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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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.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and gratitude towards my research supervisor Professor Dr. Michihiko Ike for introducing me to the research fields in water science and his helpful comments to give the experiments a meaningful direction. Furthermore, his guidance and careful reading of the manuscripts and thesis has greatly improved its quality. I am equally thankful to Prof. Kazuhito Fujiyama of International Biotechnology Center, Osaka University, and Prof. Hajime Watanabe of Division of Advance Science and Biotechnology, Graduate School of Engineering, Osaka University, for reviewing my thesis and pointing out the critical changes needed to give this thesis a better outcome.

I want to sincerely thank Asst. Professor Dr. Kazunari Sei and Researcher Dr. Daisuke Inoue for their consistent motivation during various stages in my research. I am thankful to both of them for providing me valuable assistance from teaching me experimental techniques, planning the experiments, oral discussions and reviewing the manuscripts and thesis. I am equally thankful to Mr. Masaki Inaba for helping me in the laboratory experiments and sharing his results which are important in making this thesis. I also want to thank Assoc. Professor Dr. Satoshi Soda and Ms. Yuriko Yamaoka for their help during my stay in Osaka University. I am thankful to various other colleagues at Professor Ike laboratory for stimulating and inspiring me in many ways.

Finally I would like to thank the Department of Biotechnology, Osaka University and the Monbukagakusho MEXT (The Ministry of Education, Science, Technology and Sports, Japan) for financially supporting me during my stay in Japan and providing me an opportunity to study in Japan. I am also thankful to my parents, friends and well-wishers for providing a conducing environment suitable for my study.

Rahul R. Upadhye
February, 2011

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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 km^2. 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 km^2. 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

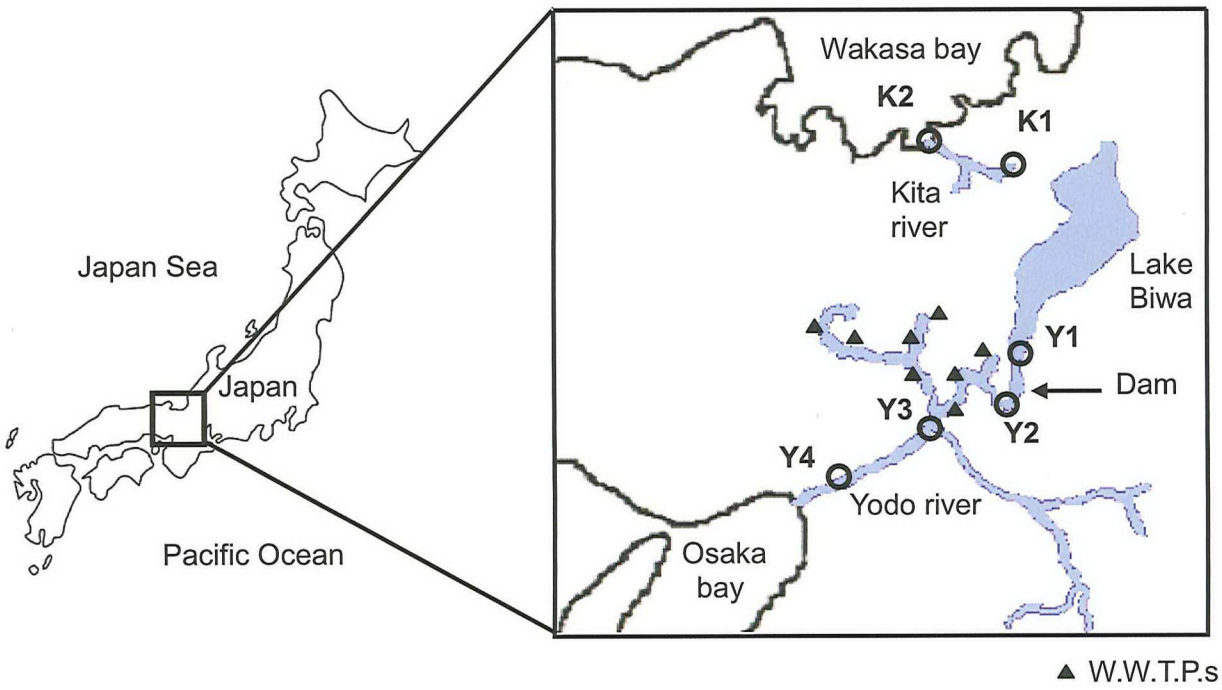


Fig. 2-1 Location of sampling stations

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 (*ortho*)	CACCTATTCGTACTTCGATTCGACCCAGTCCGAGTTCAACCTGCGCCGGCGTATCATCAC	D37783
		GATGGGAAGTACAGCGGTTTCCATGACGACATCCCGACTGATTTTTATCGAGGGAAGCTC	D16356
		TGCACTTCAAGGTGCGCAAGGACGGTTTCGAACCGTTGACCACGCAATACTACTTCGAAG	M16964
		TACTCCTTCTTTGATAAGAGCCAAACCCCTTACAATTTGCGCCGCACCATTATCGCTGAC	M57500
		CTCAATTTATTGGGTCGACATGGTAACCGCCCTTCGCATGTTCACTACTTTGTCTCTGCG	Z36909
		CGAATGGAACCTCCGCGGCTCCATCATTGCCGATGACCAGGGCAACTTCCAGATCAACAC	M94318
C23O DNA	Catechol oxidation (*meta*)	GTCCTAACCTGGATGAAACTCTGAATTTGTTCCGTGATGTGCTCGGTTTTGACCTCGCTG	AB008831
		AGAGCCGAATCAAGAAAAAGGGAAGCTTCATCATCTTTGCTATTGGTATGGCATCCCGCA	X67860
		GCTAGCTCTAACTTGCGGGATTTTATGAAAGCTAAAGCTGAGCGATGCGAGTGAACGTCG	D37828
		GCCTAGATTTCTATTTAAGTGAGCAAGGTTTGGTTGGACCCGATGCATCCACTCAAGCGG	U01826
		TCGACTAGATCACCTCAACATCACAGCGAAGGACGTTGACGACGCCCGTAACTGGTATTC	X69504
		CCATGGGATCACACGCGGGCAGACGATCTACTTCTTCGACCCCTCGGGCAACCGGAACGA	U23375
		GACTCACGGCAAGACCATCTACTTCTTCGACCCTTCGGGCAACCGCAACGAGGTGTTCTG	X80765
		ACATCGTGCGCCAAAGAGCATCGCTAGAGAGAACAATGCAAACCAATAACAAGAGTTCGT	S78585
		CACATCGTGGTGATGGTCGACGACTACGACGAGACGATGCGCTTTTACCGCGAGGTACTC	L77225
alkB	Alkane hydroxylation (Group I)	CACCGTGATGTTGCTACACCGATGGACCCTGCGACCTCACGTATGGGAGAAAATATATAT	AJ233397
		GTGCCCCAGGAACACTCCTTATGGGCAATGTAATTTATAAGATGGCAATGCGCGAGAGAG	U40233
alkM	Alkane hydroxylation (Group II)	CAAGGCTGGGGAATGAGTGCTGCTTTTCATGCTTCTATGGTGGGAATTTTTGGTAAAAGT	AJ009585
		TATATCGAGCATTATGGTCTAAAACGCCAGAAAAGAGCGGATGGCAATTACGAACGTACC	AJ002316
		CCATGTTTGCGAAATTTGGTGCCCGTTCGATTCCGTTTCAAGTTACTCAAGCTGCGTATG	AJ009582
		GATAATGAGTTATTGCAAGGCTGGACAATGACCGCAGCATTCCATTCGTCTATGGTCGGG	AJ009584
alkB & *B1*	Alkane hydroxylation (Group III)	TATCTGCTCATTCAGGCGGTGGTCGGGTTTTCCCTGCTCGAGATCGTCAACTACATGGAG	AJ009587
		GTTTCGGCCAGTCGGTCTACCAGTTCCTGCCGCATGCCTACAAGTACAACTTCCTCAGCG	AJ009580
		GTGATCCAGGGGATCTACGGCTTCTCTCTGCTGGAGGTGGTCAACTACGTCGAGCACTAC	AJ009579
		GTGATCCAGGCCTTCATCGGGTTCTCGTTACTCGAAGCGATCAACTACCTCGAGCACTAC	AJ009586
		GTAATTCAGGCGGTGTACGGCGCGTCGCTGCTCGAAGTCGTGAACTACGTCGAGCACTAC	AJ293344
		GAGGACCCGGCGAGTTCACGGTTCGGAGAAAGCTTCTGGACGTTCCTGCCCCGCAGTGTC	AJ401611
mmoX	Methane oxidation	GGTCTACGAGGATTGGGGTGGAATCTGGATCGGCCGTCTCGGCAAATATGGCGTCGAGAG	X55394
pmoA	Methane oxidation	GATCTGATCGGCCTTCACTACGTCCGCACGTCGATGCCGGAATATATCCGCATGGTCGAG	U31651
		AACTTCTGGGGTTGGACTTACTTCCCAGTTAACATTTGCTTCCCATCTAACCTGCTGCCA	U31653
		AACTTCTGGGGATGGACTTACTTCCCAGTTAACTTCGTGTTCCCATCACAATTCGCTCCA	U31654

Table 2-2 –*Continued*

mcrA	Methanogenesis	TGGCTAGGATCTTACATGTCAGGTGGAGTAGGATTCACACAATATGCTACAGCATCGTAT	J03375
		GACGACTTCACCTACTTCGGTAAGGAGTACGTGGAGGACAAATATGGACTCTGTGAGGCA	U10036
		TACATGTCTGGTGGCGTAGGTTTCACACAATACGCTACAGCAGCATACACCGACGATATC	M16893
amoA	Ammonia oxidation	CGTACAGGTACACCCGAGTATGTTCGTCATATTGAGCAAGGTTCACTGCGTACCTTTGGT	L08050
napA	Nitrate reduction	TATGCCTTCATGCGCACCTTCGGCATGGACGAGCCGATGGGCTGCTATGACGATTTCGAG	Z36773
narG	Nitrate reduction	GGACCTACTTCCACGCAGGTATCCTCGACAACACTGATCCGGACACCGCAGAGAAGATAC	AJ277440
		AAATGGCATCGATCTGTATAAAGAAGCTGAAAAAGCAGGGGCAGCAACACCTGAAGACGT	X91819
		CAGGAATTGCGCCAGGGCGACCTCTACGACCTTCAGGAACGCTATGTGCTGCTGTTCGAC	Z26255
		CCATGAATTCTTCCTCAAGTATCTCTTAGGCACGAAGAATGGCCTGTTCGCCGAGGAAGA	AB096694
		CGGCAAAGGTCATGAATATTTTCTGAAGCACTTGCTCGGCACGACTAACGGACTGATGAA	AB087407
		CTGGCGTTCTAACCTGCTCGGTTCTTCCGGTAAAGGTCATGAGTTTATGCTCAAGTACCT	X16181
nirK	Dissimilatory itrite reduction	CTCACCGCCAAGGTCGGCGAGACCGTGCTGCTGATCCACTCGCAGGCCAATCGCGACACC	AF051831
		TTACTACACGTTCCAGCAGCCCGGTATCTATGCGTATGTAAACCACAATCTGATCGAGGC	M97294
		CTATGACACCGTCTATTACATCGGCGAGAGCGACCACTACATCCCGAAGGACGAGGACGG	U62291
		TATATCCCCAAGGACAAGGACGGCCACTACAAGGACTACCCGGACCTGGCGTCCAGTTAC	Z21945
		CTGACGTACGACAAGATCTACTATGTCGGCGAGCAGGACTTCTACGTGCCGAAGGACGAG	Z48635
nirS	Dissimilatory itrite reduction	GAACGTCAAGGAAACGGGCAAGATCCTGCTGGTGGACTATACCGACCTCGACAACCTCAA	AJ401462
		CAGATCATGCTGGTCGACTACACCGACATCAAGAACCTCAAGACCACCACCATCGAATCC	M80653
		GAGTTCATCGTCAACGTGAAGGAGACCGGCAAGGTCCTGCTGGTCAACTACAAGGATATC	X16452
		CAAGATCCTGATGGTCAACTACTCGGACTTGTCCAACCTGAAGACCACCACCATCGATTC	X91394
qnorB	Dissimilatory nitric oxide reduction	TATGAGTATGTCGACCTGGGCCGGCTGTGGCAGATCGGCAAGTTCGCCGGCATCCTGATC	AF002661
cnorB	Dissimilatory nitric oxide reduction	GATCTTCTCGAGCTTCGAGATCGTGCCCTTCTTCGCCATGATGTCATTCGCCTTCGTCAT	U28078
nosZ	Nitrous oxide reduction	GTCCACATGTCCTTTACCGAGGGCAAGTATGACGGCCGCTTCCTGTTCATGAACGACAAG	AJ440508
		GGAAGGCACCTATGACGGGCGCTATCTCTACGCCAACGACAAGGCCAATACGCGTGTCTG	AJ440509
nifH	Nitrogen fixation	CGATGCCTATTCGCGAAAACAAGGCTCAGGAAATCTACATCGTCATGTCCGGTGAGATGA	V01215
nrfA	Nitrite reduction	GGAATATGAAACCTGGACAGCGGGCATTCACGGTAAAAACAACGTGACCTGTATCGACTG	X72298
16S rDNA	Anammox	TGCATTGATAACCTGCCTTTGAGATGGGAATAACTGCGTTTCGAGCAATCGGAACTACCG	AJ250882
dsrAB	Dissimilatory (bi)sulfate reduction	AAAGATGGCATTCATATCTTCCGGGTACAATCCCGAAAAGCCGATGGCAAACCGTATCAC	U16723
soxB	Thiosulfate oxidation	ACTGCCTTCAAAACTATCGGTCTGGAGTTCGAGAGAGGTGAGTGGAATTCCGAGTGTAGA	Y16933
ferA	Iron reduction	CAACAGCATCAATGATCAGTACGACCTCTGCACCAGCTGCCACACCGTCAACACCATGAC	AY033095
mofA	Manganese oxidation	GTCTACGAGACGGTGCAGGATCCGAACCAGATGAACGGTTTCAACTCGGTCGGCCGCTGG	Z25774

Table 2-2 *–Continued*

merA	Mercuric reduction	CAGCAAGGTCACGGCCCTGGCGCGCAATACCTTGTTCTTCCGTGAAGACCCGGCCATCGG	AJ418049
		GTTGTGCACGGTGAGGCGCGCTTCAAGGACGACCAGAGCCTTACTGTCCGTTTGAACGAG	AJ418052
		AGTGCGGCCGTGCCGCCGATTCCCGGACTGCAAGATACCCCCTTTTGGAACTCGGAAAAG	AJ418056
		CATGGAAGGCATCCTGGAAGAGCTATGGCCATCACCATTTTGCCCGGCTACGCCCGTTTC	AJ418057
tpm	Selenium methylation	TTGAAGGCCGGATTGGAGCGTATGGATGAGCACGTTTATGTGTTGGAACGTGTGTAACTC	L49178
cadA	Cadmium resistance	ACTACACTCCGATCATTATGGTTATTGCAGCCTTGGTTGCAGTCGTTCCACCCCTATTCT	J04551
pcoR	Copper resistance	GATCCGTTCGGGGAAGAAGATCCATCTCACCGGTAAAGAATACGTTCTGCTTGAGTTGCT	X83541
phaZ	PHB depolymerization	TCTATCGCAACGGCAACAAGGCCAACGCGCTCCCGGTCTCTGCCACCAGCTATGTCGATG	U16275
		CCGTATCGGCGACGACGAAATCGGCCTTCACCTGTACTGCCACCACGGCCAGCAACTACG	J04223
		GTCACGGCCACCACCTACACCGACACAGGCCTGACCACCGGTACGGCCTACTCCTACACC	U58990
		CTATACCGACACCGGCCTGATTGCTGGTACCACCTACAGCTACACCGTAACCGAGATCGA	D25315
apr	Hydrolysis of peptidic compound	GGCAACGACACCCTGGACTTCTCCGGTTTCACCCAGAACCAGAAGATCAACCTCAATGAG	AB013895
npr	Hydrolysis of peptidic compound	AACAATGCATTCTGGAACGGATCACAGATGGTATACGGAGATGGTGATGGTGTAACGTTT	M83910
sub	Hydrolysis of peptidic compound	TACTGGTTCTACAGCGCTGAAAACAGTAGTTGATAAAGCGGTTTCCAGCGGTATCGTCGT	S51909_1
sub	Hydrolysis of peptidic compound	TTCAACAGGAAGCGGCCAATATAGCTGGATTATTAACGGCATTGAGTGGGCCATTTCCAA	S51909_2
chiA	Chitin depolymerition	GTGAAAGAGTTCCTGCAGACCTGGAAGTTCTTCGATGGCGTGGATATCGACTGGGAGTTC	AY040610
		CAGCAATTGGCGTTGGTTACGACAAAATTGAAGACGTTGATTACGCAGATGCTGTGCAGT	AF193498
		ATAACGGTATCCAACTTCTTCTTGCTCAAGGCGTTCCTGCGAACAAGCTAGTCCTTGGTA	AB004935
		CTGAAACAGGCCGTACTTACGAACTGACATCGGCAGTAGGTGTAGGCTACGACAAGATTG	AF193500
		CATATGGGTTTGATGGCGTGGATTTAGACTGGGAATATCCGGGCGTTGAAACGATTCCTG	AY129671
		TAAACCGCAGATCGACGTTCAGGAAGAAATCCGCAGTCGCGTAGATTTCCTGAAACAGTA	AB110082
		CTCCAACAGATGACATTACGCCTACACCTACACCTACTCTTGAACCAACGCCTGAACCAA	Z68924
		GGGCATTCATGGGCTGGTTGCAATGCAGATAATGATGGGAAAATGGTTGTTGAGAATGGT	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; *Spiralbacteria*, 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 $1/10^{th}$ 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 eubacterial16S 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-μl PCR tubes 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 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× 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 × *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-μl hybridization buffer (5× 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 μl of 5 M NaCl was applied to avoid drying. Following hybridization, cover-slips were removed by immersion in 2× SSC, 0.2% SDS at 37°C. Arrays were washed with 2× SSC, 0.2% SDS and with 2× 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 *et al.*, 1999
C23O DNA	AAGAGGCATGGGGGCGCACCGGTTCGATCA	CCAGCAAACACCTCGTTGCGGTTGCC	60	Sei *et al.*, 1999
alkB	CATAATAAAGGGCATCACCGT	GATTTCATTCTCGAAACTCCAAAC	40	Kohno *et al.*, 2002
alkM	GAGACAAATCGTCTAAAACGTAA	TTGTTATTATTCCAACTATGCTC	40	Kohno *et al.*, 2002
alkB I alkB1	TCGAGCACATCCGCGGCCACCA	CCGTAGTGCTCGACGTAGTT	40	Kohno *et al.*, 2002
mmoX	GGCTCCAAGTTCAAGGTCGAGC	TGGCACTCGTAGCGCTCCGGCTCG	55	McDonald *et al.*, 1995
pmoA	GGGGGAACTTCTGGGGITGGAC	GGGGGRCIACGTCITTACCGAA	45	Cheng *et al.*, 1999
mcrA	TAYGAYCARATHTGGYT	ACRTTCATNGCNGCRTARTT	45	Springer *et al.*, 1995
amoA	GGGGTTTCTACTGGTGGT	CCCCTCKGSAAAGCCTTCTTC	55	Rotthauwe *et al.*, 1997
napA	TAYTTYYTNHSNAARATHATGTAYGG	DATNGGRTGCATYTCNGCCATRTT	45	Flanagan *et al.*, 1999
narG	TAYGTSGGSCARGARAA	TTYTCRTACCABGTBGC	60	Philippot *et al.*, 2002
nirK	GGSGCGGTCATGGTGCTGCC	TCGAAGGCCTCGATCAG	65	This study
nirS	TAYCACCCCGAGCCGCGCGT	CTTRAGYTYSAGBGTCTTGTCGTC	65	This study
qnorB	GGNCAYCARGGNTAYGA	ACCCANAGRTGNCANACCCACCA	55	Braker and Tiedje, 2003
cnorB	GACAAGNNNTACTGGTGGT	GAANCCCCANACNCCNGC	55	Braker and Tiedje, 2003
nosZ	CGGYTGGGGAMWKACCAA	ATRTCGATCARYTGNTCRTT	55	Nogales *et al.*, 2002
nifH	AAAGGYGGWATCGGYAARTCCACCAC	TTGTTSGCSGCRTACATSGCCATCAT	60	Rösch *et al.*, 2002
nrfA	GCNTGYTGGWSNTGYAA	TWNGGCATRTGRCARTC	45	Mohan *et al.*, 2004
16S rDNA (anammox)	GGATTAGGCATGCAAGTC	AAAACCCCTCTACTTAGTGCCC	60	Egli *et al.*, 2001
dsrAB	ATCGGWACCTGGAAGGAYGACATCAA	GGGCACATSGTGTAGCAGTTACCGCA	60	Karkhoff-Schweizer *et al.*, 1995
soxB	GAYGGNGGNGAYACNTGG	CATGTCNCCNCCRTGYTG	60	Petri *et al.*, 2001
ferA	ACARMARSGRTGTYGGSTGC	TGGATYMCSRGARGTYGYAGTG	45	Neal *et al.*, 2004
nofA	GGCTTCACCGAGTTCACGCA	CCAGCGGGGTGTCCATCCAG	60	Siering and Ghiorse, 1997
merA	TTGGAGAACGTGC	ACGTCCTTGGTGAAGGTCTG	55	Felske *et al.*, 2003
tpm	CAGTCAGAGGTCAATAAGG	GAGTTACACACGTTCCAACA	40	Cournoyer *et al.*, 1998
cadA	CAAAYTGYGCRGGHAARTTYGA	AACTAATGCACAAGGACA	55	Oger *et al.*, 2001
pcoR	CAGGTCGTTACCTGCAGCAG	CTCTGATCTCCAGGACATATC	55	Trajanovska *et al.*, 1997
phaZ	CGTCTACCGCAACGGCACCAAGG	TGGGCGTAGTTGCTGGCCGT	55	Sei *et al.*, 2001
apr	TAYGGBTTCAAYTCCAAYAC	VGCGATSGAMACRTTRCC	55	Bach *et al.*, 2001
npr	GTDGAYGCHCAYTAYTAYGC	ACMGCATGBGTYADYTCATG	55	Bach *et al.*, 2001
sub	ATGSAYRTTRYYAAYATGAG	GWGWHGCCATNGAYGTWC	55	Bach *et al.*, 2001
sub	GNACHCAYGTDGCHGGHAC	GWGWHGCCATNGAYGTWC	55	Bach *et al.*, 2001
chiA	GGIGGITGGACIYTIWSIGAYCCITT	ATRTCICCRTTRTCIGCRTC	40	LeCleir *et al.*, 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:

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

$$Pi = n_i / N$$

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 access 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

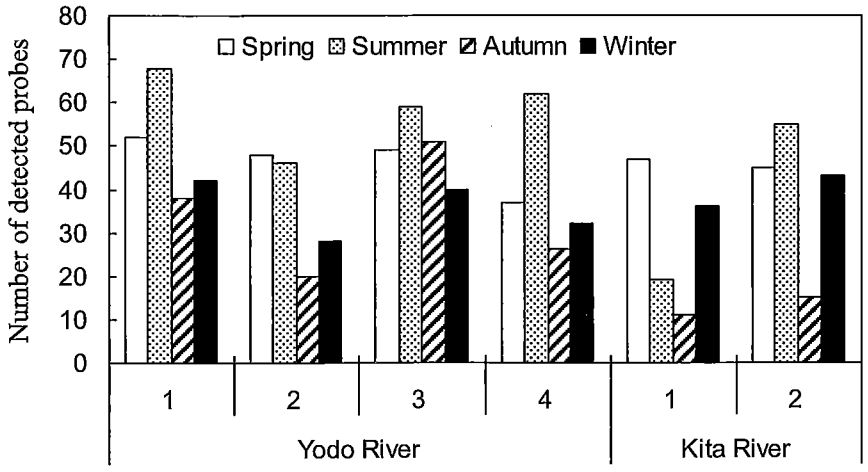


Fig. 3-1 Positive number of genes detected in river water samples collected from the Yodo and Kita Rivers. Probe with signal intensity ≥ 2500 was judged as positive.

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

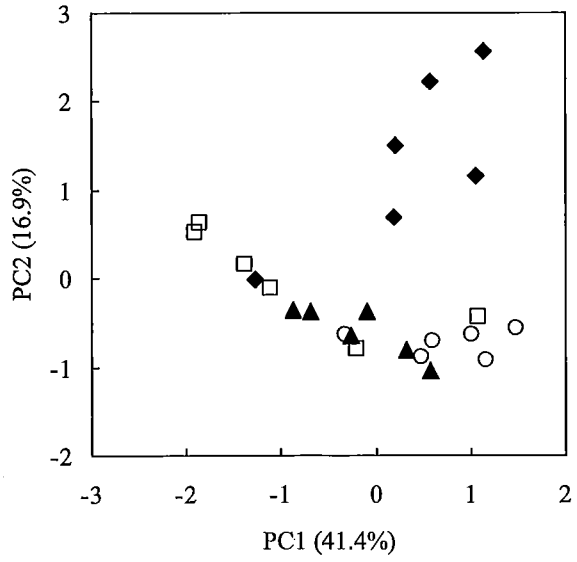


Fig. 3-2 Ordination produced from PCA based on gene profiles in river water samples obtained from Yodo and Kita Rivers in spring (open circle), summer (closed diamond), autumn (open square), and winter (closed triangle).

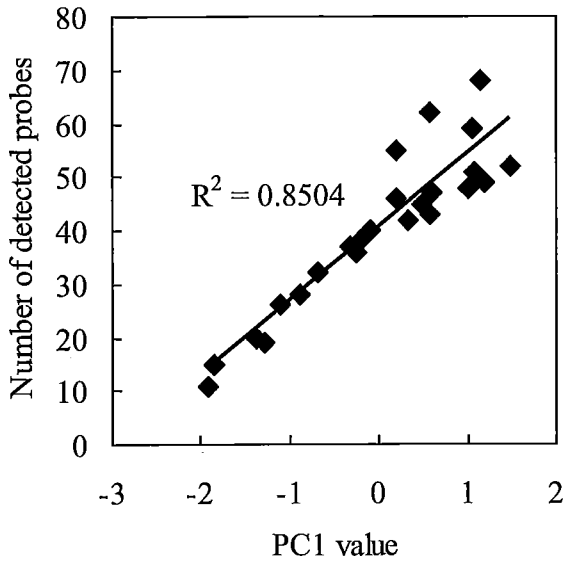


Fig. 3-3 Correlation between the value of principal component analysis factor 1 (PC1) and the number of detected gene probes in river water samples.

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

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

++, 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 (LeCleir 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*, *alk B & 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	*mmoX*
alkB	*alkM*	*mcrA*
alkB&B1	*amoA*	*qnorB*
pmoA	*napA*	*cnorB*
nirK	*narG*	16S rDNA (anammox)
tpm	*nirS*	*soxB*
phaZ	*nosZ*	*npr*
chiA	*nifH*	*nrfA*
	dsrAB	*mofA*
	ferA	*cadA*
	merA	*pcoR*
	apr	*sub*

(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

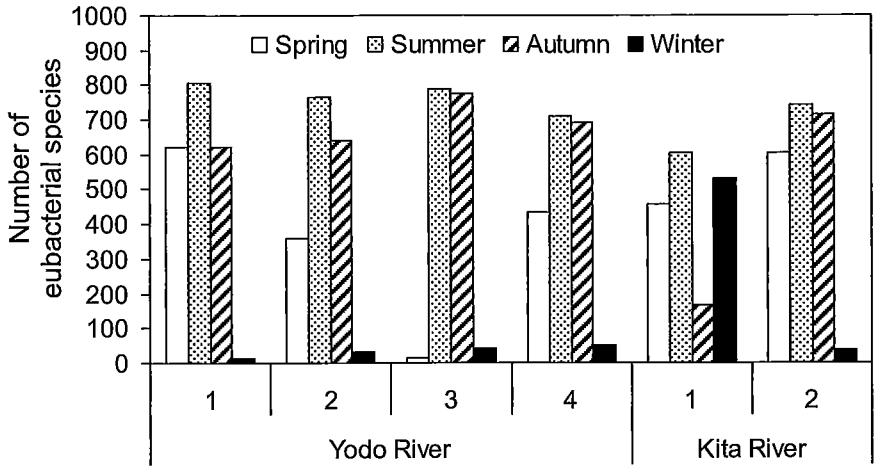


Fig. 4-1 Number of bacterial species detected in river water samples collected from Yodo and Kita Rivers. Probes with relative signal intensity > 0.1 of Cy3 control spot were judged as positive.

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).

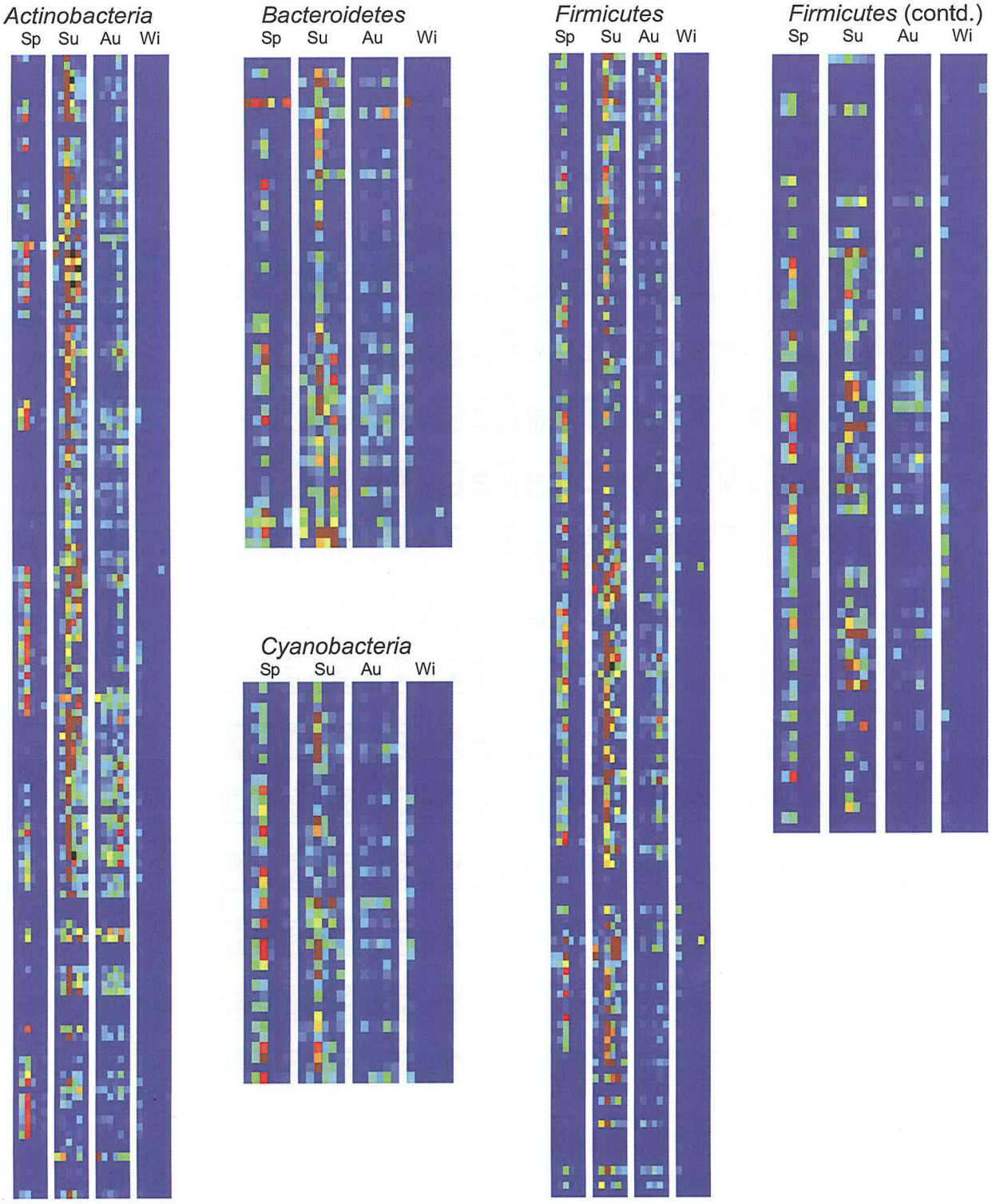
In the Kita River, fewer bacterial species were found at upstream station K1 than at downstream station K2 in spring, summer, and autumn (Fig. 4-1 and Table 4-2), and a very sharp increase in the numbers of almost all phyla was observed between K1 and K2 in autumn. In contrast, in winter the community 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

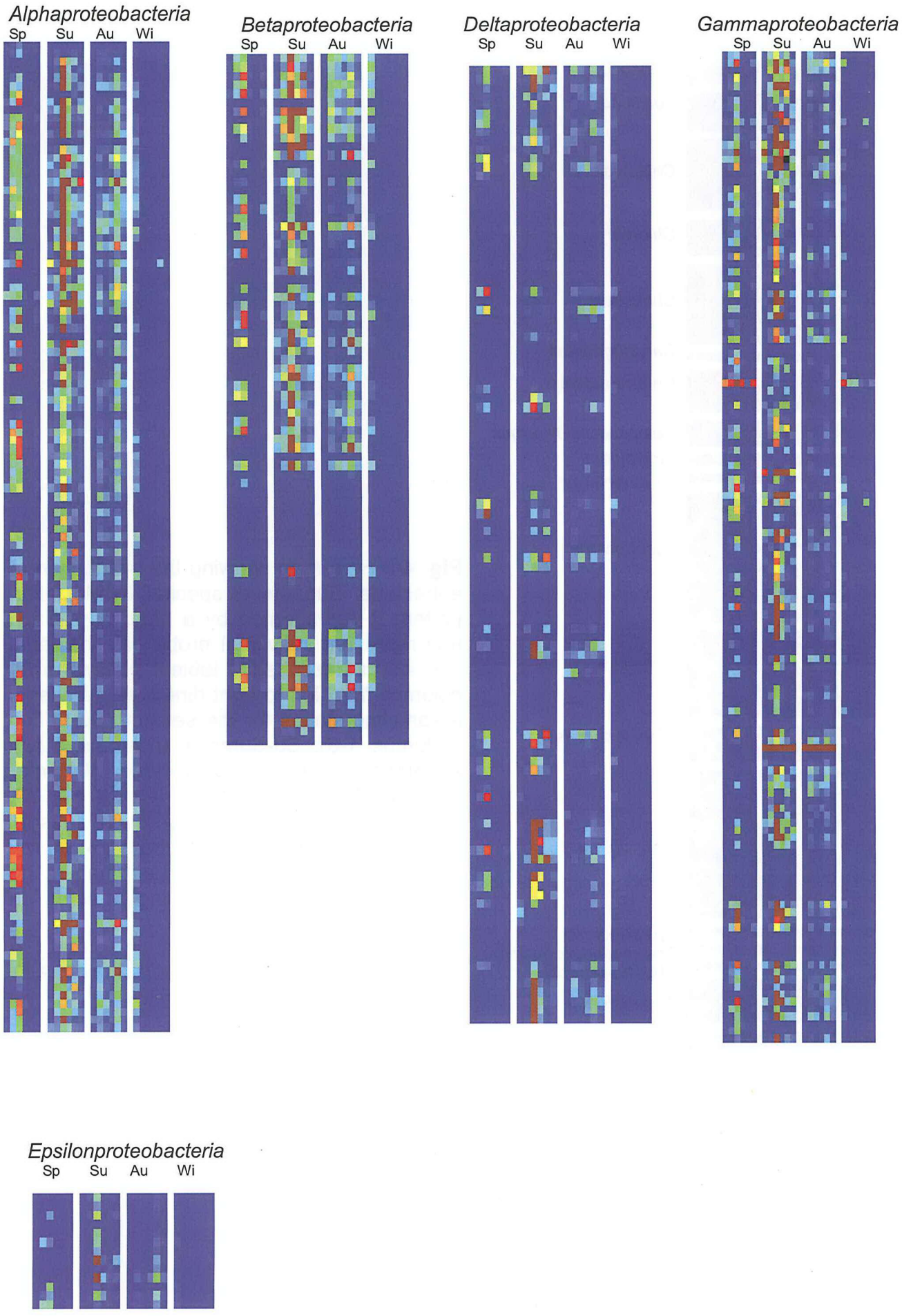
Table 4-2 Detailed distribution of bacterial species in each sample

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
Actinobacteria (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]
Bacteroidetes (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]
Cyanobacteria (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]
Firmicutes (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]
Proteobacteria (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]
- *Alpha* (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]
- *Beta* (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]
-*Gamma* (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]
- *Delta* (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]
- *Epsilon* (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
Cyanobacteria
Sp Su Au Wi
Firmicutes
Sp Su Au Wi
Firmicutes (contd.)
Sp Su Au Wi


Alphaproteobacteria
Sp Su Au Wi
Betaproteobacteria
Sp Su Au Wi
Deltaproteobacteria
Sp Su Au Wi
Gammaproteobacteria
Sp Su Au Wi
Epsilonproteobacteria
Sp Su Au Wi

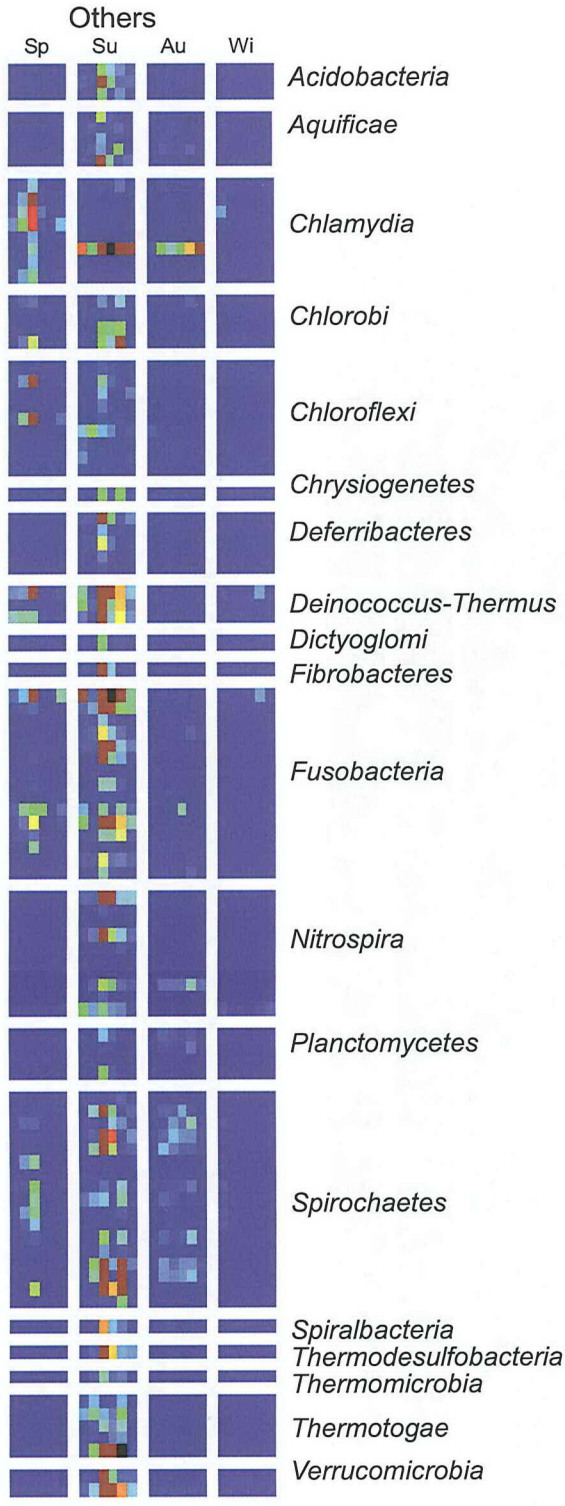


Fig. 4-2 Heat map showing the distribution of eubacteria. Eubacterial species' hybridization pattern is represented by a heat map. Each row represent individual probe of a bacterial species as described in table 4-2, each of 24 columns from left to right direction represents a sampling station for the 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:

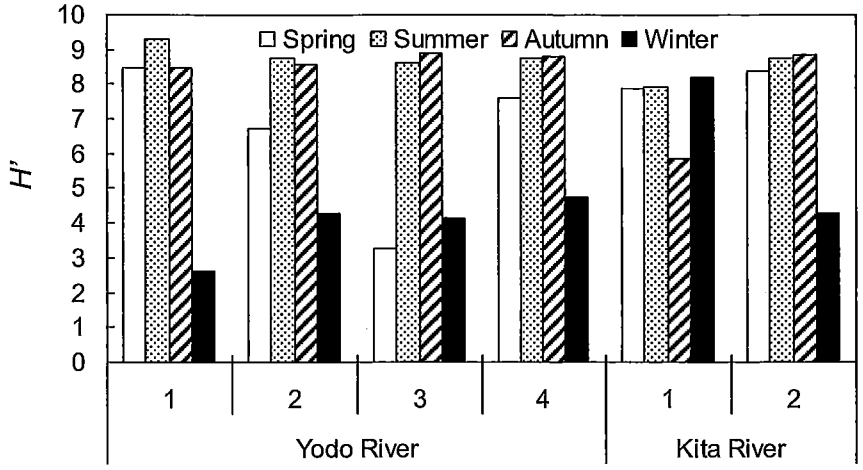


Fig. 4-3 Shannon-Weaver's diversity index (*H'*) calculated from the data of eubacterial microarray analyses. Calculations were carried out for spots whose relative signal intensity was > 0.1 of control Cy3 spot.

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	
Actinobacteria (152)	70 [46][b]	51 [34]	5 [3]	0 [0]	3 [2]	2 [1]	21 [14]
Bacteroidetes (48)	28 [58]	15 [31]	1 [2]	0 [0]	2 [4]	1 [2]	1 [2]
Cyanobacteria (39)	33 [85]	4 [10]	1 [3]	0 [0]	1 [3]	0 [0]	0 [0]
Firmicutes (226)	129 [57]	31 [14]	3 [1]	1 [0]	31 [14]	5 [2]	26 [12]
Proteobacteria (455)	230 [51]	93 [20]	10 [2]	5 [1]	14 [3]	10 [2]	93 [20]
– *Alpha* (123)	98 [80]	17 [14]	0 [0]	0 [0]	2 [2]	4 [3]	2 [2]
– *Beta* (78)	31 [40]	20 [26]	2 [3]	2 [3]	1 [1]	4 [5]	18 [23]
– *Gamma* (133)	63 [47]	36 [27]	6 [5]	0 [0]	8 [6]	1 [1]	19 [14]
– *Delta* (108)	33 [31]	13 [12]	2 [2]	3 [3]	3 [3]	1 [1]	53 [49]
– *Epsilon* (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

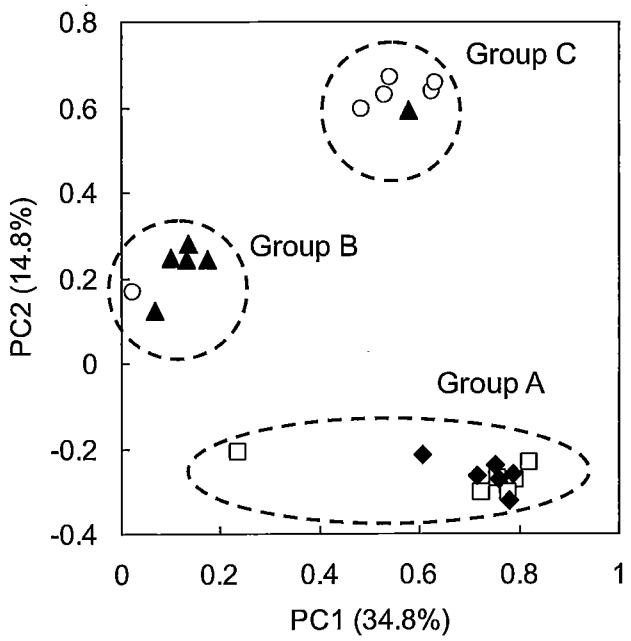


Fig. 4-4 Ordination produced from PCA based on microarray profiles in river water eubacterial community obtained from Yodo and Kita Rivers in spring (open circle), summer (closed diamond), autumn (open square) and winter (closed triangle).

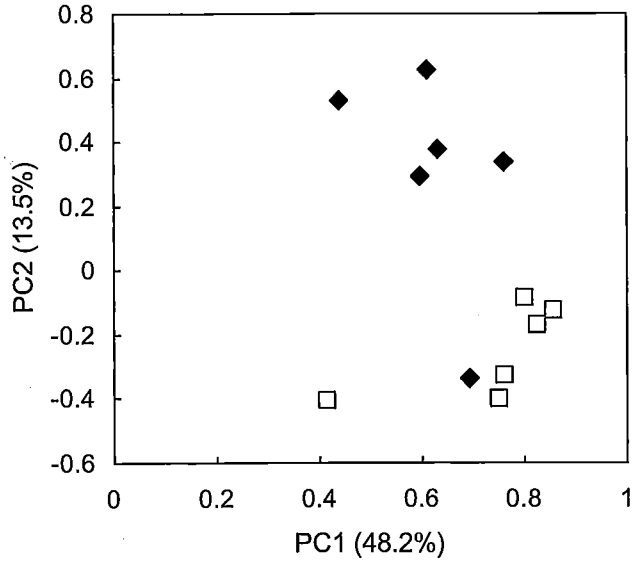


Fig. 4-5 PCA Ordination produced for group A in Fig. 4-4, with river samples obtained from Yodo and Kita Rivers in summer (closed diamond) and autumn (open square).

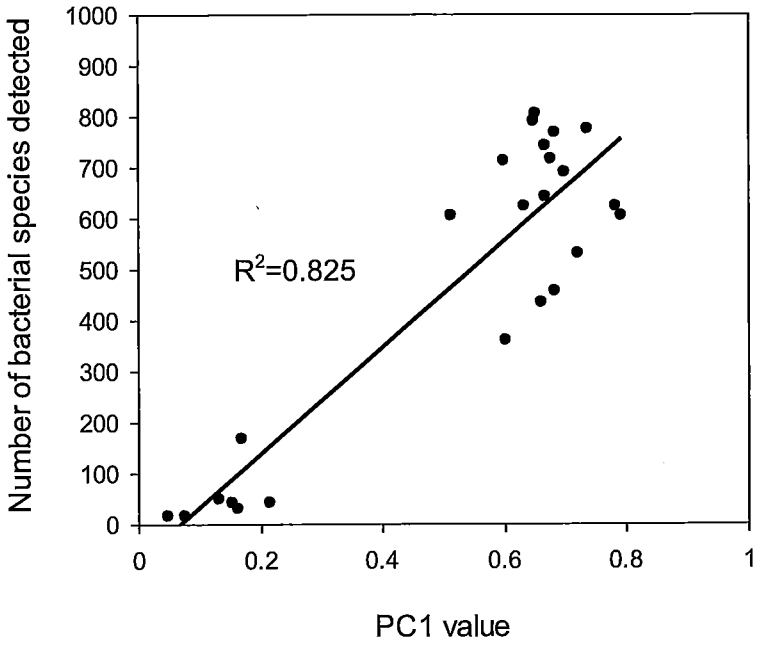


Fig. 4-6 Correlation between the value of principal component factor 1 (PC1) and the number of detected EU bacteria in river water samples.

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 (Meyer1994; 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 (Crump 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 sonne*, *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
Acetivibrio cellulosolvens	0.62	0.25	0.51	0.30		0.60					0.31	1.42												
Acinetobacter aceti							0.30																	
Acinetobacter anitratum						0.56																		
Acinetobacter baumannii						0.56																		
Acinetobacter haemolyticus						0.54																		
Acinetobacter johnsoni						0.61																		
Acinetobacter junii						0.57																		
Acinetobacter lwoffii						0.41																		
Acinetobacter radioresistens						0.25																		
Actinobacillus muris													0.61	0.43	0.64	0.62	0.87	0.65	0.57	0.56	0.63	0.81	0.57	
Actinobacillus pleuropneumoniae													1.06	0.76	0.95	0.82	0.82	0.79	1.05	0.58	0.65	0.93	0.63	0.57
Actinomadura spp.	0.71					0.57																		0.83
Aegyptianella pullorum	0.29					0.49																		
Agrobacterium tumefaciens group						0.43																		
Anaplasma marginale/centrale	0.35					0.29																		
Anaplasma phagocytophila	0.30					0.37																		
Arcobacter genus	0.68	0.26	0.60	0.61		0.59																		
Arcobacter sp.	0.28																							
Bacteroides distasonis																						0.47	0.30	0.62
Bacteroides fragilis																								0.97
Bacteroides urealyticus	0.66	0.54	0.58	0.69		0.64					0.54													
Balneatrix alpica													0.57	0.49	0.90	0.62	0.79	0.63	0.54	0.55	0.64	0.58	0.59	0.97
Bordetella avium group	0.26																							
Borrelia burgdorfii/valaisiana									0.44															
Brevundimonas diminuta	0.75	0.37	0.63	0.56		0.66					0.72													
Brevundimonas group	0.68	0.43	0.58	0.62		0.63					0.49													
Campylobacter concisus	0.71	0.52	0.63	0.30		0.54																		
Campylobacter fetus group	0.33		0.26			0.34																		
Campylobacter jejuni group	0.69		0.61	0.27		0.58																		
Campylobacter rectus									0.57			0.50												
Campylobacter sputorum	0.59					0.40																		
Centipeda periodontii																					0.39			
Chromobacterium violaceum									0.42															
Chryseobacterium meningosepticum group (1)[b]	0.59	0.33	0.52	0.30		0.56					0.29													
Chryseobacterium meningosepticum group (2)								0.27	0.95															
Chryseobacterium proteolyticum						0.36																		
Chryseobacterium scophthalmum						0.30																		
Clavibacter michiganensis							1.19	1.19	0.91	1.42		1.35												
Corynebacterium mycetoides						0.31																		
Eggerthella lenta group										0.33														
Eperythrozoon spp.							1.15	1.13	1.30	0.61		1.04												
Erysipelothrix spp.						0.31																		
Erysipelothrix rhusiopathiae	1.29	0.38	0.63	0.81		0.67					0.65													
Erysipelothrix tonsillarum	0.65	0.29	0.37	0.66		0.57					0.56													

Table 5-1 (continued)

Eubacterium combesii								0.37																
Ewingella americana						1.17	1.17	1.03	1.33		1.37													
Haemophilus haemolyticus											0.33													
Haemophilus influenzae (1)												0.87	0.82	0.65	0.63	0.73	0.73	0.62	0.64	0.66	0.58	1.04	0.81	
H. influenzae (2)												1.06	0.56	0.86	0.82	0.74	0.79	1.04	0.55	1.05	0.97	0.64	0.76	
Haemophilus parasuis (1)												0.89	0.76	0.90	0.56	0.59	0.65	0.98	0.61	1.05	0.61	1.01	0.94	
H. parasuis (2)												0.63	0.68	0.62	0.81	0.78	0.78	0.57	0.58	0.63	0.96	0.61	0.58	
Hafnia alvei						1.22		0.38	0.38		0.89													
Klebsiella oxytoca group						0.38		0.26			0.23													
Kluyvera ascobata	0.68	0.50	0.56	0.51	0.59					0.32														
Kluyvera cryocrescens	0.62		0.32	0.28	0.57					0.28														
Lactobacillus spp.	0.30		0.26																					
Legionella brunensis									0.27															
Legionella spiritensis									0.31															
Leptospira noguchii						0.46	1.22	1.46	1.24		1.36													
Leptospira parva						0.28		0.44	0.29															
Leptospira santarosai								0.49			0.32													
Mannheimia granulomatis												0.97	0.81	0.89	0.53	0.46	0.69	0.57	0.57	0.63	0.57	0.60	0.55	
Mannheimia haemolytica												1.06	0.89	0.94	0.85	0.77	0.79	0.63	1.02	1.07	0.61	1.07	0.77	
Marinospirillum megaterium												1.03	0.74	0.94	0.77	0.63	0.60	0.60	1.01	1.01	0.80	1.04	0.93	
Moraxella caviae												1.06	0.58	0.64	0.94	0.86	0.62	0.65	1.05	1.07	0.90	1.06	0.94	
Moraxella lacunata group												1.03	0.89	0.51	0.50	0.41	0.34	1.04	1.02	1.05	0.89	1.06	0.94	
Mycobacterium mucogenicum												0.27		0.34					0.33		0.80	0.35	0.69	
Mycobacterium nonchromogenicum								0.42																
Mycoplasma kahnei					0.34																			
Olsenella uli	1.11		0.31		0.48																			
Pasteurella bettyae												1.06	0.58	0.97	0.74	0.54	0.71	0.85	1.01	1.05	1.02	1.06	0.85	
Pasteurella caballi													0.99	0.85	0.57	0.44	0.65	0.82	0.94	0.91	0.59	1.00	0.85	
Pasteurella pneumotropica (1)												1.06	0.92	0.96	0.80	0.78	0.83	1.04	1.02	1.05	0.96	1.06	0.84	
Pasteurella pneumotropica (2)												1.03	1.05	0.47	0.58	0.80	0.79	0.99	0.65	1.06	0.64	1.02	0.89	
Peptoniphilus asaccharolyticus									0.30															
Porphyromonas cangingivalis							0.81																	
Proteus vulgaris						0.26																		
Pseudoalteromonas atlantica group							0.31																	
Rhodococcus equi							0.40																	
Selenomonas reminantium											1.37													
Slackia heliotrinreducens	0.39																							
Sphingobacterium multivorum								0.29																
Sphingobacterium thalpophilum				0.26																				
Sphingomonas paucimobilis	0.28																							
Staphylococcus capitis/caprae									0.27															
Treponema denticola						0.35	0.51	1.33	0.67		1.14													
Vibrio cholerae/mimicus																								0.46

[a] Blank indicates the negative result (relative signal intensity <0.25).

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

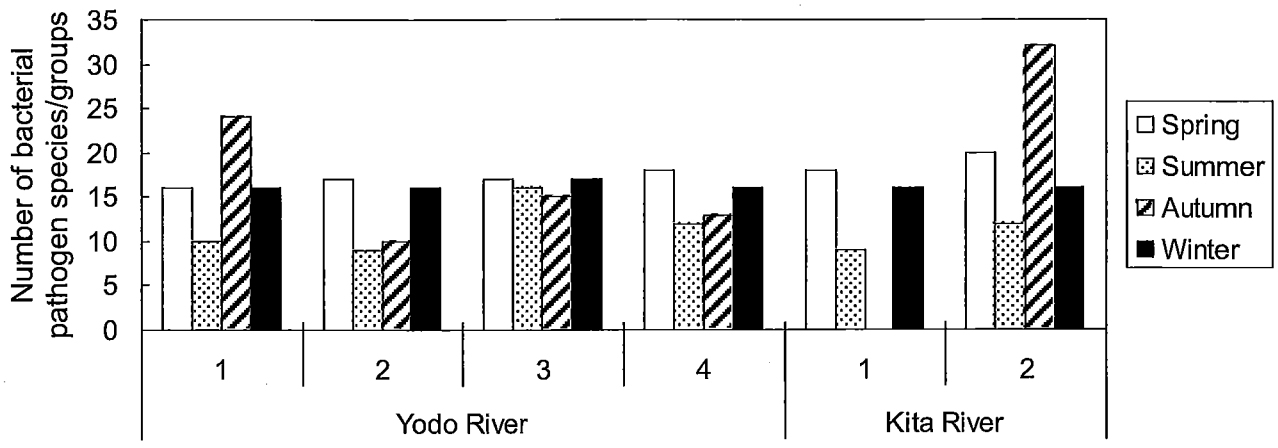


Fig. 5-1 Spatial and temporal variations in the number of pathogenic bacterial species in the Yodo and Kita rivers. Probes with relative intensity > 0.25 in the microarray analysis were judged as positive.

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*,

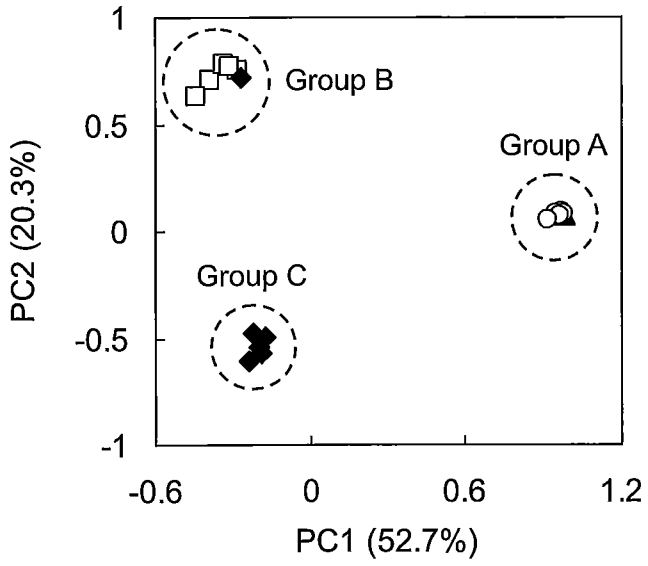


Fig. 5-2 Ordination produced from a principal component analysis based on pathogen profiles of river water samples collected from the Yodo and Kita rivers in spring (open circles), summer (closed diamonds), autumn (open squares), and winter (closed triangles).

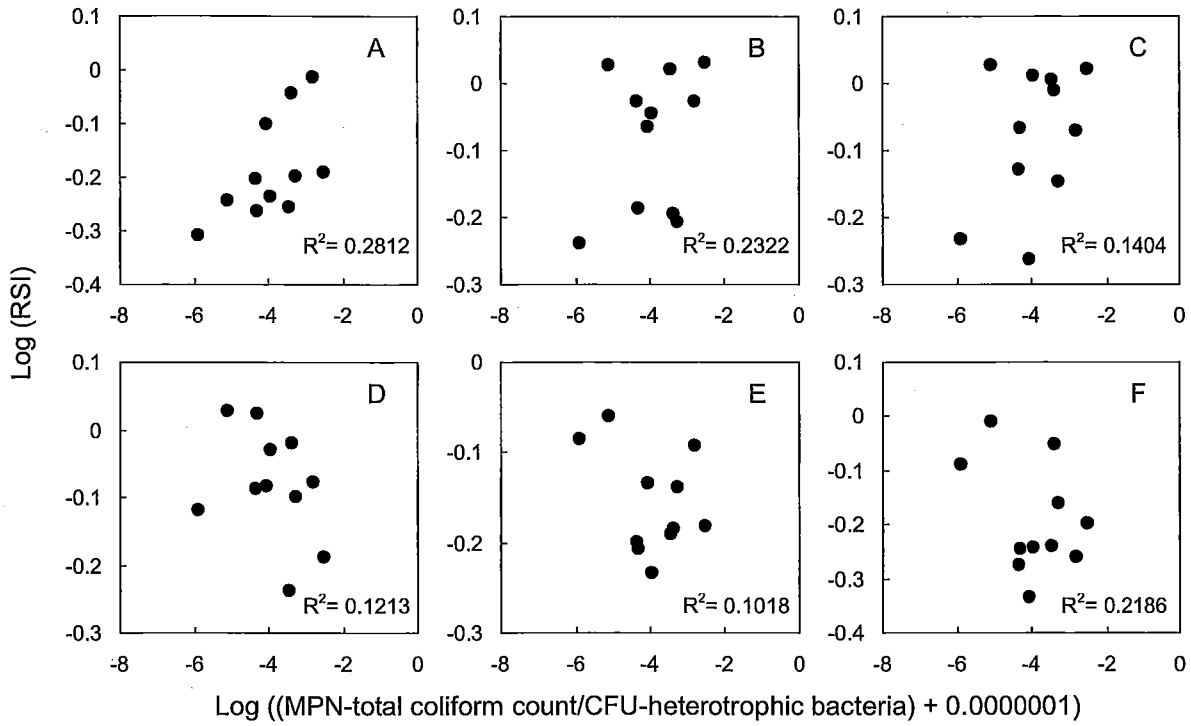


Fig. 5-3 Examples of correlations between the coliform count and the relative signal intensity of pathogen probe detected. The total coliform count relative to the total number of heterotrophic bacteria was used. A, *Balneatrix alpica*; B, *Moraxella caviae*; C, *Pasteurella bettyae*; D, *Actinobacillus 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	*Campylobacter rectus*	2
	Klebsiella oxytoca group	2
	Leptospira noguchii	2
	Leptospira parva	—
	Leptospira santarosai	—
Non-fecal	*Actinobacillus pleuropneumoniae*	—
	Eperythrozoon spp.	—
	Haemophilus influenzae (serotype A)	2
	Haemophilus parasuis (serotype B)	2
	Mannheimia granulomatis	2
	Pasteurella pneumotropica (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

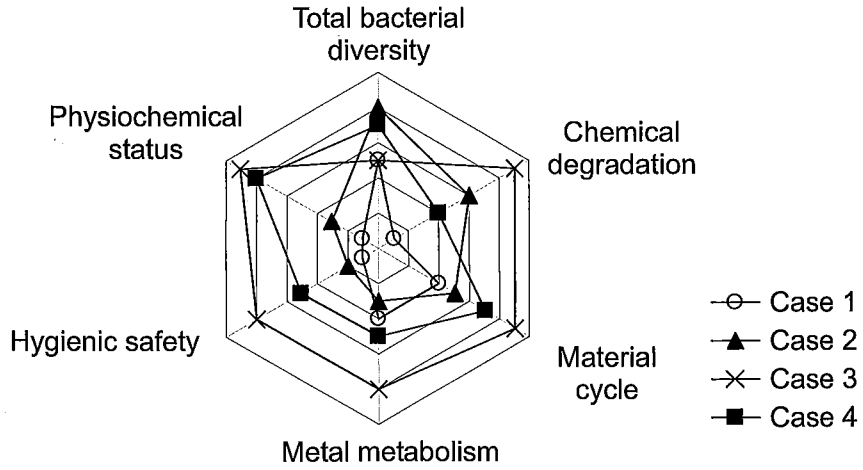


Fig. 6-1 Proposed scheme for evaluation of the soundness of riverine environment

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 log-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

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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 exihibited 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*, *Deinococcoccus-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

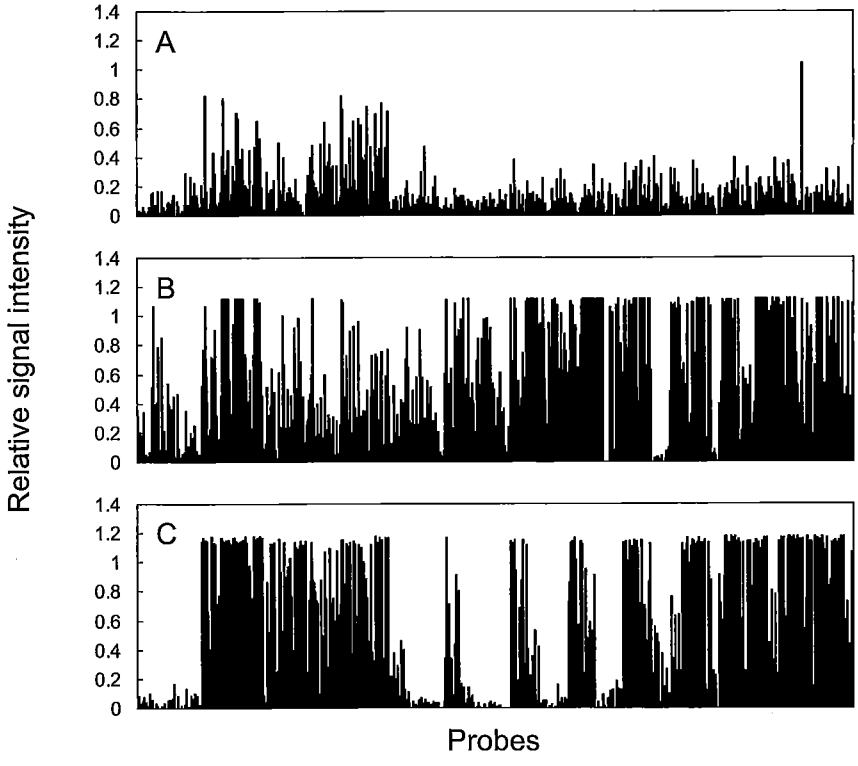

Fig. A-1 Relative signal intensities of eubacterial populations in leachate samples A, B and C obtained from DNA microarray analysis

Table A-2 Distribution of eubacterial species in leachate samples

Phylum[a]	Sample[b]		
	A	B	C
Actinobacteria (152)	126 [82]	123 [81]	106 [70]
Bacteroidetes (48)	45 [94]	41 [85]	37 [77]
Cyanobacteria (39)	35 [90]	36 [92]	36 [92]
Firmicutes (226)	169 [75]	174 [77]	151 [67]
Proteobacteria (455)	362 [80]	363 [80]	335 [74]
- *Alpha* (123)	119 [97]	121 [98]	108 [88]
- *Beta* (78)	62 [79]	64 [82]	59 [76]
-*Gamma* (133)	111 [83]	116 [87]	105 [79]
- *Delta* (108)	58 [54]	51 [47]	51 [47]
- *Epsilon* (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).

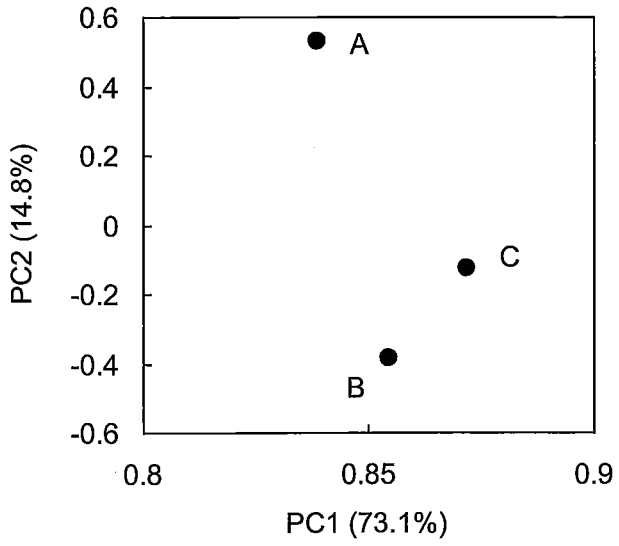

Fig. A-2 Ordination produced from principal component analysis for eubacterial community in three leachate samples.

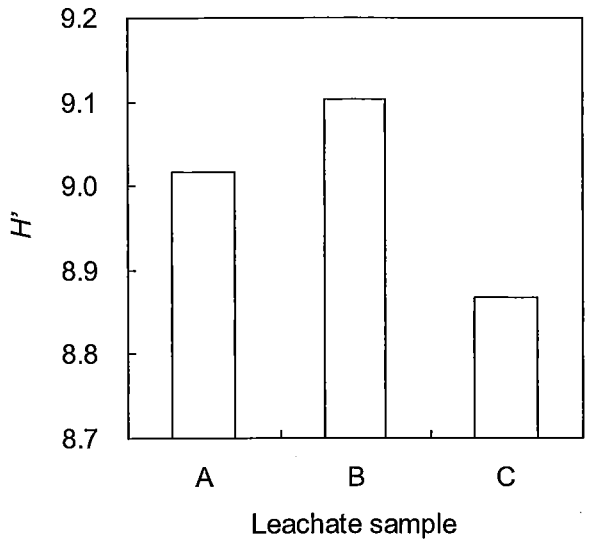

Fig. A-3 Shannon-Weaver diversity index (*H'*) calculated from DNA microarray analyses of eubacterial community in three leachate samples.

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

[a]Biosafety level classified by Japanese Society for Bacteriology in 2007.
[b]Numbers for each pathogen/sample indicate relative signal intensities in microarray analysis.
[c]–, Biosafety level is not identified.
[d]Different biosafety levels are set for pathogens within the same group.
[e]ND, not detected.

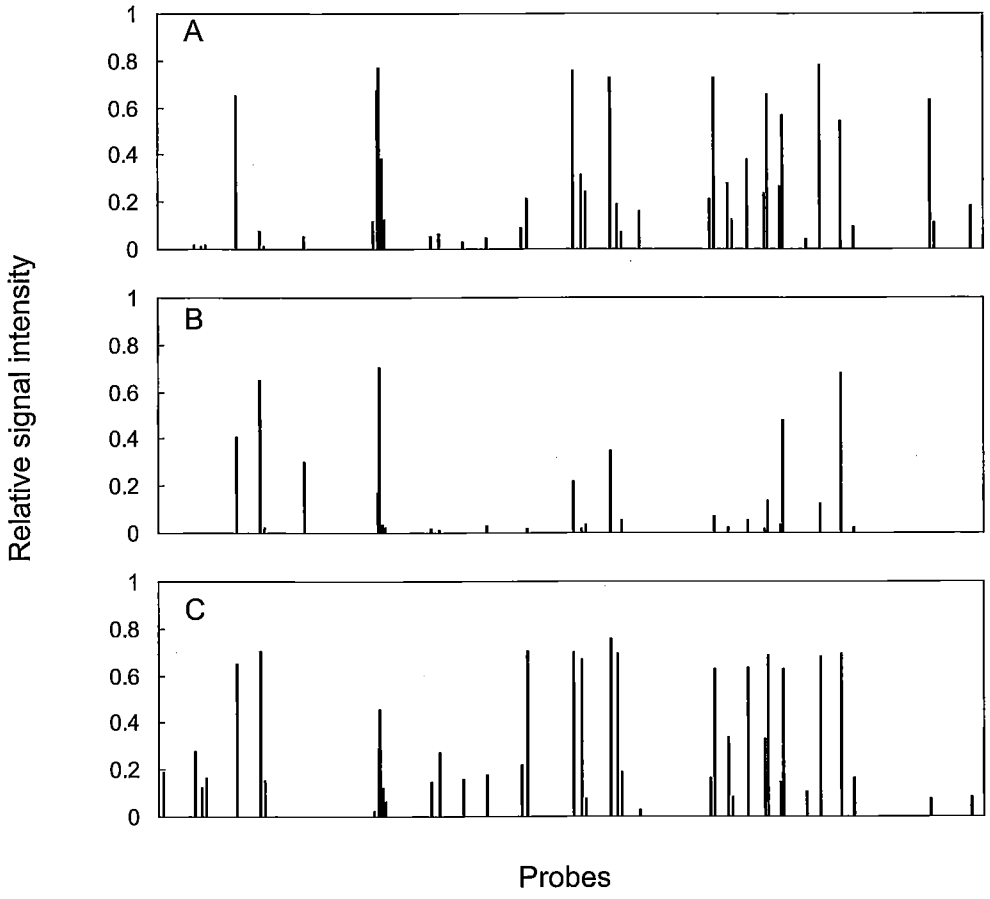

Fig. A-4 Relative signal intensities of bacterial pathogens in leachate samples A, B and C obtained from DNA microarray analysis

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 addtion, 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	Acidimicrobidae	Acidimicrobiales	Acidimicrobineae	Acidimicrobium	acidimicrobium
Actinobacteria	Actinobacteridae	Actinobacteriales	Corynebacteriaceae	Corynebacterium	corynebacterium
Actinobacteria	Actinobacteridae	Actinobacteriales	Corynebeacteriaceae	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	Nocardiaecae	Rhodococcus	rhodochronus2
Actinobacteria	Actinobacteridae	Actinobacteriales	Nocardiaecae	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	Nocardiopsis	nocardiopsis

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	kutzuneria
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiaceae	Prauserella	prauserella
Actinobacteria	Actinobacteridae	Actinobacteriales	Pseudonocardiacea	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	Thermocrispum	thermocrispum
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	streptosporanngium
Actinobacteria	Actinobacteridae	Actinobacteriales	Symbiobacterium	Symbiobacterium	symbiobacterium
Actinobacteria	Actinobacteridae	Actinobacteriales	Sporichthyaceae	Acidothermus	acidothermus
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	Porphylomonadaceae	Dysgonomonas	dysgonomonas
Bacteroidetes	Bacteroidetes	Bacteroidales	Porphylomonadaceae	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
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Gelidibacter	gelidibacter
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Myroides	myroides
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Ornithobacterium	ornithobacterium
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Polaribacter	polaribacter
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Psychroflexus	psychroflexus
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Psychroserpens	psychroserpens
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Riemerella	riemerella
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Salegentibacter	salegentibacter
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Weeksella	weeksella
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Crenotrichaceae	Chitinophaga	chitinophaga
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Crenotrichaceae	Rhodothermus	rhodothermus
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flammeovirgaceae	Flammeovirga	flammeovirga
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flammeovirgaceae	Flexithrix	flexithrix
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flammeovirgaceae	Persicobacter	persicobacter
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flammeovirgaceae	Thermonema	thermonema
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Cyclobacterium	cyclobacterium
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Cytophaga	cytophaga
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Dyadobacter	dyadobacter
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Flectobacillus	flectobacillus
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Flexibacter	flexibacter
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Hymenobacter	hymenobacter
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Microscilla	microscilla
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Runella	runella
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Spirosoma	spirosoma
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacterraceae	Sporocytophaga	sporocytophaga
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sapraspiraceae	Haliscomenobacter	haliscomenobacter
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sapraspiraceae	Lewinella	lewinella
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sapraspiraceae	Saprospira	saprospira
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	pedobacter
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium	sphingobacterium
Chlamydia	Chlamydiae	Chlamydiales	Chlamydiaceae	Chlamydia	chlamydia
Chlamydia	Chlamydiae	Chlamydiales	Chlamydiaceae	Chlamydophila	chlamydophila
Chlamydia	Chlamydiae	Chlamydiales	Chlamydiaceae	Chlamydophila	chlamydophila
Chlamydia	Chlamydiae	Chlamydiales	Chlamydiaceae	Chlamydophila	chlamydophila
Chlamydia	Chlamydiae	Chlamydiales	Chlamydiaceae	Chlamydophila	chlamydophila
Chlamydia	Chlamydiae	Chlamydiales	Parachlamydiaceae	Parachlamydia	parachlamydia
Chlamydia	Chlamydiae	Chlamydiales	Simkaniaceae	Simkaniaceae	simkaniaceae
Chlamydia	Chlamydiae	Chlamydiales	Waddliaceae	Waddlia	waddlia
Chlorobi	Chlorobia	Chlorobiales	Chlorobiaceae	Chlorobium	chlorobium
Chlorobi	Chlorobia	Chlorobiales	Chlorobiaceae	Chloroherpeton	chloroherpeton

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

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

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

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

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	Coprothermobacer	coprothermobacer
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
Firmicutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Mycoplasma	mycoplasma
Firmicutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	Haemobartonella	haemobartonella
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Cetobacterium	cetobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	fusobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	fusobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	fusobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	fusobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	fusobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	fusobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	fusobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	fusobacterium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Ilyobacter	ilyobacter
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Leptotrichia	leptotrichia
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Propionigenium	propionigenium
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Sebaldella	sebaldella
Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	Streptobacillus	streptobacillus
Fusobacteria	Fusobacteria	Fusobacterales	Fusobacteriaceae	Streptobacillus	streptobacillus
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Leptospirillum	leptospirillum
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Leptospirillum	
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Magnetobacterium	
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrosospira	nitrosospira
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrosospira	
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrosospira	briensis
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrosospira	multiformis
Nitrospirae	Nitrospirae	Nitrospirales	Nitrospiraceae	Nitrospira	nitrospira
Nitrospira	Nitrospira	Nitrospirales	Nitrospiraceae	Thermodesulfovibrio	thermodesulfovibrio
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Thermodesulfovibrio	
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetacea	Gemmata	gemmata
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetacea	Isosphaera	isosphaera
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetacea	Pirellula	pirellula
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetacea	Planctomyces	planctomyces
Proteobacteria	Alphaproteobacteria	Family unknwon	Family unknwon	Bactoderma	bactoderma
Proteobacteria	Alphaprotepbacteria	Bradyrhizobiaceae	Bradyrhizobiaceae	Bradyrhizobium	
Proteobacteria	Alphaprotepbacteria	Bradyrhizobiaceae	Bradyrhizobiaceae	Nitrobacter	
Proteobacteria	Alphaproteobacteria	Caulobacteriales	Caulobacteraceae	Asticcacaulis	asticcacaulis
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	brevundimonas
Proteobacteria	Alphaproteobacteria	Caulobacteriales	Caulobacteraceae	Caulobacter	caulobacter
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	phaeospirillum
Proteobacteria	Alphaproteobacteria	Defluvibacter	Phyllobacteriaceae	Defluvibacter	defluvibacter
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bartonellaceae	Bartonella	bartonella

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

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

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

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

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

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

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

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

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

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

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

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

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 (*ortho*)	CACCTATTCGTACTTCGATTCGACCCAGTCCGAGTTCAACCTGCGCCGGCGTATCATCAC	D37783
		GATGGGAAGTACAGCGGTTTCCATGACGACATCCCGACTGATTTTTATCGAGGGAAGCTC	D16356
		TGCACTTCAAGGTGCGCAAGGACGGTTTCGAACCGTTGACCACGCAATACTACTTCGAAG	M16964
		TACTCCTTCTTTGATAAGAGCCAAACCCCTTACAATTTGCGCCGCACCATTATCGCTGAC	M57500
		CTCAATTTATTGGGTCGACATGGTAACCGCCCTTCGCATGTTCACTACTTTGTCTCTGCG	Z36909
		CGAATGGAACCTCCGCGGCTCCATCATTGCCGATGACCAGGGCAACTTCCAGATCAACAC	M94318
C23O DNA	Catechol oxidation (*meta*)	GTCCTAACCTGGATGAAACTCTGAATTTGTTCCGTGATGTGCTCGGTTTTGACCTCGCTG	AB008831
		AGAGCCGAATCAAGAAAAAGGGAAGCTTCATCATCTTTGCTATTGGTATGGCATCCCGCA	X67860
		GCTAGCTCTAACTTGCGGGATTTTATGAAAGCTAAAGCTGAGCGATGCGAGTGAACGTCG	D37828
		GCCTAGATTTCTATTTAAGTGAGCAAGGTTTGGTTGGACCCGATGCATCCACTCAAGCGG	U01826
		TCGACTAGATCACCTCAACATCACAGCGAAGGACGTTGACGACGCCCGTAACTGGTATTC	X69504
		CCATGGGATCACACGCGGGCAGACGATCTACTTCTTCGACCCCTCGGGCAACCGGAACGA	U23375
		GACTCACGGCAAGACCATCTACTTCTTCGACCCTTCGGGCAACCGCAACGAGGTGTTCTG	X80765
		ACATCGTGCGCCAAAGAGCATCGCTAGAGAGAACAATGCAAACCAATAACAAGAGTTCGT	S78585
		CACATCGTGGTGATGGTCGACGACTACGACGAGACGATGCGCTTTTACCGCGAGGTACTC	L77225
alkB	Alkane hydroxylation (Group I)	CACCGTGATGTTGCTACACCGATGGACCCTGCGACCTCACGTATGGGAGAAAATATATAT	AJ233397
		GTGCCCCAGGAACACTCCTTATGGGCAATGTAATTTATAAGATGGCAATGCGCGAGAGAG	U40233
alkM	Alkane hydroxylation (Group II)	CAAGGCTGGGGAATGAGTGCTGCTTTTCATGCTTCTATGGTGGGAATTTTTGGTAAAAGT	AJ009585
		TATATCGAGCATTATGGTCTAAAACGCCAGAAAAGAGCGGATGGCAATTACGAACGTACC	AJ002316
		CCATGTTTGCGAAATTTGGTGCCCGTTCGATTCCGTTTCAAGTTACTCAAGCTGCGTATG	AJ009582
		GATAATGAGTTATTGCAAGGCTGGACAATGACCGCAGCATTCCATTCGTCTATGGTCGGG	AJ009584
alkB & B1	Alkane hydroxylation (Group III)	TATCTGCTCATTCAGGCGGTGGTCGGGTTTTCCCTGCTCGAGATCGTCAACTACATGGAG	AJ009587
		GTTTCGGCCAGTCGGTCTACCAGTTCCTGCCGCATGCCTACAAGTACAACTTCCTCAGCG	AJ009580
		GTGATCCAGGGGATCTACGGCTTCTCTCTGCTGGAGGTGGTCAACTACGTCGAGCACTAC	AJ009579
		GTGATCCAGGCCTTCATCGGGTTCTCGTTACTCGAAGCGATCAACTACCTCGAGCACTAC	AJ009586
		GTAATTCAGGCGGTGTACGGCGCGTCGCTGCTCGAAGTCGTGAACTACGTCGAGCACTAC	AJ293344
		GAGGACCCGGCGAGTTCACGGTTCGGAGAAAGCTTCTGGACGTTCCTGCCCCGCAGTGTC	AJ401611
mmoX	Methane oxidation	GGTCTACGAGGATTGGGGTGGAATCTGGATCGGCCGTCTCGGCAAATATGGCGTCGAGAG	X55394
pmoA	Methane oxidation	GATCTGATCGGCCTTCACTACGTCCGCACGTCGATGCCGGAATATATCCGCATGGTCGAG	U31651
		AACTTCTGGGGTTGGACTTACTTCCCAGTTAACATTTGCTTCCCATCTAACCTGCTGCCA	U31653
		AACTTCTGGGGATGGACTTACTTCCCAGTTAACTTCGTGTTCCCATCACAATTCGCTCCA	U31654

mcrA	Methanogenesis	TGGCTAGGATCTTACATGTCAGGTGGAGTAGGATTCACACAATATGCTACAGCATCGTAT	J03375
		GACGACTTCACCTACTTCGGTAAGGAGTACGTGGAGGACAAATATGGACTCTGTGAGGCA	U10036
		TACATGTCTGGTGGCGTAGGTTTCACACAATACGCTACAGCAGCATACACCGACGATATC	M16893
amoA	Ammonia oxidation	CGTACAGGTACACCCGAGTATGTTCGTCATATTGAGCAAGGTTCACTGCGTACCTTTGGT	L08050
napA	Nitrate reduction	TATGCCTTCATGCGCACCTTCGGCATGGACGAGCCGATGGGCTGCTATGACGATTTCGAG	Z36773
narG	Nitrate reduction	GGACCTACTTCCACGCAGGTATCCTCGACAACACTGATCCGGACACCGCAGAGAAGATAC	AJ277440
		AAATGGCATCGATCTGTATAAAGAAGCTGAAAAAGCAGGGGCAGCAACACCTGAAGACGT	X91819
		CAGGAATTGCGCCAGGGCGACCTCTACGACCTTCAGGAACGCTATGTGCTGCTGTTCGAC	Z26255
		CCATGAATTCTTCCTCAAGTATCTCTTAGGCACGAAGAATGGCCTGTTCGCCGAGGAAGA	AB096694
		CGGCAAAGGTCATGAATATTTTCTGAAGCACTTGCTCGGCACGACTAACGGACTGATGAA	AB087407
		CTGGCGTTCTAACCTGCTCGGTTCTTCCGGTAAAGGTCATGAGTTTATGCTCAAGTACCT	X16181
nirK	Dissimilatory itrite reduction	CTCACCGCCAAGGTCGGCGAGACCGTGCTGCTGATCCACTCGCAGGCCAATCGCGACACC	AF051831
		TTACTACACGTTCCAGCAGCCCGGTATCTATGCGTATGTAAACCACAATCTGATCGAGGC	M97294
		CTATGACACCGTCTATTACATCGGCGAGAGCGACCACTACATCCCGAAGGACGAGGACGG	U62291
		TATATCCCCAAGGACAAGGACGGCCACTACAAGGACTACCCGGACCTGGCGTCCAGTTAC	Z21945
		CTGACGTACGACAAGATCTACTATGTCGGCGAGCAGGACTTCTACGTGCCGAAGGACGAG	Z48635
nirS	Dissimilatory itrite reduction	GAACGTCAAGGAAACGGGCAAGATCCTGCTGGTGGACTATACCGACCTCGACAACCTCAA	AJ401462
		CAGATCATGCTGGTCGACTACACCGACATCAAGAACCTCAAGACCACCACCATCGAATCC	M80653
		GAGTTCATCGTCAACGTGAAGGAGACCGGCAAGGTCCTGCTGGTCAACTACAAGGATATC	X16452
		CAAGATCCTGATGGTCAACTACTCGGACTTGTCCAACCTGAAGACCACCACCATCGATTC	X91394
qnorB	Dissimilatory nitric oxide reduction	TATGAGTATGTCGACCTGGGCCGGCTGTGGCAGATCGGCAAGTTCGCCGGCATCCTGATC	AF002661
cnorB	Dissimilatory nitric oxide reduction	GATCTTCTCGAGCTTCGAGATCGTGCCCTTCTTCGCCATGATGTCATTCGCCTTCGTCAT	U28078
nosZ	Nitrous oxide reduction	GTCCACATGTCCTTTACCGAGGGCAAGTATGACGGCCGCTTCCTGTTCATGAACGACAAG	AJ440508
		GGAAGGCACCTATGACGGGCGCTATCTCTACGCCAACGACAAGGCCAATACGCGTGTCTG	AJ440509
nifH	Nitrogen fixation	CGATGCCTATTCGCGAAAACAAGGCTCAGGAAATCTACATCGTCATGTCCGGTGAGATGA	V01215
nrfA	Nitrite reduction	GGAATATGAAACCTGGACAGCGGGCATTCACGGTAAAAACAACGTGACCTGTATCGACTG	X72298
16S rDNA	Anammox	TGCATTGATAACCTGCCTTTGAGATGGGAATAACTGCGTTTCGAGCAATCGGAACTACCG	AJ250882
dsrAB	Dissimilatory (bi)sulfate reduction	AAAGATGGCATTCATATCTTCCGGGTACAATCCCGAAAAGCCGATGGCAAACCGTATCAC	U16723
soxB	Thiosulfate oxidation	ACTGCCTTCAAAACTATCGGTCTGGAGTTCGAGAGAGGTGAGTGGAATTCCGAGTGTAGA	Y16933
ferA	Iron reduction	CAACAGCATCAATGATCAGTACGACCTCTGCACCAGCTGCCACACCGTCAACACCATGAC	AY033095
mofA	Manganese oxidation	GTCTACGAGACGGTGCAGGATCCGAACCAGATGAACGGTTTCAACTCGGTCGGCCGCTGG	Z25774

merA	Mercuric reduction	CAGCAAGGTCACGGCCCTGGCGCGCAATACCTTGTTCTTCCGTGAAGACCCGGCCATCGG	AJ418049
		GTTGTGCACGGTGAGGCGCGCTTCAAGGACGACCAGAGCCTTACTGTCCGTTTGAACGAG	AJ418052
		AGTGCGGCCGTGCCGCCGATTCCCGGACTGCAAGATACCCCCTTTTGGAACTCGGAAAAG	AJ418056
		CATGGAAGGCATCCTGGAAGAGCTATGGCCATCACCATTTTGCCCGGCTACGCCCGTTTC	AJ418057
tpm	Selenium methylation	TTGAAGGCCGGATTGGAGCGTATGGATGAGCACGTTTATGTGTTGGAACGTGTGTAACTC	L49178
cadA	Cadmium resistance	ACTACACTCCGATCATTATGGTTATTGCAGCCTTGGTTGCAGTCGTTCCACCCCTATTCT	J04551
pcoR	Copper resistance	GATCCGTTCGGGGAAGAAGATCCATCTCACCGGTAAAGAATACGTTCTGCTTGAGTTGCT	X83541
phaZ	PHB depolymerization	TCTATCGCAACGGCAACAAGGCCAACGCGCTCCCGGTCTCTGCCACCAGCTATGTCGATG	U16275
		CCGTATCGGCGACGACGAAATCGGCCTTCACCTGTACTGCCACCACGGCCAGCAACTACG	J04223
		GTCACGGCCACCACCTACACCGACACAGGCCTGACCACCGGTACGGCCTACTCCTACACC	U58990
		CTATACCGACACCGGCCTGATTGCTGGTACCACCTACAGCTACACCGTAACCGAGATCGA	D25315
apr	Hydrolysis of peptidic compound	GGCAACGACACCCTGGACTTCTCCGGTTTCACCCAGAACCAGAAGATCAACCTCAATGAG	AB013895
npr	Hydrolysis of peptidic compound	AACAATGCATTCTGGAACGGATCACAGATGGTATACGGAGATGGTGATGGTGTAACGTTT	M83910
sub	Hydrolysis of peptidic compound	TACTGGTTCTACAGCGCTGAAAACAGTAGTTGATAAAGCGGTTTCCAGCGGTATCGTCGT	S51909_1
sub	Hydrolysis of peptidic compound	TTCAACAGGAAGCGGCCAATATAGCTGGATTATTAACGGCATTGAGTGGGCCATTTCCAA	S51909_2
chiA	Chitin depolymerition	GTGAAAGAGTTCCTGCAGACCTGGAAGTTCTTCGATGGCGTGGATATCGACTGGGAGTTC	AY040610
		CAGCAATTGGCGTTGGTTACGACAAAATTGAAGACGTTGATTACGCAGATGCTGTGCAGT	AF193498
		ATAACGGTATCCAACTTCTTCTTGCTCAAGGCGTTCCTGCGAACAAGCTAGTCCTTGGTA	AB004935
		CTGAAACAGGCCGTACTTACGAACTGACATCGGCAGTAGGTGTAGGCTACGACAAGATTG	AF193500
		CATATGGGTTTGATGGCGTGGATTTAGACTGGGAATATCCGGGCGTTGAAACGATTCCTG	AY129671
		TAAACCGCAGATCGACGTTCAGGAAGAAATCCGCAGTCGCGTAGATTTCCTGAAACAGTA	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 *et al.*, 1999
C23O DNA	AAGAGGCATGGGGGCGCACCGGTTCGATCA	CCAGCAAACACCTCGTTGCGGTTGCC	60	Sei *et al.*, 1999
alkB	CATAATAAAGGGCATCACCGT	GATTTCATTCTCGAAACTCCAAAC	40	Kohno *et al.*, 2002
alkM	GAGACAAATCGTCTAAAACGTAA	TTGTTATTATTCCAACTATGCTC	40	Kohno *et al.*, 2002
alkB / alkB1	TCGAGCACATCCGCGGCCACCA	CCGTAGTGCTCGACGTAGTT	40	Kohno *et al.*, 2002
mmoX	GGCTCCAAGTTCAAGGTCGAGC	TGGCACTCGTAGCGCTCCGGCTCG	55	McDonald *et al.*, 1995
pmoA	GGGGGAACTTCTGGGGITGGAC	GGGGGRCIACGTCITTACCGAA	45	Cheng *et al.*, 1999
mcrA	TAYGAYCARATHTGGYT	ACRTTCATNGCNGCRTARTT	45	Springer *et al.*, 1995
amoA	GGGGTTTCTACTGGTGGT	CCCCTCKGSAAAGCCTTCTTC	55	Rotthauwe *et al.*, 1997
napA	TAYTTYYTNHSNAARATHATGTAYGG	DATNGGRTGCATYTCNGCCATRTT	45	Flanagan *et al.*, 1999
narG	TAYGTSGGSCARGARAA	TTYTCRTACCABGTBGC	60	Philippot *et al.*, 2002
nirK	GGSGCGGTCATGGTGCTGCC	TCGAAGGCCTCGATCAG	65	This study
nirS	TAYCACCCCGAGCCGCGCGT	CTTRAGYTYSAGBGTCTTGTCGTC	65	This study
qnorB	GGNCAYCARGGNTAYGA	ACCCANAGRTGNCANACCCACCA	55	Braker and Tiedje, 2003
cnorB	GACAAGNNNTACTGGTGGT	GAANCCCCANACNCCNGC	55	Braker and Tiedje, 2003
nosZ	CGGYTGGGGAMWKACCAA	ATRTCGATCARYTGNTCRTT	55	Nogales *et al.*, 2002
nifH	AAAGGYGGWATCGGYAARTCCACCAC	TTGTTSGCSGCRTACATSGCCATCAT	60	Rösch *et al.*, 2002
nrfA	GCNTGYTGGWSNTGYAA	TWNGGCATRTGRCARTC	45	Mohan *et al.*, 2004
16S rDNA (anammox)	GGATTAGGCATGCAAGTC	AAAACCCCTCTACTTAGTGCCC	60	Egli *et al.*, 2001
dsrAB	ATCGGWACCTGGAAGGAYGACATCAA	GGGCACATSGTGTAGCAGTTACCGCA	60	Karkhoff-Schweizer *et al.*, 1995
soxB	GAYGGNGGNGAYACNTGG	CATGTCNCCNCCRTGYTG	60	Petri *et al.*, 2001
ferA	ACARMARSGRTGTYGGSTGC	TGGATYMCSRGARGTYGYAGTG	45	Neal *et al.*, 2004
nofA	GGCTTCACCGAGTTCACGCA	CCAGCGGGGTGTCCATCCAG	60	Siering and Ghiorse, 1997
merA	TTGGAGAACGTGC	ACGTCCTTGGTGAAGGTCTG	55	Felske *et al.*, 2003
tpm	CAGTCAGAGGTCAATAAGG	GAGTTACACACGTTCCAACA	40	Cournoyer *et al.*, 1998
cadA	CAAAYTGYGCRGGHAARTTYGA	AACTAATGCACAAGGACA	55	Oger *et al.*, 2001
pcoR	CAGGTCGTTACCTGCAGCAG	CTCTGATCTCCAGGACATATC	55	Trajanovska *et al.*, 1997
phaZ	CGTCTACCGCAACGGCACCAAGG	TGGGCGTAGTTGCTGGCCGT	55	Sei *et al.*, 2001
apr	TAYGGBTTCAAYTCCAAYAC	VGCGATSGAMACRTTRCC	55	Bach *et al.*, 2001
npr	GTDGAYGCHCAYTAYTAYGC	ACMGCATGBGTYADYTCATG	55	Bach *et al.*, 2001
sub	ATGSAYRTTRYYAAYATGAG	GWGWHGCCATNGAYGTWC	55	Bach *et al.*, 2001
sub	GNACHCAYGTDGCHGGHAC	GWGWHGCCATNGAYGTWC	55	Bach *et al.*, 2001
chiA	GGIGGITGGACIYTIWSIGAYCCITT	ATRTCICCRTTRTCIGCRTC	40	LeCleir *et al.*, 2004

B.3 Pathogen bacterial probes

Genus	Species Epithet
Abiotrophia	*defectiva B*
Abiotrophia	*defectiva A*
Acetobacter	*orientalis*
Acetobacter	*aceti*
Acetobacter	*pasteurianus*
Acholeplasma	*laidlawii*
Acholeplasma	*oculi*
Acholeplasma	*axanthum*
Acholeplasma	*modicum*
Achromobacter	*xylosoxidans A*
Achromobacter	*piechaudii*
Achromobacter	*xylosoxydans B*
Acidaminococcus	*fermentans A*
Acidaminococcus	*fermentans B*
Acidovorax	*anthurii*
Acidovorax	*avenae180*
Acidovorax	*avenae group*
Acinetobacter	*lwoffii140*
Acinetobacter	*johnsoni 140*
Acinetobacter	*haemolyticus 140*
Acinetobacter	*calcoaceticus B140*
Acinetobacter	*baumannii A450*
Acinetobacter	*radioresistens 450*
Acinetobacter	*calcoaceticus B450*
Acinetobacter	*johnsoni 450*
Acinetobacter	*calcoaceticus A450*
Acinetobacter	*baumannii 140*
Acinetobacter	*anitratum140*
Acinetobacter	*calcoaceticusA140*
Acinetobacter	*junii140*
Acinetobacter	*radioresistens140*
Acinetobacter	*iwoffii 450*
Acinetobacter	*haemolyticus*
Acinetobacter	*junii 450*
Acinetobacter	*anitratum 450*
Acinetobacter	*baumannii B450*
Anaplasma	*ovis*
Anaplasma	*marginale centrale*
Anaerococcus	*prevotii B*
Anaerococcus	*vaginalis*
Anaerorhabdus	*furcosus*
Arcanobacterium	*phocae*
Arcanobacterium	*pyogenes*

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

Genus	Species Epithet
Aerococcus	*viridans¡¡ subsp. Homari*
Aerococcus	*urinae*
Aerococcus	*urinaehominis*
Aeromonas	*hydrohylla450*
Aeromonas	*punctata 450*
Aeromonas	*punctata 130*
Aeromonas	*caviae group 130*
Aeromonas	*hydrohylla group 130*
Aeromonas	*veronii group 130*
Aeromonas	*salmonicida450*
Aeromonas	*veronii 450*
Aeromonas	*media 450*
Aeromonas	*simiae 450*
Aeromonas	*simiae 130*
Aeromonas	*salmonicida group 130*
Aeromonas	*sobria group130*
Afipia	*broomeae group*
Afipia	*genospecies*
Afipia	*felis*
Agrobacterium	*rubi*
Agrobacterium	*tumofaciens group*
Alcaligenes	species
Alcaligenes	*faecalis*
Alkalibacterium	*olivoapovliticus*
Alloiococcus	*otitis*
Alloiococcus	spp.
Allomonas	*enterica*
Anaerobiospirillum	*thomasii*
Anaerobiospirillum	*succiniciproducens*
Anaerococcus	*lactolyticus*
Anaerococcus	*prevotii A*
Anaerococcus	*tetradius*
Anaerococcus	*hydrogenalis*
Anaerococcus	*octavius*
Anaplasma	*phagocytophila*
Anaplasma	*bovis*
Brevibacterium	*linens*
Brenneria	*nigrifluens*
Brevibacillus	*agri*
Brevibacillus	*brevis*
Brevibacillus	*laterosporus*
Brevibacterium	*mcbrellneri*
Brevibacterium	*otitidis*

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	*thetaiotaomicron 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	*halobacteroide*
Caulobacter	*intermedius*
Cedecea	*davisae-neteri*
Centipeda	*periodontii*
Chlamydia	*trachomatis*
Chlamydia	*suis*
Chlamydophila	*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	*parkerituricatae*
Borrelia	*lonestari*
Borrelia	*tanukii*
Borrelia	*afzelii japonica*
Borrelia	*burgdorfii valaisiana*
Borrelia	*duttonii persica*
Borrelia	*recurrentis*
Borrelia	*sinicaandersonii*
Brachyspira	*hyodysenteriae group*
Brachyspira	spp.
Brachyspira	*aalborgi*
Brachyspira	*pilosicoli*
Brenneria	*alni*
Brenneria	*rubrifaciens*
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	*vietnumensis 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
Chlamydophila	*pneumoniae*
Chlamydophila	*abortus*
Chlamydophila	*pecorum*
Chlamydophila	*psittasi group*
Chromobacterium	*violaceum*
Chryseobacterium	*meningosepticum1(450)*
Chryseobacterium	*balustinum group*
Chryseobacterium	*leum-indologenes*
Chryseobacterium	*meningosepticum group*
Chryseobacterium	*proteolyticum(450)*
Chryseobacterium	*meningosepticum2(450)*
Chryseobacterium	*gleum*
Chryseobacterium	*indoltheticum group(450)*
Chryseobacterium	*proteolyticum*
Chryseobacterium	*scophthalmum(450)*
Chryseomonas	*luteola*
Citrobacter	*amalonaticus*
Citrobacter	*diversus*
Citrobacter	*freundii*
Citrobacter	*werkmanii*
Citrobacter	*braakii*
Citrobacter	*farmeri*
Citrobacter	*sedlakii*
Corynebacterium	*bovis*
Corynebacterium	*cystitidis*
Corynebacterium	*glutamicum*
Corynebacterium	*imitans*
Corynebacterium	*kutscheri*
Corynebacterium	*mastitidis*
Corynebacterium	*minutissimum*
Corynebacterium	*pilosum*
Corynebacterium	*pseudotuberculosis*
Corynebacterium	*seminale*
Corynebacterium	*ulcerans*
Cowdria	*ruminantium 1*
Cowdria	*ruminantium 2*
Coxiella	*burnetii*
Curtobacterium	*herbarum*
Curtobacterium	*flaccumfaciens*
Curtobacterium	*psychrophilum*
Cytophaga	*aurantiaca*
Cytophaga	*hutchinsonii*
Cytophaga	*johnsonae*
Delftia	*acidovorans*
Dermatophilus	*chelonae*
Dermatophilus	*congolensis*

Genus	Species Epithet
Clostridium	*tertium*
Clostridium	*absonum*
Clostridium	*baratii*
Clostridium	*botulinum A/F*
Clostridium	*botulinum B3*
Clostridium	*botulinum D*
Clostridium	*botulinum G*
Clostridium	*cadaveris*
Clostridium	*chauvoei*
Clostridium	*colinum*
Clostridium	*disporicum*
Clostridium	*glycolicum*
Clostridium	*histolyticum*
Clostridium	*innocuum*
Clostridium	*malenominatum*
Clostridium	*novyi B*
Clostridium	*paraputrificum*
Clostridium	*piliforme*
Clostridium	*putrifaciens*
Clostridium	*ramosum*
Clostridium	*sordellii*
Clostridium	*sporogenes A*
Clostridium	*subterminale A*
Eikenella	*corrodens*
Empedobacter	*brevis*
Enterobacter	*cloacae group*
Enterobacter	*aerogenes 450*
Enterobacter	*intermedius450*
Enterobacter	*gergoviae group*
Enterobacter	*sakazaki450*
Enterobacter	*pyrinus450*
Enterobacter	*aerogenes group*
Enterobacter	*cancerogenus450*
Enterobacter	*cloacae 450*
Enterobacter	*gergoviae 450*
Enterobacter	*nimipressuralis 450*
Enterobacter	*dissolvens450*
Enterobacter	*intermedius*
Enterococcus	*avium*
Enterococcus	*faecalis*
Enterococcus	*faecium2 durans*
Enterococcus	*haemoperoxidus*
Enterococcus	*moraviensis*
Enterococcus	*munditii*
Enterococcus	*phoeniculicola*
Enterococcus	*raffinosus*

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

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

Genus	Species Epithet
Enterococcus	saccharolyticus
Enterococcus	dispar
Enterococcus	faecium1
Enterococcus	flavescens gallinarum
Enterococcus	hirae
Enterococcus	pallens
Enterococcus	pseudoavium
Enterococcus	rottae
Enterococcus	solitarius
Eperythrozoon	coccoides
Eperythrozoon	spp.
Eperythrozoon	ovis
Eperythrozoon	wenyonii
Eperythrozoon	suis
Erwinia	amylovora
Granulicatella	adiacens group
Granulicatella	elegans
Granulicatella	balaenopterae
Haemobartonella	felis
Haemobartonella	canis
Haemophilus	somnus
Haemobartonella	muris
Haemophilus	segnis
Haemophilus	parasuisB
Haemophilus	paragallinarum
Haemophilus	influenzaeB
Haemophilus	aegypticus
Haemophilus	aphrophilus
Haemophilus	haemoglobinophilus
Haemophilus	parahemolyticus
Haemophilus	paraphrohaemolyticus
Haemophilus	piscium
Haemophilus	parasuisA
Haemophilus	paracuniculus
Haemophilus	influenzaeA
Haemophilus	actinomycetecomitans
Haemophilus	ducreyi
Haemophilus	haemolyticus
Haemophilus	parainfluenzae
Haemophilus	paraphrophilus
Hafnia	alvei
Helcococcus	kunzii
Helicobacter	bizzozeronii group
Helicobacter	cetorum
Helicobacter	cinaedi
Helicobacter	hepaticus

Genus	Species Epithet
Eubacterium	sulci
Ewingella	americana
Facklamia	hominis
Facklamia	sourekii
Faecalibacterium	prausnitzii A
Faecalibacterium	prausnitzii B
Filifactor	alocis A
Filifactor	villosus
Filifactor	alocis B
Finegoldia	magna A
Finegoldia	magna¡¡B
Flavimonas	oryzihabitans
Flavobacterium	columnaris
Flavobacterium	mizutaii
Flavobacterium	psychrophilum
Helicobacter	felis
Herbaspirillum	rubrisubalbicans
Herbaspirillum	seropedicae
Johnsonella	ignava
Jonesia	denitrificans
Kingella	denitrificans
Kingella	oralis
Kingella	kingae
Klebsiella	pneumonia group
Klebsiella	oxytoca group
Kluyvera	cryocrescens
Kluyvera	ascorbata
Kluyvera	georgiana
Lactobacillus	acidophilus
Lactobacillus	buchneri
Lactobacillus	delbruekii group
Lactobacillus	jensenii
Lactobacillus	plantarum
Lactobacillus	niger
Lactobacillus	brevis
Lactobacillus	casei
Lactobacillus	gasseri
Lactobacillus	rhamnosus
Lactococcus	garviae
Lactococcus	plantarum
Lactococcus	lactis
Lactococcus	raffinolactis
Lawsonia	intracellularis
Leclercia	adecarboxylata
Legionella	adelaidensis
Legionella	adelaidensis450

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	*cincinnatiensis*DN67
Legionella	*donaldsonii*
Legionella	*donaldsonii450*
Legionella	*dumofii*
Legionella	*dumofii450*
Legionella	*erythra*
Legionella	*erythra450*
Legionella	*fairfieldensis*
Legionella	*fairfieldensis450*
Legionella	*fairfieldensisGTC698*
Legionella	*feeleii*
Legionella	*feeleii 450*
Legionella	*feeleiiDN52*
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	*bozemanae group*
Legionella	*bozemanae450*
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	*weilii*
Leucothrix	*mucor*
Listeria	*innocula*
Listeria	*monocytogenes*
Listeria	*welshimeri*
Listeria	*grayi*
Listeria	*ivanovii*
Listeria	*seeligei*
Listonella	*anguillarum*
Mannheimia	*haemolytica*
Mannheimia	*granulomatis*
Marinospirillum	*megaterium*
Megasphaera	*elsdenii A*
Megasphaera	*micronuciformis*
Megasphaera	*cerevisiae*
Mycoplasma	*canis*

Genus	Species Epithet
Melissococcus	*plutonius*
Microbacterium	*schleiferi*
Microbacterium	*aurum*
Micromonas	*micros¡¡A*
Mitsuokella	*multacida*
Mitsuokella	*jalaludinii*
Mobiluncus	*mulieris*
Mobiluncus	*curtisii*
Moraxella	*bovis*
Moraxella	*ovis*
Moraxella	*osloensis*
Moraxella	*lacunata group*
Moraxella	*atlantae*
Moraxella	*catarrhalis*
Moraxella	*nonliquefaciens*
Moraxella	*caviae*
Mycobacterium	*abscessus*
Mycobacterium	*avium*
Mycobacterium	*branderi*
Mycobacterium	*celatum*
Mycobacterium	*chelonae*
Mycobacterium	*farcinogenes*
Mycobacterium	*flavescens*
Mycobacterium	*fortuitum*
Mycobacterium	*gastrii*
Mycobacterium	*genavense*
Mycobacterium	*gordonae*
Mycobacterium	*haemophilum*
Mycobacterium	*interjectum*
Mycobacterium	*intermedium*
Mycobacterium	*intracellulare*
Mycobacterium	*intracellulare A*
Mycobacterium	*intracellulare B*
Mycobacterium	*kansasii*
Mycobacterium	*kansasii group*
Mycobacterium	*leprae*
Mycobacterium	*lepraemurium*
Nocardia	*nova*
Nocardia	*otitidiscaviarum*
Nocardia	*seriolae*
Nocardiopsis	*dassonvillei*
Obesumbacterium	*proteus*
Ochrobactrum	*anthropi¡¡B*
Ochrobactrum	*intermedium*
Ochrobactrum	*anthropi¡¡A*
Ochrobactrum	*anthropi¡¡C*

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

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

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

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

Genus	Species Epithet
Pseudobutyrivibrio	*ruminis*
Pseudomonas	*putida130*
Pseudomonas	*plecoglossicida130*
Pseudomonas	*cichorii 130*
Pseudomonas	*viridiflava130*
Pseudomonas	*mendocina130*
Pseudomonas	*plecoglossicida180*
Pseudomonas	*viridiflava180*
Pseudomonas	*stutzeri B180*
Pseudomonas	*alcaligenes180*
Pseudomonas	*cichorii 180*
Pseudomonas	*stutzeri A180*
Pseudomonas	*anguilliseptica 130*
Pseudomonas	*stutzeri A130*
Pseudomonas	*alcaligenes130*
Pseudomonas	*syringe130*
Pseudomonas	*marginalis130*
Pseudomonas	*aeruginosa 130*
Pseudomonas	*fluorescens 130*
Pseudomonas	*mendocina180*
Pseudomonas	*marginalis180*
Pseudomonas	*fluorescens 180*
Pseudomonas	*syringe180*
Pseudomonas	*putida180*
Pseudomonas	*anguilliseptica 180*
Pseudomonas	*aeruginosa 180*
Pseudoramibacter	*alactolyticus*
Psychrobacter	*immobilis*
Psychrobacter	*glacialis*
Staphylococcus	*simulans*
Staphylococcus	*xylosus*
Stenotrophomonas	*maltophilia group*
Stenotrophomonas	*rhizophila*
Stenotrophomonas	*nitritireducens*
Streptobacillus	*moniliformis*
Streptococcus	*anginosus*
Streptococcus	*canis*
Streptococcus	*cristatus*
Streptococcus	*dysgalactiae*
Streptococcus	*equinus*
Streptococcus	*gordonii*
Streptococcus	*iniae*
Streptococcus	*macacae*
Streptococcus	*mutans*
Streptococcus	*parasangunis*
Streptococcus	*phocae*

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

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

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