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SHORT COMMUNICATION

THE CHOLERA-RED REACTION OF VIBRIO PARAHAEMOLYTICUS[1]

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In 1906, Paladino-Balandine stated in his report on the mechanism of the cholera-red reaction that indole and nitrite must be formed by the test organism in certain proportions and if either is in excess, the reaction is negative. This was also observed by GALLUT (1948), who reported that the color of the cholera-red reaction varied from orange to purple as the ratio of the concentrations of indole and nitrite was changed. BEAM (1959) studied the effect of excess nitrite on the cholera-red reaction and recommended that the concentration of nitrate originally present in the medium should not be greater than 0.001 per cent. During a taxonomic study of *Vibrio parahaemolyticus* (FUJINO *et al.*, 1965), we noticed that the cholera-red reaction of this bacteria was not consistantly positive. It was thought that this might be because the concentrations of tryptophan and nitrate in the media were not constant and consequently that the indole and nitrite formed were not in adequate proportions for a positive reaction.

Since the cholera-red reaction is one of the most important characters for defining the genus *Vibrio* (DAVIS and PARK, 1962), we studied the effect of the concentrations of tryptophan and nitrate in the media on the reaction.

The cholera-red reaction of one hundred and twenty strains of *Vibrio parahaemolyticus* were performed by adding concentrated sulfuric acid to 24-hour peptone water cultures of the organisms. The peptone water media containing 3% NaCl were supplemented with 0.001% $NaNO_3$ according to Beam's method (1959). (Table 1). Only two of the one hundred and twenty strains tested showed a weak positive reaction.

TABLE 1 *The cholera-red reaction of Vibrio parahaemolyticus*

	++	+	−	Total
Number of strain	0	2	118	120
Ratio in %	0	1.7	98.3	100

Peptone water containing 3% NaCl and 0.001% $NaNO_3$ was inoculated with test organisms and after incubation at 37°C for 24 hours the reactions were tested by adding a few drops of concentrated sulfuric acid. ++, + and − indicate strongly positive, weakly positive and negative reactions, respectively.

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TABLE 2 *Effect of the concentrations of tryptophan and sodium nitrate in the medium on the cholera-red reaction*

Concentration of $NaNO_3$ (M)		10^{-3}			5×10^{-4}			10^{-4}			0
Concentration of tryptophan (M)		10^{-2}	2×10^{-3}	10^{-3}	10^{-2}	2×10^{-3}	10^{-3}	10^{-2}	2×10^{-3}	10^{-3}	0
Strain	Culture time										
0- 1	24	++	+	+	+++	+	+	−	−	−	−
	72	+++	++	+	+++	+++	++	−	−	−	−
	120	+++	+++	++	+++	+++	++	−	−	−	−
0- 2	24	++	+	+	+++	−	−	−	−	−	−
	72	+++	+++	−	−	−	−	−	−	−	−
	120	+++	++	−	−	−	−	−	−	−	−
0- 3	24	++	++	++	−	−	−	−	−	−	−
	72	++	−	−	−	−	−	−	−	−	−
	120	+++	++	−	−	−	−	−	−	−	−
0- 4	24	++	+	−	−	−	−	−	−	−	−
	72	++	−	−	−	−	−	−	−	−	−
	120	+++	−	−	−	−	−	−	−	−	−
0- 5	24	++	+	++	++	−	−	−	−	−	−
	72	+++	−	−	−	−	−	−	−	−	−
	120	+++	−	−	−	−	−	−	−	−	−
0- 6	24	++	+	++	−	−	−	−	−	−	−
	72	+++	++	−	−	−	−	−	−	−	−
	120	+++	++	++	++	−	−	−	−	−	−
0- 7	24	+++	−	−	−	−	−	−	−	−	−
	72	++	−	−	−	−	−	−	−	−	−
	120	+++	−	−	−	−	−	−	−	−	−
0- 8	24	++	−	−	+++	−	−	−	−	−	−
	72	−	−	−	−	−	−	−	−	−	−
	120	+++	−	−	−	−	−	−	−	−	−
0- 9	24	+	−	−	+	−	−	−	−	−	−
	72	+++	−	−	−	−	−	−	−	−	−
	120	++	−	−	−	−	−	−	−	−	−
0-10	24	+	−	−	−	−	−	−	−	−	−
	72	+++	−	−	−	−	−	−	−	−	−
	120	+++	−	−	−	−	−	−	−	−	−

Media containing 1% Difco casamino acid, 3% NaCl and various concentrations of tryptophan and $NaNO_3$ were inoculated with test organisms and the reactions were tested after incubation at 37°C for 24, 72 and 120 hours. +++, ++ and + indicate strongly positive reactions. + and − indicate weakly positive and negative reactions, respectively.

Table 2 shows the effect on the reaction of strains of *Vibrio parahaemolyticus* of various concentrations of tryptophan and sodium nitrate. The media used contained 1% Difco casamino acid, 3% NaCl and varying concentrations of tryptophan and sodium nitrate. The pH was adjusted to 7.0. The reactions were performed by adding a few drops of concentrated sulfuric acid to 24, 72 and 120 hour cultures. The cholera-red reaction was strongly positive in the medium containing 10^{-2} M tryptophan and 10^{-3} M sodium nitrate. At lower concentrations of tryptophan and sodium nitrate, the reactions were generally negative. Kovacs indole test and the test for nitrite were positive with media which showed a negative cholera-red reaction.

TABLE 3 *The cholera-red reaction of Vibrio parahaemolyticus*

Culture time in hours	24	72	120
Number of strains giving positive reaction	101	61	51
Number of strains giving negative reaction	5	45	55
Percentage of positive strains	95.3	57.5	48.1

Media containing 1% Difco casamino acid, 3% NaCl, 10^{-2} M tryptophan and 10^{-3} M sodium nitrate were inoculated with test organisms and the reactions were tested after incubation at 37°C for 24, 72 and 120 hours.

Using media containing 10^{-2} M tryptophan and 10^{-3} M sodium nitrate, the cholera-red reaction was performed with 24, 72 and 120 hour cultures. (Table 3).

One hundred and one of the one hundred and six strains of *Vibrio parahaemolyticus* tested gave positive reactions in a 24-hour culture, showing colors varying from orange through pink and dark red to purple. Of the five strains with negative cholera-red reactions in the 24-hour culture, only one showed a positive reaction in the 72-hour culture. The number of negative strains increased as the culture was aged. All other *Vibrios* in media containing 10^{-2} M tryptophan and 10^{-3} M sodium nitrate showed strongly positive reactions. The *Vibrios* examined were as follows; *Vibrio cholerae* Ogawa, Inaba and HIKOJIMA, *Vibrio* species representing Gardner Venkatraman's group II, III, IV, V and VI, *Vibrio* species representing Heiberg's biological groups and three strains of *El Tor Vibrio*. As control *Aeromonas hydrophila and Aeromonas formicans* and five strains of *Escherichia coli* were examined, and all of them showed a negative reaction.

From these results we recommend that the cholera-red reaction of *Vibrio parahaemolyticus* should be performed using the 24-hour culture incubated in the medium supplemented with 10^{-2} M tryptophan and 10^{-3} M sodium nitrate before inoculation.

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