i>	<i>Trichlorobacter</i>	trichlorobacter
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfomonales</i>	<i>Pelobacteraceae</i>	<i>Malonomonas</i>	malonomonas
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfohalobium</i>	
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfohalobium</i>	desulfohalobium
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfomonas</i>	

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfomonas</i>	
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfomonas</i>	<i>desulfomonas</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfonatronovibrio</i>	<i>desulfonatronovibrio</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfovibrio</i>	1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfovibrio</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfohalobiaceae</i>	<i>Desulfovibrio</i>	3
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfomicrobiaceae</i>	<i>Desulfomicrobium</i>	<i>apsheronum</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfomicrobiaceae</i>	<i>Desulfomicrobium</i>	
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfomicrobiaceae</i>	<i>Desulfomicrobium</i>	<i>norvegicum</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfomicrobiaceae</i>	<i>Desulfomicrobium</i>	<i>desulfomicrobium</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfonatronumaceae</i>	<i>Desulfonatronum</i>	
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfonatronumaceae</i>	<i>Desulfonatronum</i>	<i>desulfonatronum</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Bilophila</i>	<i>bilophila</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Bilophila</i>	<i>wadsworthia</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	7
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	4
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	5
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	6
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	<i>desulfovibrio</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Lawsonia</i>	<i>lawsonia</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfurellales</i>	<i>Desulfurellaceae</i>	<i>Desulfurella</i>	
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfurellales</i>	<i>Desulfurellaceae</i>	<i>Desulfurella</i>	<i>desulfurella</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfurellales</i>	<i>Desulfurellaceae</i>	<i>Hipaea</i>	<i>hipaea</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfuromonadales</i>	<i>Desulfobulbaceae</i>	<i>Desulfocapsa</i>	1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfuromonadales</i>	<i>Desulfobulbaceae</i>	<i>Desulfocapsa</i>	2
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfuromonadales</i>	<i>Desulfomonaceae</i>	<i>Desulfuromonas</i>	<i>chloroethanica</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulfuromonadales</i>	<i>Desulfomonaceae</i>	<i>Desulfuromonas</i>	<i>acetoxidans</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Desulphovibrionales</i>	<i>Desulfovibrionaceae</i>	<i>Lawsonia</i>	<i>intracellularis</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Cystobacteraceae</i>	<i>Cystobacter</i>	<i>cystobacter</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Cystobacteriaceae</i>	<i>Angiococcus</i>	<i>angiococcus</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Cystobacteriaceae</i>	<i>Archangium</i>	<i>archangium</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Cystobacteriaceae</i>	<i>Melittangium</i>	<i>melittangium</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Cystobacteriaceae</i>	<i>Stigmatella</i>	<i>stigmatella</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Myxococcaceae</i>	<i>Myxococcus</i>	<i>myxococcus</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Nanocystaceae</i>	<i>Nannocystis</i>	<i>nannocystis</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Polyangiaceae</i>	<i>Chondromyces</i>	<i>chondromyces</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Polyangiaceae</i>	<i>Polyangium</i>	<i>polyangium</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Desulfobacca</i>	<i>desulfobacca</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Desulfomonile</i>	<i>sp.2</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Desulfomonile</i>	<i>sp.3</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Desulfomonile</i>	<i>sp.1</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Desulfomonile</i>	<i>tiedjei</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Desulfomonile</i>	<i>limimaris</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Desulfomonile</i>	<i>sp</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Desulfomonile</i>	<i>desulfomonile</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Smithella</i>	<i>smithella</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophaceae</i>	<i>Syntrophus</i>	<i>syntrophus</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophobacteraceae</i>	<i>Desulfacinum</i>	<i>desulfacinum</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophobacteraceae</i>	<i>Desulforhabdus</i>	<i>desulforhabdus</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophobacteraceae</i>	<i>Syntrophobacter</i>	<i>syntrophobacter</i>
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>	<i>Syntrophobacteraceae</i>	<i>Thermodesulforhabdus</i>	<i>thermodesulforhabdus</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Campylobacteraceae</i>	<i>Arcobacter</i>	<i>arcobacter</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Campylobacteraceae</i>	<i>Campylobacter</i>	<i>campylobacter</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Campylobacteriaceae</i>	<i>Dehalospirillum</i>	<i>multivorans</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteraceae</i>	<i>Helicobacter</i>	<i>helicobacter</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Thiovulum</i>	<i>thiovulum</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Wolinella</i>	<i>wolinella</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Wolinella</i>	<i>wolinella</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Wolinella</i>	<i>wolinella</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Wolinella</i>	<i>wolinella</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Wolinella</i>	<i>wolinella</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Wolinella</i>	<i>wolinella</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Wolinella</i>	<i>wolinella</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Helicobacteriaceae</i>	<i>Wolinella</i>	<i>wolinella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Acidithiobacillales</i>	<i>Acidithiobacillales</i>	<i>Acidithiobacillus</i>	<i>acidithiobacillus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Aeromonadales</i>	<i>Aeromonadaceae</i>	<i>Aeromonas</i>	<i>aeromonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Aeromonadales</i>	<i>Aeromonadaceae</i>	<i>Tolumonas</i>	<i>tolumonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Aeromonadales</i>	<i>Succinivibrionaceae</i>	<i>Ruminobacter</i>	<i>ruminobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Aeromonadales</i>	<i>Succinivibrionaceae</i>	<i>Succinimonas</i>	<i>succinimonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Aeromonadales</i>	<i>Succinivibrionaceae</i>	<i>Succinivibrio</i>	<i>succinivibrio</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Alteromonas</i>	<i>alteromonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Colwellia</i>	<i>colwellia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Ferrimonas</i>	<i>ferrimonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Glaciecola</i>	<i>glaciecola</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Idiomarina</i>	<i>idiomarina</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Marinobacter</i>	<i>marinobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Marinobacterium</i>	<i>marinobacterium</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Moritella</i>	<i>moritella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Pseudoalteromonas</i>	<i>pseudoalteromonas</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>Shewanella</i>	<i>shewanella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Psychromonadaceae</i>	<i>Psychromonas</i>	<i>psychromonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Cardiobacteriales</i>	<i>Cardiobacteriaceae</i>	<i>Cardiobacterium</i>	<i>budvicia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Cardiobacteriales</i>	<i>Cardiobacteriaceae</i>	<i>Dichelobacter</i>	<i>dichelobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Allochromatium</i>	<i>allochromatium</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Chromatium</i>	<i>chromatium</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Halochromatium</i>	<i>halochromatium</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Lamprocystis</i>	<i>lamprocystis</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Nitrosococcus</i>	<i>nitrosococcus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Pfennigia</i>	<i>pfennigia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Rhabdochromatium</i>	<i>rhabdochromatium</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Thermochromatium</i>	<i>thermochromatium</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Thioalkalicoccus</i>	<i>thioalkalicoccus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Thioflavicoccus</i>	<i>thioflavicoccus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Thiohalocapsa</i>	<i>thiohalocapsa</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Thiolamprovum</i>	<i>thiolamprovum</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Thiorhodococcus</i>	<i>thiorhodococcus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Thiorhodovibrio</i>	<i>thiorhodovibrio</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Ectothiorhodospiraceae</i>	<i>Arhodomonas</i>	<i>arhodomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Ectothiorhodospiraceae</i>	<i>Ectothiorhodospira</i>	<i>ectothiorhodospira</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Ectothiorhodospiraceae</i>	<i>Ectothiorhodospira</i>	<i>ectothiorhodospira</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Ectothiorhodospiraceae</i>	<i>Halorhodospira</i>	<i>halorhodospira</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Ectothiorhodospiraceae</i>	<i>Thiorhodospira</i>	<i>thiorhodospira</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Chromatiales</i>	<i>Nitrosococcaceae</i>	<i>Nitrosococcus</i>	
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Alterococcus</i>	<i>alterococcus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Arsenophonus</i>	<i>arsenophonus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Brenneria</i>	<i>beggiatoa</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Buchnera</i>	<i>brenneria</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Budvicia</i>	<i>buchnera</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Edwardsiella</i>	<i>edwardsiella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Enterobacter</i>	<i>aerogenes</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Escherichia</i>	<i>escherichia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Ewingella</i>	<i>ewingella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Hafnia</i>	<i>hafnia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Kluyvera</i>	<i>kluyvera</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Leminorella</i>	<i>legionella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Leminorella</i>	<i>leminorella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Morganella</i>	<i>morganella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Pantoea</i>	<i>agglomerans</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Photorhabdus</i>	<i>photorhabdus</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Plesiomonas</i>	<i>plesiomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Proteus</i>	<i>proteus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Providencia</i>	<i>providencia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Salmonella</i>	<i>salmonella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Tatumella</i>	<i>tatumella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Trabulsiella</i>	<i>trabulsiella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Wigglesworthia</i>	<i>wigglesworthia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Enterobacteriales</i>	<i>Enterobacteriaceae</i>	<i>Xenorhabdus</i>	<i>xenorhabdus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Legionellales</i>	<i>Coxiellaceae</i>	<i>Coxiella</i>	<i>coxiella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Legionellales</i>	<i>Coxiellaceae</i>	<i>Rickettsiella</i>	<i>rickettsiella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Legionellales</i>	<i>Legionellaceae</i>	<i>Legionella</i>	<i>lampropedia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylobacter</i>	<i>methylobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylocaldum</i>	<i>methylocaldum</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylococcus</i>	<i>methylococcus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylomicrobium</i>	<i>methylomicrobium</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylomonas</i>	<i>methylomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylosphaera</i>	<i>methylosphaera</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Alcanivoraceae</i>	<i>Alcanivorax</i>	<i>alcanivorax</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Alcanivoraceae</i>	<i>Fundibacter</i>	<i>fundibacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Halomonadaceae</i>	<i>Halomonas</i>	<i>halomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Halomonadaceae</i>	<i>Carnimonas</i>	<i>cardiobacterium</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Halomonadaceae;</i>	<i>Zymobacter</i>	<i>zymobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Oceanospirillaceae</i>	<i>Balneatrix</i>	<i>azotobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Oceanospirillaceae</i>	<i>Marinomonas</i>	<i>marinomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Oceanospirillaceae</i>	<i>Marinospirillum</i>	<i>marinospirillum</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Oceanospirillaceae</i>	<i>Neptunomonas</i>	<i>neptunomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Oceanospirillales</i>	<i>Oceanospirillaceae</i>	<i>Oceanospirillum</i>	<i>oceanospirillum</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pasteurellales</i>	<i>Pasteurellaceae</i>	<i>Actinobacillus</i>	<i>actinobacillus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pasteurellales</i>	<i>Pasteurellaceae</i>	<i>Haemophilus</i>	<i>haemophilus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pasteurellales</i>	<i>Pasteurellaceae</i>	<i>Lonepinella</i>	<i>lonepinella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pasteurellales</i>	<i>Pasteurellaceae</i>	<i>Pasteurella</i>	<i>pasteurella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pasteurellales</i>	<i>Pasteurellaceae</i>	<i>Phocoenobacter</i>	<i>phocoenobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Moraxellaceae</i>	<i>Acinetobacter</i>	
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Moraxellaceae</i>	<i>Acinetobacter</i>	<i>acinetobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Moraxellaceae</i>	<i>Enhydrobacter</i>	<i>enhydrobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Moraxellaceae</i>	<i>Moraxella</i>	<i>moraxella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Moraxellaceae</i>	<i>Psychrobacter</i>	<i>psychrobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Azorhizophilus</i>	<i>azomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Azotobacter</i>	<i>azorhizophilus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Cellvibrio</i>	<i>cellvibrio</i>

Phylum	Class	Order	Family	Genus	Species
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Chryseomonas</i>	<i>chryseomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Flavimonas</i>	<i>flavimonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Pseudomonas</i>	<i>pseudomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Francisellaceae</i>	<i>Francisella</i>	<i>francisella</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Piscirickettsiaceae</i>	<i>Cycloclasticus</i>	<i>cycloclasticus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Piscirickettsiaceae</i>	<i>Hydrogenovibrio</i>	<i>hydrogenovibrio</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Piscirickettsiaceae</i>	<i>Methylophaga</i>	<i>methylophaga</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Piscirickettsiaceae</i>	<i>Piscirickettsia</i>	<i>piscirickettsia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Piscirickettsiaceae</i>	<i>Thiomicrospira</i>	<i>thiomicrospira</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Beggiatoa</i>	1
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Beggiatoa</i>	2
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Beggiatoa</i>	3
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Beggiatoa</i>	4
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Beggiatoa</i>	5
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Beggiatoa</i>	6
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Beggiatoa</i>	6
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Beggiatoa</i>	<i>balneatrix</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Leucothrix</i>	<i>leucothrix</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Thioploca</i>	<i>thioploca</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Thiothrix</i>	<i>thiothrix</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Thiothrix</i>	1
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Thiothrix</i>	2
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Thiothrix</i>	3
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Thiotrichales</i>	<i>Thiotrichaceae</i>	<i>Thiothrix</i>	4
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Vibrionales</i>	<i>Vibrionaceae</i>	<i>Allomonas</i>	<i>allomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Vibrionales</i>	<i>Vibrionaceae</i>	<i>Catenococcus</i>	<i>carnimonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Vibrionales</i>	<i>Vibrionaceae</i>	<i>Catenococcus</i>	<i>catenococcus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Vibrionales</i>	<i>Vibrionaceae</i>	<i>Salinivibrio</i>	<i>salinivibrio</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Vibrionales</i>	<i>Vibrionaceae</i>	<i>Catenococcus</i>	<i>catenococcus</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Vibrionales</i>	<i>Vibrionaceae</i>	<i>Vibrio</i>	<i>vibrio</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>	<i>Xanthomonadaceae</i>	<i>Luteimonas</i>	<i>luteimonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>	<i>Xanthomonadaceae</i>	<i>Nevskia</i>	<i>nevskia</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>	<i>Xanthomonadaceae</i>	<i>Rhodanobacter</i>	<i>rhodanobacter</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>	<i>Xanthomonadaceae</i>	<i>Xanthomonas</i>	<i>xanthomonas</i>
<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>	<i>Xanthomonadaceae</i>	<i>Xylella</i>	<i>xylella</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Leptospiraceae</i>	<i>Leptonema</i>	<i>leptonema</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Leptospiraceae</i>	<i>Leptospira</i>	<i>leptospira</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Leptospiraceae</i>	<i>Leptospira</i>	<i>leptospira</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Leptospiraceae</i>	<i>Leptospira</i>	<i>leptospira</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Leptospiraceae</i>	<i>Leptospira</i>	<i>leptospira</i>

Phylum	Class	Order	Family	Genus	Species
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Borrelia</i>	<i>borrelia</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Brachyspira</i>	<i>brachyspira</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Brevinema</i>	<i>brevinema</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Cristispira</i>	<i>cristispira</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Spirochaeta</i>	<i>spirochaeta</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	<i>treponema</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	<i>treponema</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	<i>treponema</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	<i>treponema</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	<i>treponema</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	<i>treponema</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	<i>treponema</i>
<i>Spiralbacteria</i>	<i>Spirochaetes</i>	<i>Spirochaetales</i>	<i>Spirochaetaceae</i>	<i>Treponema</i>	<i>treponema</i>
<i>Thermodesulfobacteria</i>	<i>Thermodesulfobacteriales</i>	<i>Thermodesulfobacteriales</i>	<i>Thermodesulfobacteriaceae</i>	<i>Thermodesulfobacterium</i>	<i>thermodesulfobacterium</i>
<i>Thermomicrobia</i>	<i>Thermomicrobia</i>	<i>Thermomicrobiales</i>	<i>Thermomicrobiaceae</i>	<i>Thermomicrobium</i>	<i>thermomicrobium</i>
<i>Thermotogae</i>	<i>Thermotogae</i>	<i>Thermotogales</i>	<i>Thermotogaceae</i>	<i>Fervidobacterium</i>	<i>fervidobacterium</i>
<i>Thermotogae</i>	<i>Thermotogae</i>	<i>Thermotogales</i>	<i>Thermotogaceae</i>	<i>Geotoga</i>	<i>geotoga</i>
<i>Thermotogae</i>	<i>Thermotogae</i>	<i>Thermotogales</i>	<i>Thermotogaceae</i>	<i>Petrotoga</i>	<i>petrotoga</i>
<i>Thermotogae</i>	<i>Thermotogae</i>	<i>Thermotogales</i>	<i>Thermotogaceae</i>	<i>Thermosipho</i>	<i>thermosipho</i>
<i>Thermotogae</i>	<i>Thermotogae</i>	<i>Thermotogales</i>	<i>Thermotogaceae</i>	<i>Thermotoga</i>	<i>thermotoga</i>
<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	<i>Verrucomicrobiales</i>	<i>Verrucomicrobiaceae</i>	<i>Prostheco bacter</i>	<i>prostheco bacter</i>
<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	<i>Verrucomicrobiales</i>	<i>Verrucomicrobiaceae</i>	<i>Verrucomicrobium</i>	<i>verrucomicrobium</i>

B.2 DNA microarray for bacterial functions

B.2.1 Gene probes for bacterial functions

Target	Function	Sequence (5' – 3')	Accession No. used for design
C12O DNA	Catechol oxidation (<i>ortho</i>)	CACCTATTCGTACTTCGATTGACCCAGTCCGAGTTCAACCTGCGCCGGCGTATCATCAC	D37783
		GATGGGAAGTACAGCGGTTTCCATGACGACATCCCGACTGATTTTATCGAGGGAAGCTC	D16356
		TGCACTTCAAGGTGCGCAAGGACGGTTTGAACCGTTGACCACGCAATACTACTTCGAAG	M16964
		TACTCCTTCTTTGATAAGAGCCAAACCCCTTACAATTTGCGCCGACCATTATCGCTGAC	M57500
		CTCAATTTATTGGGTGACATGGTAACCGCCCTTCGCATGTTCACTACTTTGTCTCTGCG	Z36909
		CGAATGGAACCTCCGCGGCTCCATCATTGCCGATGACCAGGGCAACTTCCAGATCAACAC	M94318
C23O DNA	Catechol oxidation (<i>meta</i>)	GTCCTAACCTGGATGAAACTCTGAATTTGTTCCGTGATGTGCTCGGTTTTGACCTCGCTG	AB008831
		AGAGCCGAATCAAGAAAAAGGGAAGCTTCATCATCTTTGCTATTGGTATGGCATCCCGCA	X67860
		GCTAGCTCTAACTTGCGGGATTTTATGAAAGCTAAAGCTGAGCGATGCGAGTGAACGTCG	D37828
		GCCTAGATTTCTATTTAAGTGAGCAAGGTTTGGTTGGACCCGATGCATCCACTCAAGCGG	U01826
		TCGACTAGATCACCTCAACATCACAGCGAAGGACGTTGACGACGCCCGTAAGTGGTATTC	X69504
		CCATGGGATCACACGCGGGCAGACGATCTACTTCTTCGACCCCTCGGGCAACCGGAACGA	U23375
		GACTCACGGCAAGACCATCTACTTCTTCGACCCCTTCGGGCAACCGCAACGAGGTGTTCTG	X80765
		ACATCGTGCGCCAAAGAGCATCGCTAGAGAGAACAATGCAAACCAATAACAAGAGTTCGT	S78585
		CACATCGTGGTGATGGTTCGACGACTACGACGAGACGATGCGCTTTTACCGCGAGGTACTC	L77225
		CACCGTGATGTTGCTACACCGATGGACCCTGCGACCTCACGTATGGGAGAAAAATATATAT	AJ233397
<i>alkB</i>	Alkane hydroxylation (Group I)	GTGCCCCAGGAACACTCCTTATGGGCAATGTAATTTATAAGATGGCAATGCGCGAGAGAG	U40233
<i>alkM</i>	Alkane hydroxylation (Group II)	CAAGGCTGGGGAATGAGTGCTGCTTTTCATGCTTCTATGGTGGGAATTTTGGTAAAAAGT	AJ009585
		TATATCGAGCATTATGGTCTAAAACGCCAGAAAAGAGCGGATGGCAATTACGAACGTACC	AJ002316
		CCATGTTTGCAGAAATTTGGTGCCCGTTTCGATTCCGTTTCAAGTTACTCAAGCTGCGTATG	AJ009582
		GATAATGAGTTATTGCAAGGCTGGACAATGACCGCAGCATTCCATTCTATGGTTCGGG	AJ009584
<i>alkB</i> & <i>B1</i>	Alkane hydroxylation (Group III)	TATCTGCTCATTACAGCGGTGGTTCGGGTTTTCCCTGCTCGAGATCGTCAACTACATGGAG	AJ009587
		GTTTCGGCCAGTCGGTCTACCAAGTTCTGCGCATGCCTACAAGTACAACCTCCTCAGCG	AJ009580
		GTGATCCAGGGGATCTACGGCTTCTCTCTGCTGGAGGTGGTCAACTACGTGAGCACTAC	AJ009579
		GTGATCCAGGCCCTTCATCGGGTTCTCGTTACTCGAAGCGATCAACTACCTCGAGCACTAC	AJ009586
		GTAATTCAGGCGGTGTACGGCGCGTCGCTGCTCGAAGTCGTGAACTACGTGAGCACTAC	AJ293344
		GAGGACCCGGCGAGTTCACGGTTCGGAGAAAAGCTTCTGGACGTTCTGCCCCGAGTGTC	AJ401611
		GGTCTACGAGGATTGGGGTGGAATCTGGATCGGCCGTCTCGGCAAATATGGCGTCGAGAG	X55394
<i>mmoX</i>	Methane oxidation	GATCTGATCGGCCTTCACTACGTCCGCACGTGATGCCGGAATATATCCGCATGGTTCGAG	U31651
<i>pmoA</i>	Methane oxidation	AACTTCTGGGGTTGGACTTACTTCCCAGTTAATTTGCTTCCCATCTAACCTGCTGCCA	U31653
		AACTTCTGGGGATGGACTTACTTCCCAGTTAATTCGTGTTCCCATCACAATTCGCTCCA	U31654

<i>mcrA</i>	Methanogenesis	TGGCTAGGATCTTACATGTCAGGTGGAGTAGGATTCACACAATATGCTACAGCATCGTAT GACGACTTCACCTACTTCGGTAAGGAGTACGTGGAGGACAAATATGGACTCTGTGAGGCA TACATGTCTGGTGGCGTAGGTTTCACACAATACGCTACAGCAGCATACACCGACGATATC	J03375 U10036 M16893
<i>amoA</i>	Ammonia oxidation	CGTACAGGTACACCCGAGTATGTTTCGTCATATTGAGCAAGGTTCACTGCGTACCTTTGGT	L08050
<i>napA</i>	Nitrate reduction	TATGCCTTCATGCGCACCTTCGGCATGGACGAGCCGATGGGCTGCTATGACGATTTCGAG	Z36773
<i>narG</i>	Nitrate reduction	GGACCTACTTCCACGCAGGTATCCTCGACAACACTGATCCGGACACCGCAGAGAAGATAC AAATGGCATCGATCTGTATAAAGAAGCTGAAAAAGCAGGGGCAGCAACACCTGAAGACGT CAGGAATTGCGCCAGGGCGACCTCTACGACCTTCAGGAACGCTATGTGCTGCTGTTTCGAC CCATGAATTCTTCCTCAAGTATCTCTTAGGCACGAAGAATGGCCTGTTTCGCCGAGGAAGA CGGCAAAGGTCATGAATATTTTCTGAAGCACTTGCTCGGCACGACTAACGGACTGATGAA CTGGCGTTCTAACCTGCTCGGTTCTTCCGGTAAAGGTCATGAGTTTATGCTCAAGTACCT	AJ277440 X91819 Z26255 AB096694 AB087407 X16181
<i>nirK</i>	Dissimilatory itrite reduction	CTCACCGCCAAGGTCGGCGAGACCGTGCTGCTGATCCACTCGCAGGCCAATCGCGACACC TTACTACACGTTCCAGCAGCCCCGGTATCTATGCGTATGTAAACCACAATCTGATCGAGGC CTATGACACCGTCTATTACATCGGCGAGAGCGACCACTACATCCCGAAGGACGAGGACGG TATATCCCCAAGGACAAGGACGGCCACTACAAGGACTACCCGGACCTGGCGTCCAGTTAC CTGACGTACGACAAGATCTACTATGTCTGGCGAGCAGGACTTCTACGTGCCGAAGGACGAG	AF051831 M97294 U62291 Z21945 Z48635
<i>nirS</i>	Dissimilatory itrite reduction	GAACGTCAAGGAAACGGGCAAGATCCTGCTGGTGGACTATACCGACCTCGACAACCTCAA CAGATCATGCTGGTCGACTACACCGACATCAAGAACCTCAAGACCACCACCATCGAATCC GAGTTCATCGTCAACGTGAAGGAGACCGGCAAGGTCCTGCTGGTCAACTACAAGGATATC CAAGATCCTGATGGTCAACTACTCGGACTTGTCGAACCTGAAGACCACCACCATCGATTCTATGAGTATGTGACCTGGGCCGGCTGTGGCAGATCGGCAAGTTCGCCGGCATCCTGATC	AJ401462 M80653 X16452 X91394 AF002661
<i>qnorB</i>	Dissimilatory nitric oxide reduction	GATCTTCTCGAGCTTCGAGATCGTGCCCTTCTTCGCCATGATGTCATTGCGCTTCGTCAT	U28078
<i>cnorB</i>	Dissimilatory nitric oxide reduction	GTCCACATGTCCCTTTACCGAGGGCAAGTATGACGGCCGCTTCCTGTTTCATGAACGACAAG	AJ440508
<i>nosZ</i>	Nitrous oxide reduction	GGAAGGCACCTATGACGGGCGCTATCTCTACGCCAACGACAAGGCCAATACGCGTGTCTG	AJ440509
<i>nifH</i>	Nitrogen fixation	CGATGCCTATTTCGCGAAAAACAAGGCTCAGGAAATCTACATCGTCATGTCCGGTGAGATGA	V01215
<i>nrfA</i>	Nitrite reduction	GGAATATGAAACCTGGACAGCGGGCATTACGGTAAAAACAACGTGACCTGTATCGACTG	X72298
16S rDNA	Anammox	TGCATTGATAACCTGCCTTTGAGATGGGAATAACTGCGTTTCGAGCAATCGGAACCTACCG	AJ250882
<i>dsrAB</i>	Dissimilatory (bi)sulfate reduction	AAAGATGGCATTATATCTTCCGGGTACAATCCCGAAAAAGCCGATGGCAAACCGTATCAC	U16723
<i>soxB</i>	Thiosulfate oxidation	ACTGCCTTCAAACTATCGGTCTGGAGTTCGAGAGAGGTGAGTGGAATTCCGAGTGTAGA	Y16933
<i>ferA</i>	Iron reduction	CAACAGCATCAATGATCAGTACGACCTCTGCACCAGCTGCCACACCGTCAACACCATGAC	AY033095
<i>mofA</i>	Manganese oxidation	GTCTACGAGACGGTGCAGGATCCGAACCAGATGAACGGTTTCAACTCGGTGCGCCGCTGG	Z25774

<i>merA</i>	Mercuric reduction	CAGCAAGGTCACGGCCCTGGCGCGCAATACCTTGTTCTTCCGTGAAGACCCGGCCATCGG	AJ418049
		GTTGTGCACGGTGAGGCGCGCTTCAAGGACGACCAGAGCCTTACTGTCCGTTTGAACGAG	AJ418052
		AGTGCGGCCGTGCCGCCGATTCCCGGACTGCAAGATACCCCTTTTGGAACTCGGAAAAAG	AJ418056
		CATGGAAGGCATCCTGGAAGAGCTATGGCCATCACCATTTGCCCCGGCTACGCCCGTTTC	AJ418057
<i>tpm</i>	Selenium methylation	TTGAAGGCCGGATTGGAGCGTATGGATGAGCACGTTTATGTGTTGGAACGTGTGTAACCTC	L49178
<i>cadA</i>	Cadmium resistance	ACTACACTCCGATCATTATGGTTATTGCAGCCTTGTTGTCAGTCGTTCCACCCCTATTCT	J04551
<i>pcoR</i>	Copper resistance	GATCCGTTCCGGGAAGAAGATCCATCTCACCGGTAAAGAATACGTTCTGCTTGAGTTGCT	X83541
<i>phaZ</i>	PHB depolymerization	TCTATCGCAACGGCAACAAGGCCAACGCGCTCCCGGTCTCTGCCACCAGCTATGTCGATG	U16275
		CCGTATCGGCGACGACGAAATCGGCCTTCACCTGTACTGCCACCACGGCCAGCAACTACG	J04223
		GTCACGGCCACCACCTACACCGACACAGGCCTGACCACCGGTACGGCCTACTCCTACACC	U58990
		CTATACCGACACCGGCCTGATTGCTGGTACCACCTACAGCTACACCGTAACCGAGATCGA	D25315
<i>apr</i>	Hydrolysis of peptidic compound	GGCAACGACACCCTGGACTTCTCCGGTTTCACCCAGAACCAGAAGATCAACCTCAATGAG	AB013895
<i>npr</i>	Hydrolysis of peptidic compound	AACAATGCATTCTGGAACGGATCACAGATGGTATACGGAGATGGTGATGGTGTAACGTTT	M83910
<i>sub</i>	Hydrolysis of peptidic compound	TACTGGTTCTACAGCGCTGAAAACAGTAGTTGATAAAGCGGTTTCCAGCGGTATCGTCGT	S51909_1
<i>sub</i>	Hydrolysis of peptidic compound	TTCAACAGGAAGCGGCCAATATAGCTGGATTATTAACGGCATTGAGTGGGCCATTTCCAA	S51909_2
<i>chiA</i>	Chitin depolymerition	GTGAAAGAGTTCCTGCAGACCTGGAAGTTCTTCGATGGCGTGGATATCGACTGGGAGTTC	AY040610
		CAGCAATTGGCGTTGGTTACGACAAAATTGAAGACGTTGATTACGCAGATGCTGTGCAGT	AF193498
		ATAACGGTATCCAACCTTCTTCTTGCTCAAGGCGTTCTGCGAACAAGCTAGTCCTTGGTA	AB004935
		CTGAAACAGGCCGTACTTACGAACTGACATCGGCAGTAGGTGTAGGCTACGACAAGATTG	AF193500
		CATATGGGTTTGATGGCGTGGATTTAGACTGGGAATATCCGGGCGTTGAAACGATTCCTG	AY129671
		TAAACCGCAGATCGACGTTCAGGAAGAAATCCGCAGTCGCGTAGATTTCTGAAACAGTA	AB110082
		CTCCAACAGATGACATTACGCCTACACCTACACCTACTCTTGAACCAACGCCTGAACCAA	Z68924
		GGGCATTCATGGGCTGGTTGCAATGCAGATAATGATGGGAAAATGGTTGTTGAGAATGGT	BA000028

B.2.2 Primers used to prepare template for bacterial function gene array

Target gene	Forward primer	Reverse primer	Annealing Temp (°C)	Reference
C12O DNA	GCCAACGTCGACGTCTGGCA	CGCCTTCAAAGTTGATCTGCGTGGT	60	Sei <i>et al.</i> , 1999
C23O DNA	AAGAGGCATGGGGGCGCACCGGTTTCGATCA	CCAGCAAACACCTCGTTGCGGTTGCC	60	Sei <i>et al.</i> , 1999
<i>alkB</i>	CATAATAAAGGGCATCACCGT	GATTCATTCTCGAAACTCCAAAC	40	Kohno <i>et al.</i> , 2002
<i>alkM</i>	GAGACAAATCGTCTAAAACGTAA	TTGTTATTATTCCAACTATGCTC	40	Kohno <i>et al.</i> , 2002
<i>alkB / alkB1</i>	TCGAGCACATCCGCGGCCACCA	CCGTAGTGCTCGACGTAGTT	40	Kohno <i>et al.</i> , 2002
<i>mmoX</i>	GGCTCCAAGTTCAAGGTCGAGC	TGGCACTCGTAGCGCTCCGGCTCG	55	McDonald <i>et al.</i> , 1995
<i>pmoA</i>	GGGGGAACCTTCTGGGGITGGAC	GGGGGRCIACGTCITTACCGAA	45	Cheng <i>et al.</i> , 1999
<i>mcrA</i>	TAYGAYCARATHTGGYT	ACRTTCATNGCNGCRTARTT	45	Springer <i>et al.</i> , 1995
<i>amoA</i>	GGGGTTTCTACTGGTGGT	CCCCTCKGSAAAGCCTTCTTC	55	Rotthauwe <i>et al.</i> , 1997
<i>napA</i>	TAYTTYTNHSNAARATHATGTAYGG	DATNGGRTGCATYTCNGCCATRTT	45	Flanagan <i>et al.</i> , 1999
<i>narG</i>	TAYGTSGGSCARGARAA	TTYTCRTACCABGTBGC	60	Philippot <i>et al.</i> , 2002
<i>nirK</i>	GGSGCGGTCATGGTGCTGCC	TCGAAGGCCTCGATCAG	65	This study
<i>nirS</i>	TAYCACCCCGAGCCGCGCGT	CTTRAGYTYAGBTCTTGTCGTC	65	This study
<i>qnorB</i>	GGNCAYCARGGNTAYGA	ACCCANAGRTGNCANACCCACCA	55	Braker and Tiedje, 2003
<i>cnorB</i>	GACAAGNNNTACTGGTGGT	GAANCCCCANACNCCNGC	55	Braker and Tiedje, 2003
<i>nosZ</i>	CGGYTGGGGAMWKACCAA	ATRTCGATCARYTGNTCRTT	55	Nogales <i>et al.</i> , 2002
<i>nifH</i>	AAAGGYGGWATCGGYAARTCCACCAC	TTGTTSGCSGCRATATSGCCATCAT	60	Rösch <i>et al.</i> , 2002
<i>nrfA</i>	GCNTGYTGWSNTGYAA	TWNGGCATRTGRCARTC	45	Mohan <i>et al.</i> , 2004
16S rDNA (<i>anammox</i>)	GGATTAGGCATGCAAGTC	AAAACCCCTCTACTTAGTGCCC	60	Egli <i>et al.</i> , 2001
<i>dsrAB</i>	ATCGGWACCTGGAAGGAYGACATCAA	GGGCACATSGTGTAGCAGTTACCGCA	60	Karkhoff-Schweizer <i>et al.</i> , 1995
<i>soxB</i>	GAYGGNGGNGAYACNTGG	CATGTCNCCNCCRTGYTG	60	Petri <i>et al.</i> , 2001
<i>ferA</i>	ACARMARSGRTGTYGGSTGC	TGGATYMCSRARGTYGYAGTG	45	Neal <i>et al.</i> , 2004
<i>nofA</i>	GGCTTCACCGAGTTCACGCA	CCAGCGGGGTGTCCATCCAG	60	Siering and Ghiorse, 1997
<i>merA</i>	TTGGAGAACGTGC	ACGTCCTTGGTGAAGGTCTG	55	Felske <i>et al.</i> , 2003
<i>tpm</i>	CAGTCAGAGGTCAATAAGG	GAGTTACACACGTTCCAACA	40	Courmoyer <i>et al.</i> , 1998
<i>cadA</i>	CAAAYTYGCRGGHAARTTYGA	AACTAATGCACAAGGACA	55	Oger <i>et al.</i> , 2001
<i>pcoR</i>	CAGGTCGTTACCTGCAGCAG	CTCTGATCTCCAGGACATATC	55	Trajanovska <i>et al.</i> , 1997
<i>phaZ</i>	CGTCTACCGCAACGGCACCAAGG	TGGGCGTAGTTGCTGGCCGT	55	Sei <i>et al.</i> , 2001
<i>apr</i>	TAYGGBTTCAAYTCCAAYAC	VGCGATSGAMACRTTRCC	55	Bach <i>et al.</i> , 2001
<i>npr</i>	GTDGAYGCHCAYTAYTAYGC	ACMGCATGBGTYADYTCATG	55	Bach <i>et al.</i> , 2001
<i>sub</i>	ATGSAYRTTRYAAYATGAG	GWGWHGCCATNGAYGTWC	55	Bach <i>et al.</i> , 2001
<i>sub</i>	GNACHCAYGTDGCHGGHAC	GWGWHGCCATNGAYGTWC	55	Bach <i>et al.</i> , 2001
<i>chiA</i>	GGIGGITGACIYTIWSIGAYCCITT	ATRTCICCRTTRTCIGCRTC	40	LeClerc <i>et al.</i> , 2004

B.3 Pathogen bacterial probes

Genus	Species Epithet
<i>Abiotrophia</i>	<i>defectiva</i> B
<i>Abiotrophia</i>	<i>defectiva</i> A
<i>Acetobacter</i>	<i>orientalis</i>
<i>Acetobacter</i>	<i>aceti</i>
<i>Acetobacter</i>	<i>pasteurianus</i>
<i>Acholeplasma</i>	<i>laidlawii</i>
<i>Acholeplasma</i>	<i>oculi</i>
<i>Acholeplasma</i>	<i>axanthum</i>
<i>Acholeplasma</i>	<i>modicum</i>
<i>Achromobacter</i>	<i>xylosoxidans</i> A
<i>Achromobacter</i>	<i>piechaudii</i>
<i>Achromobacter</i>	<i>xylosoxydans</i> B
<i>Acidaminococcus</i>	<i>fermentans</i> A
<i>Acidaminococcus</i>	<i>fermentans</i> B
<i>Acidovorax</i>	<i>anthurii</i>
<i>Acidovorax</i>	<i>avenae</i> 180
<i>Acidovorax</i>	<i>avenae</i> group
<i>Acinetobacter</i>	<i>lwoffii</i> 140
<i>Acinetobacter</i>	<i>johnsoni</i> 140
<i>Acinetobacter</i>	<i>haemolyticus</i> 140
<i>Acinetobacter</i>	<i>calcoaceticus</i> B140
<i>Acinetobacter</i>	<i>baumannii</i> A450
<i>Acinetobacter</i>	<i>radioresistens</i> 450
<i>Acinetobacter</i>	<i>calcoaceticus</i> B450
<i>Acinetobacter</i>	<i>johnsoni</i> 450
<i>Acinetobacter</i>	<i>calcoaceticus</i> A450
<i>Acinetobacter</i>	<i>baumannii</i> 140
<i>Acinetobacter</i>	<i>anitratum</i> 140
<i>Acinetobacter</i>	<i>calcoaceticus</i> A140
<i>Acinetobacter</i>	<i>junii</i> 140
<i>Acinetobacter</i>	<i>radioresistens</i> 140
<i>Acinetobacter</i>	<i>iwofii</i> 450
<i>Acinetobacter</i>	<i>haemolyticus</i>
<i>Acinetobacter</i>	<i>junii</i> 450
<i>Acinetobacter</i>	<i>anitratum</i> 450
<i>Acinetobacter</i>	<i>baumannii</i> B450
<i>Anaplasma</i>	<i>ovis</i>
<i>Anaplasma</i>	<i>marginale</i> centrale
<i>Anaerococcus</i>	<i>prevotii</i> B
<i>Anaerococcus</i>	<i>vaginalis</i>
<i>Anaerorhabdus</i>	<i>furcosus</i>
<i>Arcanobacterium</i>	<i>phocae</i>
<i>Arcanobacterium</i>	<i>pyogenes</i>

Genus	Species Epithet
<i>Acrobacter</i>	<i>butzleri</i>
<i>Acrobacter</i>	<i>genus</i> 450
<i>Acrobacter</i>	<i>cryaerophilus</i>
<i>Acrobacter</i>	spp.
<i>Actinobacillus</i>	<i>equuli</i>
<i>Actinobacillus</i>	<i>lignieresii</i> A
<i>Actinobacillus</i>	<i>scotiae</i>
<i>Actinobacillus</i>	<i>suis</i>
<i>Actinobacillus</i>	<i>rossii</i>
<i>Actinobacillus</i>	<i>pleuropneumoniae</i>
<i>Actinobacillus</i>	<i>capsulatus</i>
<i>Actinobacillus</i>	<i>hominis</i>
<i>Actinobacillus</i>	<i>lignieresii</i> B
<i>Actinobacillus</i>	<i>seminis</i>
<i>Actinobacillus</i>	<i>porcitonsillarum</i>
<i>Actinobacillus</i>	<i>muris</i>
<i>Actinobacillus</i>	<i>arthritidis</i>
<i>Actinobaculum</i>	<i>schaalii</i>
<i>Actinobaculum</i>	<i>urinae</i>
<i>Actinobaculum</i>	<i>suis</i>
<i>Actinomadura</i>	<i>madurae</i>
<i>Actinomadura</i>	<i>pelletieri</i>
<i>Actinomadura</i>	spp.
<i>Actinomyces</i>	<i>gerencseriae</i>
<i>Actinomyces</i>	<i>hyovaginalis</i>
<i>Actinomyces</i>	<i>meyeri</i> group
<i>Actinomyces</i>	<i>radigae</i>
<i>Actinomyces</i>	<i>viscosus</i> group
<i>Actinomyces</i>	<i>bovis</i>
<i>Actinomyces</i>	<i>hordeovulneris</i>
<i>Actinomyces</i>	<i>israelii</i>
<i>Actinomyces</i>	<i>neuii</i>
<i>Actinomyces</i>	<i>turicensis</i>
<i>Aegyptianella</i>	<i>pullorum</i>
<i>Aeromonas</i>	<i>sobria</i> 450
<i>Aeromonas</i>	<i>caviae</i> 450
<i>Bacteroides</i>	<i>gracilis</i>
<i>Bacteroides</i>	<i>putredinis</i>
<i>Bacteroides</i>	<i>stercoris</i>
<i>Bacteroides</i>	<i>uniformis</i>
<i>Bacteroides</i>	<i>ureolyticus</i> B
<i>Bacteroides</i>	<i>vulgatus</i> group
<i>Balnearia</i>	<i>alpica</i>

Genus	Species Epithet
<i>Aerococcus</i>	<i>viridans</i> jj subsp. <i>Homari</i>
<i>Aerococcus</i>	<i>urinae</i>
<i>Aerococcus</i>	<i>urinaehominis</i>
<i>Aeromonas</i>	<i>hydrophylla</i> 450
<i>Aeromonas</i>	<i>punctata</i> 450
<i>Aeromonas</i>	<i>punctata</i> 130
<i>Aeromonas</i>	<i>caviae</i> group 130
<i>Aeromonas</i>	<i>hydrophylla</i> group 130
<i>Aeromonas</i>	<i>veronii</i> group 130
<i>Aeromonas</i>	<i>salmonicida</i> 450
<i>Aeromonas</i>	<i>veronii</i> 450
<i>Aeromonas</i>	<i>media</i> 450
<i>Aeromonas</i>	<i>simiae</i> 450
<i>Aeromonas</i>	<i>simiae</i> 130
<i>Aeromonas</i>	<i>salmonicida</i> group 130
<i>Aeromonas</i>	<i>sobria</i> group 130
<i>Afipia</i>	<i>broomeae</i> group
<i>Afipia</i>	<i>genospecies</i>
<i>Afipia</i>	<i>felis</i>
<i>Agrobacterium</i>	<i>rubi</i>
<i>Agrobacterium</i>	<i>tumofaciens</i> group
<i>Alcaligenes</i>	<i>species</i>
<i>Alcaligenes</i>	<i>faecalis</i>
<i>Alkalibacterium</i>	<i>olivoapovlitis</i>
<i>Alloiococcus</i>	<i>otitis</i>
<i>Alloiococcus</i>	spp.
<i>Allomonas</i>	<i>enterica</i>
<i>Anaerobiospirillum</i>	<i>thomasi</i>
<i>Anaerobiospirillum</i>	<i>succiniciproducens</i>
<i>Anaerococcus</i>	<i>lactolyticus</i>
<i>Anaerococcus</i>	<i>prevotii</i> A
<i>Anaerococcus</i>	<i>tetradius</i>
<i>Anaerococcus</i>	<i>hydrogenalis</i>
<i>Anaerococcus</i>	<i>octavius</i>
<i>Anaplasma</i>	<i>phagocytophila</i>
<i>Anaplasma</i>	<i>bovis</i>
<i>Brevibacterium</i>	<i>linens</i>
<i>Brenneria</i>	<i>nigrifluens</i>
<i>Brevibacillus</i>	<i>agri</i>
<i>Brevibacillus</i>	<i>brevis</i>
<i>Brevibacillus</i>	<i>laterosporus</i>
<i>Brevibacterium</i>	<i>mcbrellneri</i>
<i>Brevibacterium</i>	<i>otitidis</i>

Genus	Species Epithet
Arcanobacterium	bovis
Arcanobacterium	haemolyticum
Arthrobacter	cumminsii
Arthrobacter	ilicis
Arthrobacter	albus
Arthrobacter	globiformis
Arthrobacter	woluwensis
Atopobium	minutum
Atopobium	rimae
Atopobium	fossor
Atopobium	parvulum
Bacillus	anthracis
Bacillus	coagulans
Bacillus	mycoides
Bacillus	sphaericus
Bacillus	Thuringensis
Bacillus	Cereus
Bacillus	Megaterium
Bacillus	Pumilus
Bacillus	Subtilis
Bacteroides	capillosus
Bacteroides	distasonis B
Bacteroides	fragilis group
Bacteroides	ovatus
Bacteroides	splanchnicus
Bacteroides	thetaitaomicron group
Bacteroides	urealyticum A
Bacteroides	vulgatus
Bacteroides	caccae
Bacteroides	distasonis A
Bacteroides	fragilis A
Cardiobacterium	valvarum
Cardiobacterium	hominis
Cardiobacterium	divergens
Catonella	SP A.
Catonella	morbi
Catonella	SP B
Caulobacter	fusiformis
Caulobacter	henricii
Caulobacter	halobacterioide
Caulobacter	intermedius
Cedecea	davisae-neteri
Centipeda	periodontii
Chlamydia	trachomatis
Chlamydia	suis
Chlamydomphila	caviae

Genus	Species Epithet
Bartonella	henselae 150
Bartonella	quintana 150
Bartonella	group 210
Bartonella	bacilliformis 150
Bartonella	vinsonii vinsonii 150
Bergeyella	zoohelcum
Bifidobacterium	animalis
Bifidobacterium	dentium
Bifidobacterium	pseudolongum
Bifidobacterium	adolescentis
Bifidobacterium	breve
Bifidobacterium	infantis group
Bilophila	wadsworthia
Bordetella	pertussis group
Bordetella	avium group
Borrelia	anserina
Borrelia	coriaceae
Borrelia	parkeritunicatae
Borrelia	lonestari
Borrelia	tanukii
Borrelia	afzelii japonica
Borrelia	burgdorffii valaisiana
Borrelia	duttonii persica
Borrelia	recurrentis
Borrelia	sinicaandersonii
Brachyspira	hyodysenteriae group
Brachyspira	spp.
Brachyspira	aalborgi
Brachyspira	pilosicoli
Brenneria	alni
Brenneria	rubrificans
Citrobacter	youngae
Clavibacter	michiganensis
Clostridium	ghoni
Clostridium	hastiforme
Clostridium	indolis
Clostridium	limosum
Clostridium	novyi A
Clostridium	oroticum
Clostridium	perfringens
Clostridium	puniceum
Clostridium	putrificum
Clostridium	septicum
Clostridium	sphenoides
Clostridium	sporogenes B
Clostridium	subterminale B

Genus	Species Epithet
Brevinema	andersonii
Brevundimonas	diminuta
Brevundimonas	group
Brucella	melitensis
Burkholderia	gladioli group 450
Burkholderia	mallei
Burkholderia	vietnamsensis 450
Burkholderia	cepacia 450
Burkholderia	glumae 450
Burkholderia	uvoniae 450
Butyrivibrio	fibrisolvens
Campylobacter	fetus group 400
Campylobacter	gracilis
Campylobacter	jejuni coli
Campylobacter	lari
Campylobacter	rectus
Campylobacter	upsaliensis
Campylobacter	curvus
Campylobacter	jejuni group 400
Campylobacter	jejuni coli lari
Campylobacter	mucosalis
Campylobacter	sputorum
Campylobacter	concisus
Campylobacter	fetus group 400
Capnocytophaga	canimorsus
Capnocytophaga	gingivalis
Capnocytophaga	haemolytica
Capnocytophaga	sputigena
Capnocytophaga	cynodegmi
Capnocytophaga	granulosa
Capnocytophaga	ochracea
Clostridium	symbiosum
Clostridium	tetani
Clostridium	argentinense
Clostridium	bifermentans
Clostridium	botulinum B
Clostridium	botulinum E
Clostridium	botulinum C haemolyticum
Clostridium	butyricum
Clostridium	carnis
Clostridium	clostridiiforme
Clostridium	difficile
Clostridium	fallax
Collinsella	aerofaciens
Comamonas	denitrificans
Comamonas	terrigena

Genus	Species Epithet
<i>Chlamydophila</i>	<i>pneumoniae</i>
<i>Chlamydophila</i>	<i>abortus</i>
<i>Chlamydophila</i>	<i>pecorum</i>
<i>Chlamydophila</i>	<i>psittaci</i> group
<i>Chromobacterium</i>	<i>violaceum</i>
<i>Chryseobacterium</i>	<i>meningosepticum</i> 1(450)
<i>Chryseobacterium</i>	<i>balustinum</i> group
<i>Chryseobacterium</i>	<i>leum-indologenes</i>
<i>Chryseobacterium</i>	<i>meningosepticum</i> group
<i>Chryseobacterium</i>	<i>proteolyticum</i> (450)
<i>Chryseobacterium</i>	<i>meningosepticum</i> 2(450)
<i>Chryseobacterium</i>	<i>gleum</i>
<i>Chryseobacterium</i>	<i>indoltheticum</i> group(450)
<i>Chryseobacterium</i>	<i>proteolyticum</i>
<i>Chryseobacterium</i>	<i>scophthalmum</i> (450)
<i>Chryseomonas</i>	<i>luteola</i>
<i>Citrobacter</i>	<i>amalonaticus</i>
<i>Citrobacter</i>	<i>diversus</i>
<i>Citrobacter</i>	<i>freundii</i>
<i>Citrobacter</i>	<i>werkmanii</i>
<i>Citrobacter</i>	<i>braakii</i>
<i>Citrobacter</i>	<i>farmeri</i>
<i>Citrobacter</i>	<i>sedlakii</i>
<i>Corynebacterium</i>	<i>bovis</i>
<i>Corynebacterium</i>	<i>cystitidis</i>
<i>Corynebacterium</i>	<i>glutamicum</i>
<i>Corynebacterium</i>	<i>imitans</i>
<i>Corynebacterium</i>	<i>kutscheri</i>
<i>Corynebacterium</i>	<i>mastitidis</i>
<i>Corynebacterium</i>	<i>minutissimum</i>
<i>Corynebacterium</i>	<i>pilosum</i>
<i>Corynebacterium</i>	<i>pseudotuberculosis</i>
<i>Corynebacterium</i>	<i>seminale</i>
<i>Corynebacterium</i>	<i>ulcerans</i>
<i>Cowdria</i>	<i>ruminantium</i> 1
<i>Cowdria</i>	<i>ruminantium</i> 2
<i>Coxiella</i>	<i>burnetii</i>
<i>Curtobacterium</i>	<i>herbarum</i>
<i>Curtobacterium</i>	<i>flaccumfaciens</i>
<i>Curtobacterium</i>	<i>psychrophilum</i>
<i>Cytophaga</i>	<i>aurantiaca</i>
<i>Cytophaga</i>	<i>hutchinsonii</i>
<i>Cytophaga</i>	<i>johnsonae</i>
<i>Delftia</i>	<i>acidovorans</i>
<i>Dermatophilus</i>	<i>cheloniae</i>
<i>Dermatophilus</i>	<i>congolensis</i>

Genus	Species Epithet
<i>Clostridium</i>	<i>tertiium</i>
<i>Clostridium</i>	<i>absonum</i>
<i>Clostridium</i>	<i>baratii</i>
<i>Clostridium</i>	<i>botulinum</i> A/F
<i>Clostridium</i>	<i>botulinum</i> B3
<i>Clostridium</i>	<i>botulinum</i> D
<i>Clostridium</i>	<i>botulinum</i> G
<i>Clostridium</i>	<i>cadaveris</i>
<i>Clostridium</i>	<i>chauvoei</i>
<i>Clostridium</i>	<i>colinum</i>
<i>Clostridium</i>	<i>disporicum</i>
<i>Clostridium</i>	<i>glycolicum</i>
<i>Clostridium</i>	<i>histolyticum</i>
<i>Clostridium</i>	<i>innocuum</i>
<i>Clostridium</i>	<i>malenominatum</i>
<i>Clostridium</i>	<i>novyi</i> B
<i>Clostridium</i>	<i>paraputrificum</i>
<i>Clostridium</i>	<i>piliforme</i>
<i>Clostridium</i>	<i>putrificans</i>
<i>Clostridium</i>	<i>ramosum</i>
<i>Clostridium</i>	<i>sordellii</i>
<i>Clostridium</i>	<i>sporogenes</i> A
<i>Clostridium</i>	<i>subterminale</i> A
<i>Eikenella</i>	<i>corrodens</i>
<i>Empedobacter</i>	<i>brevis</i>
<i>Enterobacter</i>	<i>cloacae</i> group
<i>Enterobacter</i>	<i>aerogenes</i> 450
<i>Enterobacter</i>	<i>intermedius</i> 450
<i>Enterobacter</i>	<i>gergoviae</i> group
<i>Enterobacter</i>	<i>sakazaki</i> 450
<i>Enterobacter</i>	<i>pyrinus</i> 450
<i>Enterobacter</i>	<i>aerogenes</i> group
<i>Enterobacter</i>	<i>cancerogenus</i> 450
<i>Enterobacter</i>	<i>cloacae</i> 450
<i>Enterobacter</i>	<i>gergoviae</i> 450
<i>Enterobacter</i>	<i>nimipressuralis</i> 450
<i>Enterobacter</i>	<i>dissolvens</i> 450
<i>Enterobacter</i>	<i>intermedius</i>
<i>Enterococcus</i>	<i>avium</i>
<i>Enterococcus</i>	<i>faecalis</i>
<i>Enterococcus</i>	<i>faecium</i> 2 <i>durans</i>
<i>Enterococcus</i>	<i>haemoperoxidus</i>
<i>Enterococcus</i>	<i>moraviensis</i>
<i>Enterococcus</i>	<i>munditii</i>
<i>Enterococcus</i>	<i>phoeniculicola</i>
<i>Enterococcus</i>	<i>raffinosis</i>

Genus	Species Epithet
<i>Comamonas</i>	<i>aquatica</i>
<i>Comamonas</i>	<i>kerstersii</i>
<i>Comamonas</i>	<i>testosteloni</i>
<i>Coprobacillus</i>	<i>catenaformis</i>
<i>Coprococcus</i>	<i>catus</i>
<i>Coprococcus</i>	<i>eutactus</i>
<i>Corynebacterium</i>	<i>accolens</i>
<i>Corynebacterium</i>	<i>Amycolatum</i>
<i>Corynebacterium</i>	<i>auris</i>
<i>Corynebacterium</i>	<i>coyleae</i>
<i>Corynebacterium</i>	<i>diphtheriae</i>
<i>Corynebacterium</i>	<i>hoagii</i>
<i>Corynebacterium</i>	<i>jeikeium</i> group
<i>Corynebacterium</i>	<i>lipophiloflavum</i>
<i>Corynebacterium</i>	<i>matruchotii</i>
<i>Corynebacterium</i>	<i>mycetoides</i>
<i>Corynebacterium</i>	<i>propinquum</i> group
<i>Corynebacterium</i>	<i>renale</i>
<i>Corynebacterium</i>	<i>striatum</i>
<i>Corynebacterium</i>	<i>ureolyticum</i>
<i>Corynebacterium</i>	<i>afermentans</i>
<i>Corynebacterium</i>	<i>argentoratense</i>
<i>Corynebacterium</i>	<i>auriscanis</i>
<i>Erwinia</i>	<i>bulbicola</i>
<i>Erwinia</i>	<i>mallotivora</i>
<i>Erwinia</i>	<i>ananas</i>
<i>Erwinia</i>	<i>herbicola</i>
<i>Erysipelothrix</i>	spp.
<i>Erysipelothrix</i>	<i>tonsillarum</i> 450
<i>Erysipelothrix</i>	<i>rhusiopathiae</i> 450
<i>Eubacterium</i>	<i>combesii</i>
<i>Eubacterium</i>	<i>infirmum</i>
<i>Eubacterium</i>	<i>minutum</i>
<i>Eubacterium</i>	<i>nitritogenes</i>
<i>Eubacterium</i>	<i>saphenum</i>
<i>Eubacterium</i>	<i>tenue</i>
<i>Eubacterium</i>	<i>ventriosum</i>
<i>Eubacterium</i>	<i>rectale</i>
<i>Eubacterium</i>	<i>brachy</i>
<i>Eubacterium</i>	<i>contortum</i>
<i>Eubacterium</i>	<i>limosum</i>
<i>Eubacterium</i>	<i>moniliforme</i>
<i>Eubacterium</i>	<i>nodatum</i>
<i>Eubacterium</i>	<i>sulci</i>
<i>Eubacterium</i>	<i>tortuosum</i>
<i>Eubacterium</i>	<i>yurii</i> subsp <i>yurii</i>

Genus	Species Epithet
<i>Desulfovibrio</i>	<i>hydrothermalis</i>
<i>Desulfovibrio</i>	<i>intestinalis</i>
<i>Dialister</i>	<i>pneumosintes</i>
<i>Dialister</i>	<i>invisus</i>
<i>Dichelobacter</i>	<i>nodosus</i>
<i>Dolosigranulum</i>	<i>pigrum</i>
<i>Edwardsiella</i>	<i>tarda-ictalui</i> group
<i>Eggerthella</i>	<i>lenta</i> group
<i>Ehrlichia</i>	<i>chaffeensis</i>
<i>Ehrlichia</i>	<i>ewingii</i>
<i>Ehrlichia</i>	<i>risticii</i>
<i>Ehrlichia</i>	<i>canis</i>
<i>Ehrlichia</i>	<i>equi</i>
<i>Ehrlichia</i>	<i>muris</i>
<i>Ehrlichia</i>	<i>sennetsu</i>
<i>Flavobacterium</i>	<i>columnaris</i>
<i>Flavobacterium</i>	<i>branchiophilum</i>
<i>Flavobacterium</i>	<i>johnsoniae</i>
<i>Flavobacterium</i>	<i>gelidilacus</i>
<i>Flexibacter</i>	<i>flexilis</i>
<i>Flexibacter</i>	<i>aggregans</i>
<i>Flexibacter</i>	<i>ovolyticus</i>
<i>Francisella</i>	group
<i>Francisella</i>	<i>tularensis</i> group 450
<i>Francisella</i>	<i>philomiragia</i> 450
<i>Fusobacterium</i>	<i>gonidiaforme</i>
<i>Fusobacterium</i>	<i>naviforme</i>
<i>Fusobacterium</i>	<i>necrophorum</i> A
<i>Fusobacterium</i>	<i>nucleatum</i> A
<i>Fusobacterium</i>	<i>periodonticum</i>
<i>Fusobacterium</i>	<i>simiae</i>
<i>Fusobacterium</i>	<i>varium</i>
<i>Fusobacterium</i>	<i>equinum</i>
<i>Fusobacterium</i>	<i>mortiferum</i>
<i>Fusobacterium</i>	<i>necrogenes</i>
<i>Fusobacterium</i>	<i>necrophorum</i> B
<i>Fusobacterium</i>	<i>nucleatum</i> B
<i>Fusobacterium</i>	<i>russii</i>
<i>Fusobacterium</i>	<i>ulcerans</i>
<i>Gardnerella</i>	<i>vaginalis</i>
<i>Gemella</i>	<i>Morbillosum</i>
<i>Gemella</i>	<i>palaticanis</i>
<i>Gemella</i>	<i>haemolysans</i>
<i>Gemella</i>	<i>bergeri</i>
<i>Gemella</i>	<i>sanguinis</i>
<i>Geobacillus</i>	<i>stearothermophilus</i>

Genus	Species Epithet
<i>Enterococcus</i>	<i>saccharolyticus</i>
<i>Enterococcus</i>	<i>dispar</i>
<i>Enterococcus</i>	<i>faecium</i> 1
<i>Enterococcus</i>	<i>flavescens gallinarum</i>
<i>Enterococcus</i>	<i>hirae</i>
<i>Enterococcus</i>	<i>pallens</i>
<i>Enterococcus</i>	<i>pseudoavium</i>
<i>Enterococcus</i>	<i>rottae</i>
<i>Enterococcus</i>	<i>solitarius</i>
<i>Eperythrozoon</i>	<i>coccoides</i>
<i>Eperythrozoon</i>	spp.
<i>Eperythrozoon</i>	<i>ovis</i>
<i>Eperythrozoon</i>	<i>wenyonii</i>
<i>Eperythrozoon</i>	<i>suis</i>
<i>Erwinia</i>	<i>amylovora</i>
<i>Granulicatella</i>	<i>adiacens</i> group
<i>Granulicatella</i>	<i>elegans</i>
<i>Granulicatella</i>	<i>balaenopterae</i>
<i>Haemobartonella</i>	<i>felis</i>
<i>Haemobartonella</i>	<i>canis</i>
<i>Haemophilus</i>	<i>somnus</i>
<i>Haemobartonella</i>	<i>muris</i>
<i>Haemophilus</i>	<i>segnis</i>
<i>Haemophilus</i>	<i>parasuis</i> B
<i>Haemophilus</i>	<i>paragallinarum</i>
<i>Haemophilus</i>	<i>influenzae</i> B
<i>Haemophilus</i>	<i>aegypticus</i>
<i>Haemophilus</i>	<i>aphrophilus</i>
<i>Haemophilus</i>	<i>haemoglobinophilus</i>
<i>Haemophilus</i>	<i>parahemolyticus</i>
<i>Haemophilus</i>	<i>paraphrohaemolyticus</i>
<i>Haemophilus</i>	<i>piscium</i>
<i>Haemophilus</i>	<i>parasuis</i> A
<i>Haemophilus</i>	<i>paracuniculus</i>
<i>Haemophilus</i>	<i>influenzae</i> A
<i>Haemophilus</i>	<i>actinomycescomitans</i>
<i>Haemophilus</i>	<i>ducreyi</i>
<i>Haemophilus</i>	<i>haemolyticus</i>
<i>Haemophilus</i>	<i>parainfluenzae</i>
<i>Haemophilus</i>	<i>paraphrophilus</i>
<i>Hafnia</i>	<i>alvei</i>
<i>Helcococcus</i>	<i>kunzii</i>
<i>Helicobacter</i>	<i>bizzozeronii</i> group
<i>Helicobacter</i>	<i>cetorum</i>
<i>Helicobacter</i>	<i>cinaedi</i>
<i>Helicobacter</i>	<i>hepaticus</i>

Genus	Species Epithet
<i>Eubacterium</i>	<i>sulci</i>
<i>Ewingella</i>	<i>americana</i>
<i>Facklamia</i>	<i>hominis</i>
<i>Facklamia</i>	<i>sourekii</i>
<i>Faecalibacterium</i>	<i>prausnitzii</i> A
<i>Faecalibacterium</i>	<i>prausnitzii</i> B
<i>Filifactor</i>	<i>alocis</i> A
<i>Filifactor</i>	<i>villosus</i>
<i>Filifactor</i>	<i>alocis</i> B
<i>Finegoldia</i>	<i>magna</i> A
<i>Finegoldia</i>	<i>magna</i> jjB
<i>Flavimonas</i>	<i>oryzihabitans</i>
<i>Flavobacterium</i>	<i>columnaris</i>
<i>Flavobacterium</i>	<i>mizutaii</i>
<i>Flavobacterium</i>	<i>psychrophilum</i>
<i>Helicobacter</i>	<i>felis</i>
<i>Herbaspirillum</i>	<i>rubrisubalbicans</i>
<i>Herbaspirillum</i>	<i>seropedicae</i>
<i>Johnsonella</i>	<i>ignava</i>
<i>Jonesia</i>	<i>denitrificans</i>
<i>Kingella</i>	<i>denitrificans</i>
<i>Kingella</i>	<i>oralis</i>
<i>Kingella</i>	<i>kingae</i>
<i>Klebsiella</i>	<i>pneumonia</i> group
<i>Klebsiella</i>	<i>oxytoca</i> group
<i>Kluyvera</i>	<i>cryocrescens</i>
<i>Kluyvera</i>	<i>ascorbata</i>
<i>Kluyvera</i>	<i>georgiana</i>
<i>Lactobacillus</i>	<i>acidophilus</i>
<i>Lactobacillus</i>	<i>buchneri</i>
<i>Lactobacillus</i>	<i>delbruekii</i> group
<i>Lactobacillus</i>	<i>jensenii</i>
<i>Lactobacillus</i>	<i>plantarum</i>
<i>Lactobacillus</i>	<i>niger</i>
<i>Lactobacillus</i>	<i>brevis</i>
<i>Lactobacillus</i>	<i>casei</i>
<i>Lactobacillus</i>	<i>gasseri</i>
<i>Lactobacillus</i>	<i>ramnosus</i>
<i>Lactococcus</i>	<i>garviae</i>
<i>Lactococcus</i>	<i>plantarum</i>
<i>Lactococcus</i>	<i>lactis</i>
<i>Lactococcus</i>	<i>raffinolactis</i>
<i>Lawsonia</i>	<i>intracellularis</i>
<i>Leclercia</i>	<i>adecarboxylata</i>
<i>Legionella</i>	<i>adelaidensis</i>
<i>Legionella</i>	<i>adelaidensis</i> 450

Genus	Species Epithet
Geobacillus	thermoleovorans
Gordonia	aichiensis
Gordonia	amaraeii A
Gordonia	amaraeii B
Gordonia	bronchialis
Gordonia	sputi
Gordonia	terrae
Legionella	brunensis450
Legionella	busanensis
Legionella	busanensis450
Legionella	cherii
Legionella	cherii450
Legionella	cherriiDN56
Legionella	cincinnatiensis
Legionella	cincinnatiensis450
Legionella	cincinnatiensisDN67
Legionella	donaldsonii
Legionella	donaldsonii450
Legionella	dumofii
Legionella	dumofii450
Legionella	erythra
Legionella	erythra450
Legionella	fairfieldensis
Legionella	fairfieldensis450
Legionella	fairfieldensisGTC698
Legionella	feeleeii
Legionella	feeleeii 450
Legionella	feeleeiiDN52
Legionella	geestiana
Legionella	geestiana450
Legionella	gormanii
Legionella	gormanii 450
Legionella	gratiana
Legionella	gratiana450
Legionella	hackeliae
Legionella	hackeliae450
Legionella	israelensis
Legionella	israelensis450
Legionella	israelensisDN61
Legionella	jamestowniensis
Legionella	jamestowniensis450
Legionella	jordanis
Legionella	jordanis450
Legionella	lansingensis
Legionella	lansingensis450
Megasphaera	elsdenii B

Genus	Species Epithet
Helicobacter	pullorum
Helicobacter	pylori
Helicobacter	bilis
Helicobacter	canis
Helicobacter	cholecystus
Helicobacter	heilmannii
Helicobacter	mustelae
Legionella	londiniensis
Legionella	londiniensis450
Legionella	longbeachae
Legionella	longbeachae450
Legionella	lytica
Legionella	lytica450
Legionella	maceachernii
Legionella	maceachernii450
Legionella	micdadei
Legionella	micdadei450
Legionella	moravica
Legionella	moravica450
Legionella	nautarum
Legionella	nautarum450
Legionella	oakridgensis
Legionella	oakridgensis450
Legionella	parisiensis
Legionella	parisiensis450
Legionella	pneumophila
Legionella	pneumophila
Legionella	pneumophila 450
Legionella	fraseri.pasculle
Legionella	pascurilli,fraserii
Legionella	fraseri450
Legionella	quateirensis
Legionella	quateirensis450
Legionella	quinlivanii
Legionella	quinlivanii450
Legionella	rubrilucens
Legionella	rubrilucens450
Legionella	sainthelensi
Legionella	sainthelensi450
Legionella	santicrucis
Legionella	santicrucis450
Legionella	santicrucisDN63
Legionella	shakespearei
Legionella	shakespearei450
Legionella	shakespeareiGTC701
Mycobacterium	malmoense

Genus	Species Epithet
Legionella	anisa
Legionella	anisa450
Legionella	birminghamensis
Legionella	birminghamensis450
Legionella	bozemaniae group
Legionella	bozemaniae450
Legionella	brunensis
Legionella	spiritensis
Legionella	spiritensis450
Legionella	steigerwaltii450
Legionella	tucsonensis450
Legionella	tucsonensisDN68
Legionella	wadsworthii
Legionella	wadsworthii450
Legionella	wadsworthiiDN51
Legionella	worsleiensis
Legionella	worsleiensis450
Leifsonia	xyli
Leptospira	biflexa
Leptospira	fainei
Leptospira	inadai
Leptospira	kirschneri
Leptospira	noguchii
Leptospira	santarosai
Leptospira	wolbachii
Leptospira	borgpetersenii
Leptospira	illini
Leptospira	interrogans
Leptospira	meyeri
Leptospira	parva
Leptospira	weillii
Leucothrix	mucor
Listeria	innocula
Listeria	monocytogenes
Listeria	welshimeri
Listeria	grayi
Listeria	ivanovii
Listeria	seeligeri
Listonella	anguillarum
Mannheimia	haemolytica
Mannheimia	granulomatis
Marinospirillum	megaterium
Megasphaera	elsdenii A
Megasphaera	micronuciformis
Megasphaera	cerevisiae
Mycoplasma	canis

Genus	Species Epithet
<i>Melissococcus</i>	<i>plutonius</i>
<i>Microbacterium</i>	<i>schleiferi</i>
<i>Microbacterium</i>	<i>aurum</i>
<i>Micromonas</i>	<i>microsjjA</i>
<i>Mitsuokella</i>	<i>multacida</i>
<i>Mitsuokella</i>	<i>jalaludinii</i>
<i>Mobiluncus</i>	<i>mulieris</i>
<i>Mobiluncus</i>	<i>curtisii</i>
<i>Moraxella</i>	<i>bovis</i>
<i>Moraxella</i>	<i>ovis</i>
<i>Moraxella</i>	<i>osloensis</i>
<i>Moraxella</i>	<i>lacunata</i> group
<i>Moraxella</i>	<i>atlantae</i>
<i>Moraxella</i>	<i>catarrhalis</i>
<i>Moraxella</i>	<i>nonliquefaciens</i>
<i>Moraxella</i>	<i>caviae</i>
<i>Mycobacterium</i>	<i>abscessus</i>
<i>Mycobacterium</i>	<i>avium</i>
<i>Mycobacterium</i>	<i>branderi</i>
<i>Mycobacterium</i>	<i>celatum</i>
<i>Mycobacterium</i>	<i>chelonae</i>
<i>Mycobacterium</i>	<i>farcinogenes</i>
<i>Mycobacterium</i>	<i>flavescens</i>
<i>Mycobacterium</i>	<i>fortuitum</i>
<i>Mycobacterium</i>	<i>gastrii</i>
<i>Mycobacterium</i>	<i>genavense</i>
<i>Mycobacterium</i>	<i>gordonae</i>
<i>Mycobacterium</i>	<i>haemophilum</i>
<i>Mycobacterium</i>	<i>interjectum</i>
<i>Mycobacterium</i>	<i>intermedium</i>
<i>Mycobacterium</i>	<i>intracellulare</i>
<i>Mycobacterium</i>	<i>intracellulare A</i>
<i>Mycobacterium</i>	<i>intracellulare B</i>
<i>Mycobacterium</i>	<i>kansasii</i>
<i>Mycobacterium</i>	<i>kansasii</i> group
<i>Mycobacterium</i>	<i>leprae</i>
<i>Mycobacterium</i>	<i>lepraemurium</i>
<i>Nocardia</i>	<i>nova</i>
<i>Nocardia</i>	<i>otitidiscaviarum</i>
<i>Nocardia</i>	<i>seriolae</i>
<i>Nocardioopsis</i>	<i>dassonvillei</i>
<i>Obesumbacterium</i>	<i>proteus</i>
<i>Ochrobactrum</i>	<i>anthropijjB</i>
<i>Ochrobactrum</i>	<i>intermedium</i>
<i>Ochrobactrum</i>	<i>anthropijjA</i>
<i>Ochrobactrum</i>	<i>anthropijjC</i>

Genus	Species Epithet
<i>Mycobacterium</i>	<i>marinum ulcerans</i>
<i>Mycobacterium</i>	<i>mucogenicum</i>
<i>Mycobacterium</i>	<i>nonchromogenicum</i>
<i>Mycobacterium</i>	<i>paratuberculosis</i>
<i>Mycobacterium</i>	<i>peregrinum</i> group
<i>Mycobacterium</i>	<i>porcinum</i>
<i>Mycobacterium</i>	<i>scroflaceum</i>
<i>Mycobacterium</i>	<i>scrofulaceum</i>
<i>Mycobacterium</i>	<i>shimoidei</i>
<i>Mycobacterium</i>	<i>simiae</i>
<i>Mycobacterium</i>	<i>smegmatis</i>
<i>Mycobacterium</i>	<i>sphagni</i>
<i>Mycobacterium</i>	<i>szulgai</i>
<i>Mycobacterium</i>	<i>tuberculosis</i>
<i>Mycobacterium</i>	<i>tuberculosis</i> complex
<i>Mycobacterium</i>	<i>vaccae</i>
<i>Mycobacterium</i>	<i>xenophi</i>
<i>Mycoplasma</i>	<i>dispar</i>
<i>Mycoplasma</i>	<i>faucium</i>
<i>Mycoplasma</i>	<i>fermentans</i>
<i>Mycoplasma</i>	<i>gallinarum</i>
<i>Mycoplasma</i>	<i>genitalium</i>
<i>Mycoplasma</i>	<i>haemofelis</i>
<i>Mycoplasma</i>	<i>hyopneumoniae</i>
<i>Mycoplasma</i>	<i>hyosynoviae</i>
<i>Mycoplasma</i>	<i>kahnei</i>
<i>Mycoplasma</i>	<i>meleagridis</i>
<i>Mycoplasma</i>	<i>neurolyticum</i>
<i>Mycoplasma</i>	<i>penetrans</i>
<i>Mycoplasma</i>	<i>primum</i>
<i>Mycoplasma</i>	<i>putrificans</i>
<i>Mycoplasma</i>	<i>salivarium</i>
<i>Mycoplasma</i>	<i>wenyonii</i>
<i>Mycoplasma</i>	<i>agalactiae</i>
<i>Mycoplasma</i>	<i>arthritidis</i>
<i>Mycoplasma</i>	<i>bovirhinis</i>
<i>Mycoplasma</i>	<i>bovoculi</i>
<i>Peptoniphilus</i>	<i>indolicus</i>
<i>Peptoniphilus</i>	<i>lacrimalis</i>
<i>Peptoniphilus</i>	<i>asaccharolyticus A</i>
<i>Peptoniphilus</i>	<i>harei</i>
<i>Peptoniphilus</i>	<i>ivorii</i>
<i>Peptostreptococcus</i>	<i>anaerobius</i>
<i>Photobacterium</i>	<i>phosphoreum</i>
<i>Photobacterium</i>	<i>damselae</i>
<i>Piscirickettsia</i>	<i>salmonis</i>

Genus	Species Epithet
<i>Mycoplasma</i>	<i>columbinasale</i>
<i>Mycoplasma</i>	<i>falcon</i>
<i>Mycoplasma</i>	<i>felis</i>
<i>Mycoplasma</i>	<i>flocculare</i>
<i>Mycoplasma</i>	<i>gallisepticum</i>
<i>Mycoplasma</i>	<i>haemocanis</i>
<i>Mycoplasma</i>	<i>hominis</i>
<i>Mycoplasma</i>	<i>hyorhinis</i>
<i>Mycoplasma</i>	<i>iowae</i>
<i>Mycoplasma</i>	<i>lipophilium</i>
<i>Mycoplasma</i>	<i>mycoides</i>
<i>Mycoplasma</i>	<i>oralis</i>
<i>Mycoplasma</i>	<i>pneumoniae</i>
<i>Mycoplasma</i>	<i>pullorum</i>
<i>Mycoplasma</i>	<i>pulmonis</i>
<i>Mycoplasma</i>	<i>synoviae</i>
<i>Mycoplasma</i>	<i>alkalescens</i>
<i>Mycoplasma</i>	<i>bovigenitalium</i>
<i>Mycoplasma</i>	<i>bovis</i>
<i>Mycoplasma</i>	<i>buccale</i>
<i>Mycoplasma</i>	<i>capricolum</i> subsp.
<i>Mycoplasma</i>	<i>conjunctivae</i>
<i>Myroides</i>	<i>odoratus</i>
<i>Neisseria</i>	<i>flavescensjj</i> group
<i>Neisseria</i>	<i>iguanae</i>
<i>Neisseria</i>	<i>meningitidis</i> group
<i>Neisseria</i>	<i>sicca</i>
<i>Neisseria</i>	<i>elongata ii</i>
<i>Neisseria</i>	<i>gonorrhea</i>
<i>Neisseria</i>	<i>lactamica</i>
<i>Neisseria</i>	<i>mucosa</i>
<i>Neisseria</i>	<i>weaveri</i>
<i>Neorickettsia</i>	<i>sennetsu</i>
<i>Neorickettsia</i>	<i>helminthoeca</i>
<i>Nocardia</i>	<i>asteroids</i>
<i>Nocardia</i>	<i>brasiliensis</i>
<i>Nocardia</i>	<i>farcinica</i>
<i>Prevotella</i>	<i>tannerae</i>
<i>Propionibacterium</i>	<i>acnes</i>
<i>Propionibacterium</i>	<i>propionicus</i>
<i>Proteus</i>	<i>mirabilis</i>
<i>Proteus</i>	<i>vulgaris</i>
<i>Providencia</i>	<i>alcalifaciens</i>
<i>Providencia</i>	<i>stuartii</i>
<i>Pseudoalteromonas</i>	<i>piscicida</i>
<i>Pseudoalteromonas</i>	<i>atlantica</i> group

Genus	Species Epithet
<i>Ochrobactrum</i>	<i>tritici</i>
<i>Olsenella</i>	<i>uliiiA</i>
<i>Olsenella</i>	<i>uliiiB</i>
<i>Orientia</i>	<i>tsutsugamushi</i>
<i>Ornithobacterium</i>	<i>rhinotracheale</i>
<i>Paenibacillus</i>	<i>polymyxa</i> jj
<i>Paenibacillus</i>	<i>larvae</i>
<i>Paenibacillus</i>	<i>thiaminolyticus</i>
<i>Pantoea</i>	<i>agglomerans</i>
<i>Pantoea</i>	<i>ananatis</i>
<i>Pasteurella</i>	<i>bettyae</i>
<i>Pasteurella</i>	<i>multocida</i>
<i>Pasteurella</i>	<i>pneumotropica</i> B
<i>Pasteurella</i>	<i>testudinis</i>
<i>Pasteurella</i>	<i>mairii</i>
<i>Pasteurella</i>	<i>dagmatis</i>
<i>Pasteurella</i>	<i>aerogenes</i>
<i>Pasteurella</i>	<i>caballi</i>
<i>Pasteurella</i>	<i>pneumotropica</i> A
<i>Pasteurella</i>	<i>pneumotropica</i> C
<i>Pasteurella</i>	<i>stomatis</i>
<i>Pasteurella</i>	<i>gallinarum</i>
<i>Pasteurella</i>	<i>canis</i>
<i>Pectobacterium</i>	<i>chrysanthemi</i> B
<i>Pectobacterium</i>	<i>cacticida</i>
<i>Pectobacterium</i>	<i>carotovorum</i> subsp.
<i>Pectobacterium</i>	<i>carotovorum</i> subsp.
<i>Pectobacterium</i>	<i>chrysanthemi</i> A
<i>Peptoniphilus</i>	<i>asaccharolyticus</i> B
<i>Psychrobacter</i>	<i>phenylpyruvicus</i>
<i>Ralstonia</i>	<i>pickettii</i>
<i>Ralstonia</i>	<i>solanacearum</i>
<i>Raoultella</i>	<i>planticola</i>
<i>Rathayibacter</i>	<i>rathayi</i> A
<i>Rathayibacter</i>	<i>toxicus</i>
<i>Rathayibacter</i>	<i>iranicus</i> group
<i>Rathayibacter</i>	<i>rathayi</i> B
<i>Rathayibacter</i>	<i>tritici</i>
<i>Renibacterium</i>	<i>salmoninarum</i> A
<i>Renibacterium</i>	<i>salmoninarum</i> B
<i>Rhizobium</i>	<i>radiobacter</i>
<i>Rhizobium</i>	<i>rhizogenes</i>
<i>Rhodococcus</i>	<i>equi</i>
<i>Rhodococcus</i>	<i>erythropolis</i>
<i>Rhodococcus</i>	<i>fascians</i>
<i>Rhodococcus</i>	<i>gordoniae</i>

Genus	Species Epithet
<i>Plesiomonas</i>	<i>shigelloides</i>
<i>Porphyomonas</i>	<i>asaccharolyticus</i>
<i>Porphyomonas</i>	<i>endodontalis</i>
<i>Porphyomonas</i>	<i>levii</i>
<i>Porphyomonas</i>	<i>catoniae</i>
<i>Porphyomonas</i>	<i>gingivalis</i>
<i>Porphyomonas</i>	<i>cansulci</i>
<i>Porphyomonas</i>	<i>macacae salivosa</i>
<i>Porphyomonas</i>	<i>canis</i>
<i>Porphyomonas</i>	<i>circumdentaria</i>
<i>Porphyomonas</i>	<i>gulae</i>
<i>Porphyomonas</i>	<i>cangingivalis</i>
<i>Porphyomonas</i>	<i>canoris</i>
<i>Prevotella</i>	<i>bryantii</i>
<i>Prevotella</i>	<i>buccalis</i>
<i>Prevotella</i>	<i>bivia</i>
<i>Prevotella</i>	<i>buccae</i>
<i>Prevotella</i>	<i>dentalis</i>
<i>Prevotella</i>	<i>corporis</i>
<i>Prevotella</i>	<i>denticola</i>
<i>Prevotella</i>	<i>disiens</i>
<i>Prevotella</i>	<i>intermedia</i>
<i>Prevotella</i>	<i>melaninogenicus</i>
<i>Prevotella</i>	<i>oralis</i>
<i>Prevotella</i>	<i>ruminicola</i>
<i>Prevotella</i>	<i>veroralis</i>
<i>Prevotella</i>	<i>loescheii</i>
<i>Prevotella</i>	<i>nigrescens</i>
<i>Prevotella</i>	<i>oris</i>
<i>Sarcina</i>	<i>ventriculi</i> B
<i>Sarcina</i>	<i>ventriculi</i> A
<i>Selenomonas</i>	<i>flueggei</i> group
<i>Selenomonas</i>	<i>ruminantium</i> B
<i>Selenomonas</i>	<i>ruminantium</i> A
<i>Selenomonas</i>	<i>sputigena</i>
<i>Serratia</i>	<i>grimesii</i>
<i>Serratia</i>	<i>marcescens</i>
<i>Serratia</i>	<i>rubidaea</i>
<i>Serratia</i>	<i>liquifaciens</i>
<i>Serratia</i>	<i>proteamaculans</i> subsp.
<i>Shewanella</i>	<i>putrefaciens</i>
<i>Shewanella</i>	<i>algae</i>
<i>Slackia</i>	<i>exigua</i>
<i>Slackia</i>	<i>heliotrinreducens</i>
<i>Sphingobacterium</i>	<i>spiritivorum</i>
<i>Sphingobacterium</i>	<i>mizutae</i>

Genus	Species Epithet
<i>Pseudobutyrvibrio</i>	<i>ruminis</i>
<i>Pseudomonas</i>	<i>putida</i> 130
<i>Pseudomonas</i>	<i>plecoglossicida</i> 130
<i>Pseudomonas</i>	<i>cichorii</i> 130
<i>Pseudomonas</i>	<i>viridiflava</i> 130
<i>Pseudomonas</i>	<i>mendocina</i> 130
<i>Pseudomonas</i>	<i>plecoglossicida</i> 180
<i>Pseudomonas</i>	<i>viridiflava</i> 180
<i>Pseudomonas</i>	<i>stutzeri</i> B180
<i>Pseudomonas</i>	<i>alcaligenes</i> 180
<i>Pseudomonas</i>	<i>cichorii</i> 180
<i>Pseudomonas</i>	<i>stutzeri</i> A180
<i>Pseudomonas</i>	<i>anguilliseptica</i> 130
<i>Pseudomonas</i>	<i>stutzeri</i> A130
<i>Pseudomonas</i>	<i>alcaligenes</i> 130
<i>Pseudomonas</i>	<i>syringe</i> 130
<i>Pseudomonas</i>	<i>marginalis</i> 130
<i>Pseudomonas</i>	<i>aeruginosa</i> 130
<i>Pseudomonas</i>	<i>fluorescens</i> 130
<i>Pseudomonas</i>	<i>mendocina</i> 180
<i>Pseudomonas</i>	<i>marginalis</i> 180
<i>Pseudomonas</i>	<i>fluorescens</i> 180
<i>Pseudomonas</i>	<i>syringe</i> 180
<i>Pseudomonas</i>	<i>putida</i> 180
<i>Pseudomonas</i>	<i>anguilliseptica</i> 180
<i>Pseudomonas</i>	<i>aeruginosa</i> 180
<i>Pseudoramibacter</i>	<i>afactolyticus</i>
<i>Psychrobacter</i>	<i>immobilis</i>
<i>Psychrobacter</i>	<i>glacialis</i>
<i>Staphylococcus</i>	<i>simulans</i>
<i>Staphylococcus</i>	<i>xylosus</i>
<i>Stenotrophomonas</i>	<i>maltophilia</i> group
<i>Stenotrophomonas</i>	<i>rhizophila</i>
<i>Stenotrophomonas</i>	<i>nitritireducens</i>
<i>Streptobacillus</i>	<i>moniliformis</i>
<i>Streptococcus</i>	<i>anginosus</i>
<i>Streptococcus</i>	<i>canis</i>
<i>Streptococcus</i>	<i>cristatus</i>
<i>Streptococcus</i>	<i>dysgalactiae</i>
<i>Streptococcus</i>	<i>equinus</i>
<i>Streptococcus</i>	<i>gordonii</i>
<i>Streptococcus</i>	<i>iniae</i>
<i>Streptococcus</i>	<i>macacae</i>
<i>Streptococcus</i>	<i>mutans</i>
<i>Streptococcus</i>	<i>parasanguinis</i>
<i>Streptococcus</i>	<i>phocae</i>

Genus	Species Epithet
<i>Rhodococcus</i>	<i>rhodochrous</i>
<i>Rothia</i>	<i>mucilaginosa</i>
<i>Rothia</i>	<i>dentocariosa</i>
<i>Ruminococcus</i>	<i>albus</i>
<i>Ruminococcus</i>	<i>callidus</i>
<i>Ruminococcus</i>	<i>flavefaciens</i> jjB
<i>Ruminococcus</i>	<i>gravus</i>
<i>Ruminococcus</i>	<i>hansenii</i> jjB
<i>Ruminococcus</i>	<i>obeum</i> jjA
<i>Ruminococcus</i>	<i>productus</i>
<i>Ruminococcus</i>	<i>torques</i>
<i>Ruminococcus</i>	<i>bromii</i>
<i>Ruminococcus</i>	<i>flavefaciens</i> jjA
<i>Ruminococcus</i>	<i>flavefaciens</i> jjC
<i>Ruminococcus</i>	<i>hansenii</i> jj A
<i>Ruminococcus</i>	<i>lactaris</i>
<i>Ruminococcus</i>	<i>obeum</i> jjB
<i>Ruminococcus</i>	<i>schinkii</i>
<i>Salmonella</i>	<i>enterica</i> serovars
<i>Sanguibacter</i>	spp.
<i>Sarcina</i>	<i>maxima</i>
<i>Streptomyces</i>	<i>somaliensis</i>
<i>Streptomyces</i>	<i>viridocyaneus</i>
<i>Sutterella</i>	<i>wadsworthensis</i>
<i>Suttonella</i>	<i>indologenes</i>
<i>Tannerella</i>	<i>forsythus</i>
<i>Tatumella</i>	<i>ptyseos</i>
<i>Taylorella</i>	<i>asinigenitalis</i>
<i>Taylorella</i>	<i>equigenitalis</i>
<i>Tenacibaculum</i>	<i>maritimum</i>
<i>Tenacibaculum</i>	<i>ovolyticum</i>
<i>Tenacibaculum</i>	<i>maritimum</i>
<i>Tenacibaculum</i>	<i>mesophilum</i>
<i>Tissierella</i>	<i>praeacuta</i>
<i>Treponema</i>	<i>denticola</i>
<i>Treponema</i>	<i>medium</i> B

Genus	Species Epithet
<i>Sphingobacterium</i>	<i>multivorum</i>
<i>Sphingobacterium</i>	<i>thalpophilum</i>
<i>Sphingomonas</i>	<i>paucimobilis</i>
<i>Sphingomonas</i>	<i>parapaucimobilis</i>
<i>Sporomusa</i>	<i>acidovorans</i>
<i>Sporomusa</i>	<i>aerovorans</i>
<i>Staphylococcus</i>	<i>capitis/caprae</i>
<i>Staphylococcus</i>	<i>felis</i>
<i>Staphylococcus</i>	<i>hominis</i> subsp. <i>Hominis</i>
<i>Staphylococcus</i>	<i>intermedius</i>
<i>Staphylococcus</i>	<i>lugdunensis</i>
<i>Staphylococcus</i>	<i>saccharolyticus</i>
<i>Staphylococcus</i>	<i>sciuri</i>
<i>Staphylococcus</i>	<i>warneri</i>
<i>Staphylococcus</i>	<i>aureus</i> subsp. <i>aureus</i>
<i>Staphylococcus</i>	<i>epidermidis</i>
<i>Staphylococcus</i>	<i>hemolyticus</i>
<i>Staphylococcus</i>	<i>hyicus</i> subsp. <i>Hyicus</i>
<i>Staphylococcus</i>	<i>kloosii</i>
<i>Staphylococcus</i>	<i>pasteuri</i>
<i>Staphylococcus</i>	<i>saprophyticus</i>
<i>Treponema</i>	<i>pallidum</i> <i>pertenue</i>
<i>Treponema</i>	<i>vincentii</i>
<i>Treponema</i>	<i>bryantii</i>
<i>Treponema</i>	<i>medium</i> A
<i>Treponema</i>	<i>Multophilium</i>
<i>Treponema</i>	<i>socranskii</i>
<i>Trichococcus</i>	<i>pasteurii</i>
<i>Tropheryma</i>	<i>whippellii</i> 1
<i>Tsukamurella</i>	<i>inchonensis</i> <i>pulmonis</i>
<i>Tsukamurella</i>	<i>paurometabola</i>
<i>Turicella</i>	<i>otitidis</i>
<i>Ureaplasma</i>	<i>diversum</i>
<i>Ureaplasma</i>	<i>parvum</i>
<i>Ureaplasma</i>	<i>canigenitalium</i>
<i>Ureaplasma</i>	<i>felinum</i>

Genus	Species Epithet
<i>Streptococcus</i>	<i>porcinus</i>
<i>Streptococcus</i>	<i>salivarius</i>
<i>Streptococcus</i>	<i>sobrinus</i>
<i>Streptococcus</i>	<i>thermophilus</i>
<i>Streptococcus</i>	<i>vestibularis</i>
<i>Streptococcus</i>	<i>agalactiae</i>
<i>Streptococcus</i>	<i>bovis</i>
<i>Streptococcus</i>	<i>constellatus</i>
<i>Streptococcus</i>	<i>downei</i>
<i>Streptococcus</i>	<i>equi</i>
<i>Streptococcus</i>	<i>gallolyticus</i>
<i>Streptococcus</i>	<i>infantarius</i>
<i>Streptococcus</i>	<i>intermedius</i>
<i>Streptococcus</i>	<i>mitis</i>
<i>Streptococcus</i>	<i>oralis</i>
<i>Streptococcus</i>	<i>parauberis</i>
<i>Streptococcus</i>	<i>pneumoniae</i>
<i>Streptococcus</i>	<i>pyogenes</i>
<i>Streptococcus</i>	<i>sanguinis</i>
<i>Streptococcus</i>	<i>suis</i>
<i>Streptococcus</i>	<i>uberis</i>
<i>Ureaplasma</i>	<i>ureolyticum</i>
<i>Vagococcus</i>	<i>salmoninarum</i>
<i>Veillonella</i>	<i>atypica</i>
<i>Veillonella</i>	<i>parvula</i>
<i>Wolinella</i>	<i>succinogenes</i>
<i>Xanthomonas</i>	<i>axonopodis</i> group
<i>Xanthomonas</i>	<i>campestris</i> group
<i>Yersinia</i>	<i>enterocolitica</i>
<i>Yersinia</i>	<i>pestis</i> 450
<i>Yersinia</i>	<i>ruckeri</i> 450
<i>Yersinia</i>	<i>frederiksenii</i> 450
<i>Yersinia</i>	<i>intermedia</i> 450
<i>Yersinia</i>	<i>pseudotuberculosis</i> 450
<i>Yokenella</i>	<i>regensburgi</i> group