Correlations between Heterotrophic Bacterial Populations and Water Quality Variables in Stream Waters

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Heterotrophic bacterial populations in a total of 22 stream waters polluted in different degrees were investigated by analyzing correlations with water quality variables. Colony-forming units on polypeptone-yeast extract-glucose agar plates correlated significantly with the concentrations of suspended solids, chemical oxygen demand and total organic carbon. Four hundred and thirty-four strains isolated from 22 stream waters were classified into 60 types according to the characteristics of colony color, motility, Gram stain, catalase production, oxidase production and oxidation-fermentation (O-F) test. In relationships between the percentage of the 60 types of isolates and water quality variables, YA1, YI, and NPG type positively, and NPK type negatively correlated significantly with many of the variables. All of these bacterial types were Gram negative bacteria; the YA1 type was a yellow pigmented, non-motile, catalase-positive, oxidase-negative and O-F test-negative bacterium (*Flavobacterium* sp.); the YI type was a yellow pigmented, motile, catalase-positive, oxidase-negative and O-F test-negative bacterium (*Pseudomonas* sp.); the NPG type was a non-pigmented, motile, catalase- and oxidase-positive, and O-F test-oxidative bacterium (*Pseudomonas* sp.); and the NPK type, a non-pigmented, motile, catalase-negative, oxidase-positive and O-F test-negative bacterium (unknown sp.). These results suggest that YA1, YI and NPG types may be present predominantly in polluted waters and the NPK type in non-polluted waters, that is, that they may be good candidates as pollution indicators. The percentages of total YA1, YI and NPG types correlated strongly with most of the water quality variables.

Keywords — heterotrophic bacteria; *Pseudomonas*; *Flavobacterium*; yellow pigmented bacteria; stream water; water quality variable; pollution index bacteria

Introduction

Coliform bacteria in aquatic environments are generally used as indicators of human and animal fecal pollution, but investigations of other heterotrophic bacterial species as pollution indicators are limited.

There have been several studies in non-river waters on bacterial indicator organisms other than coliform. Simidu *et al.*¹⁾ and Ishida and Kadota²⁾ found that *Acinetobacter* spp. were dominant in eutrophic sea water. Guthrie *et al.*³⁾ hypothesized, from a limnological

study, that when chromogenic bacteria constituted 25% or more of the aerobic, heterotrophic bacterial populations in an aquatic environment, it indicated unpolluted conditions. In contrast, in drinking waters, Reasoner *et al.*⁴⁾ recently suggested that pigmented bacteria can be used as a marker for monitoring change in water quality, because high percentages of these bacteria were found in the absence of a free chlorine residual.

Many microbiological studies⁵⁻¹⁰⁾ have been reported in river waters, but none has been conducted to evaluate heterotrophic

bacteria as an indicator of pollution. Most of the literature has addressed bacterial populations from ecological perspectives, including studies on the correlation of physical and chemical factors with the dynamics of bacterial populations.⁶⁾ Also, most studies have utilized only a few sampling sites. We examined many sites, both clean and polluted, which receive differing quantities of domestic and industrial effluents, to determine correlations of bacterial populations with pollution.

The purpose of this study was to investigate relationships between bacterial populations and water quality variables such as concentrations of organic and inorganic contaminants in waters.

Bacterial isolates are identified generally using identification manuals, such as Cowan's manual^{11a)} and Bergey's Manual.^{11b)} In studies on bacterial flora in aquatic environments, it is difficult to identify isolates, because there is no absolute manual on environmental bacteria. Identification of the isolates, therefore, has generally been made to genus levels but

not to species levels. However, the genus *Pseudomonas*, for instance, contains both positive and negative bacteria in oxidase test, and both yellow pigmented and non-pigmented bacteria in colony color.¹¹⁾ We thus considered that analysis of bacterial flora conducted after identification of genus level alone could not be expected to provide good results. In this study, isolates were classified without referring to the identification manuals,¹¹⁾ but rather on the basis of colony color, in addition to motility, Gram stain, oxidase and catalase reaction and oxidation-fermentation (O-F) test (glucose); then, we also attempted to identify the isolates to genus levels to compare with other studies on bacterial flora.

Materials and Methods

Study Site and Sample Collection — Ten streams receiving differing degrees of domestic and sewage effluent in Fukuoka, Japan were examined (Fig. 1). S1, S2, S4 and S10 were at four tributaries of the Tikugo River which flows into the Ariake Sea. S1 and S4 are located in Kurume

Fig. 1. Location of Sampling Sites in Fukuoka, Japan

City. S3, S5 and S7 were at three streams in Omuta City which empty into the Ariake Sea, and S6, S8 and S9 were at two streams which drain into the Suo Sea.

Water samples were collected 1–4 times at each site from June 1986 through February 1987. A total of 22 samples were taken from the 10 sampling sites. Surface waters were collected in well-rinsed 10 l polyethylene buckets, and transferred to sterile 100 ml polyethylene bottles for bacteriological examination and 2 l polyethylene bottles for water quality measurements.

Water Quality Analysis — Water temperature and dissolved oxygen (DO) were measured in the field using a laboratory thermometer and a portable DO meter (Iizima Seimitu Kougyou, F 101). Biochemical oxygen demand (BOD), pH, suspended solids (SS), chemical oxygen demand (COD), ammonium-nitrogen ($\text{NH}_4\text{-N}$) and phosphate-phosphorus ($\text{PO}_4\text{-P}$) were measured by the method of the Japanese Industrial Standard Committee.¹²⁾ Nitrate-nitrogen ($\text{NO}_3\text{-N}$) and nitrite-nitrogen ($\text{NO}_2\text{-N}$) were determined on a Technicon II Auto analyzer. Methylene blue active substances (MBAS) were measured using sodium *n*-dodecylbenzene sulfonate as the standard.¹³⁾ Total organic carbon (TOC) was analyzed using a Shimadzu TOC-10B. Total nitrogen (T-N) and total phosphorus (T-P) were measured by spectrophotometric methods.¹⁴⁾

Methods for Culturing Heterotrophic Bacteria — A water sample was homogenized with a sterile Potter-Elvehjem homogenizer and then a five-fold dilution series was prepared aseptically. Subsamples (0.1 ml) of each dilution were spread on PYG agar plates in triplicate. The plate contained 1.0 g of polypeptone (Daigoeiyo), 0.5 g of yeast extract (Difco), 0.2 g of glucose (Wako) and 15 g of agar powder (Wako) in 1000 ml of distilled water, pH 7.2. The plates were incubated at 20°C in the dark and colony forming units (CFUs) were counted after 2 weeks. The growth conditions for maximum CFU were confirmed in the previous work.¹⁵⁾

Isolation and Physiological Tests of Bacteria — One of the agar plates forming about 30

–50 colonies per water sample was selected. All of the colonies were picked from this plate and purified by repeated streaking on PYG agar plates until pure colonies were obtained. Finally, 20 strains were selected at random from each sample. However, six strains of 20 isolates at S9 in July 1986 failed to grow on a subculture. Physiological tests of a total of 434 strains were made according to the methods of Cowan;^{11a)} the tests were pigmentation of colonies, Gram stain, motility by hanging drop method, oxidase production, catalase production and O-F test. For the O-F test, a medium containing 1 g of polypeptone, 0.5 g of yeast extract, 1 g of glucose, 100 ml of 0.04% bromothymol blue, and 2 g of agar in 1000 ml of distilled water was used, because many isolates did not grow in the original medium.¹⁶⁾

Classification of Bacteria — The 434 isolates were classified into bacterial types on the basis of colony color on PYG medium, Gram stain, motility, oxidase and catalase reaction, and O-F test, and then the bacterial types were identified to family or genus levels by the identification manuals¹¹⁾ to compare with other studies on aquatic bacteria.

Data Analysis — Data were analyzed by correlation analysis. CFU values were taken after log 10 transformation.

Results

Analysis of Water Quality

The results are shown in Table I, in which pH and water temperature are omitted, since they are not significant in this study. The concentrations of BOD measured in the 22 stream samples ranged from 0.4 to 7.6 mg/l, with an average of 2.55 mg/l. Pollution levels of the 22 samples were divided into 3 groups using BOD concentrations based on the water quality standard on Living Environmental Preservation by Water Pollution Control Law.¹⁷⁾ That is, relatively clean waters ($\text{BOD} < 2 \text{ mg/l}$) were reflected in 13 water samples, moderately polluted water (2–5 mg/l) characterized 5 samples and highly polluted waters ($> 5 \text{ mg/l}$) were found in 4

TABLE I. Water Quality of Stream Waters (mg/l)

Site ^{a)}	Date	DO	SS	BOD	COD	TOC	MBAS	T-N	NH ₄ -N	NO ₂ -N	NO ₃ -N	T-P	PO ₄ -P
S1	Sep '86	6.7	39	5.4	9.4	5.5	0.15	6.5	5.6	0.14	0.25	0.34	0.15
S1	Nov '86	8.9	9	7.6	10	7.0	0.36	8.7	5.2	0.37	3.1	0.79	0.36
S2	Jun '86	7.8	30	3.7	6.8	4.1	0.08	4.9	0.26	0.00	0.70	0.05	0.03
S2	Sep '86	6.4	41	2.1	8.4	4.5	0.12	2.9	0.22	0.08	1.6	0.29	0.15
S2	Nov '86	8.0	6	2.4	6.8	3.9	0.10	2.7	0.19	0.06	1.6	0.23	0.19
S2	Feb '87	8.6	2	5.5	8.8	6.4	0.20	5.4	0.70	0.08	3.5	0.24	0.24
S3	Jul '86	5.3	24	1.9	6.4	5.0	0.05	3.3	0.67	0.12	1.8	0.19	0.11
S3	Jan '87	5.3	2	5.6	6.2	4.1	0.34	2.8	1.5	0.02	0.39	0.31	0.20
S4	Jun '86	9.3	6	4.3	3.9	3.8	0.38	2.3	1.7	0.00	0.60	0.16	0.13
S5	Jul '86	6.8	10	3.3	4.6	4.9	0.42	2.1	0.27	0.06	1.7	0.08	0.05
S6	Jul '86	7.4	19	1.7	3.2	3.1	0.03	1.2	0.03	0.00	0.56	0.07	0.04
S6	Oct '86	9.4	13	1.3	3.8	2.4	0.09	0.9	0.13	0.01	0.46	0.07	0.03
S7	Jul '86	6.8	15	1.0	3.2	3.0	0.01	2.8	0.02	0.01	2.4	0.09	0.05
S7	Jan '87	9.2	0	1.5	3.0	2.4	0.07	2.2	0.06	0.01	2.1	0.04	0.02
S8	Jul '86	7.5	6	1.5	2.4	3.0	0.02	1.1	0.02	0.00	0.63	0.04	0.02
S8	Oct '86	9.5	10	1.0	2.7	2.2	0.01	0.9	0.07	0.00	0.57	0.04	0.01
S9	Jul '86	7.9	1	1.0	2.6	3.2	0.00	1.0	0.04	0.00	0.46	0.05	0.03
S9	Oct '86	9.5	0	0.5	1.8	1.9	0.00	0.3	0.02	0.00	0.21	0.02	0.01
S10	Jul '86	8.9	17	0.9	1.6	2.4	0.00	1.0	0.31	0.00	0.66	0.03	0.01
S10	Sep '86	7.8	8	0.4	1.7	2.2	0.00	1.7	0.00	0.05	0.86	0.07	0.02
S10	Nov '86	10.8	0	0.5	2.1	1.7	0.00	0.4	0.00	0.00	0.38	0.04	0.03
S10	Feb '87	11.1	0	0.7	1.7	1.3	0.00	0.7	0.07	0.00	0.48	0.02	0.02

a) See Fig. 1.

samples. Consequently, clean waters (BOD < 2 mg/l, 13 samples) were somewhat more common than polluted waters (BOD > 2 mg/l, 9 samples). On the other hand, the 10 sampling sites could be approximately classified by their BOD concentrations: the polluted sites were S1–S5, and the non-polluted sites, S6–S10. COD, TOC, MBAS, T-N and T-P increased in concentrations with BOD ($r > 0.807$). The components of T-N, that is, NH₄-N, NO₃-N and NO₂-N, behaved differently from organic pollutants; in particular, NO₃-N concentrations correlated poorly with BOD ($r = 0.422$) and COD ($r = 0.520$). NH₄-N and NO₂-N concentrations at S1 showed

extremely high values, whereas those at other sampling sites were generally low. Of all sampling sites, S1 was proved to be the most highly polluted water based on data showing the highest values of many items except for MBAS and NO₃-N. The values in November were higher than those in September at S1. The highest MBAS was obtained at S5, and that of NO₃-N in February at S2. The cleanest waters in this study were at S9 and S10.

Bacterial Number

The number of heterotrophic bacteria on PYG agar plates in the 22 samples ranged from 10³ to 10⁶ CFU/ml, the maximum being 7.6 × 10⁶ CFU/ml in June at S4, and minimum

TABLE II. Classification of Isolates in Stream Waters

Type	Gram stain	Motility	Catalase	Oxidase	O-F test	No. of strains belonging to each colony color					
						NP ^{a)}	Yellow	Pink	OR ^{b)}	PP ^{c)}	Red
A1	—	—	+	—	—	19	40				
A2	—	—	+	—	O	4	5				
B1	—	+	+	+	F	13	6	2		2	
B2	—	—	+	+	F	3	1	1			
C	—	+	+	+	—	58	7	4			1
D	—	+	+	—	F	14	19	1			
E	—	—	+	—	F	6	26	1			
F	—	—	+	+	—	10					
G	—	+	+	+	O	21	2				
H	—	+	+	—	O	9	6	2			
I	—	+	+	—	—	33	25	1			
J	—	+	—	—	—	10	2	1			
K	—	+	—	+	—	24	1	1			
L	—	—	—	—	—	12	1				
M	—	—	—	+	O			1			
N	—	+	—	+	F	2					
O	—	+	—	+	O	7					
P	—	+	—	—	F		1				
Q	—	—	—	+	—	4					
R	—	+	—	—	O		1	1			
S	—	—	—	—	F	1	1				
T	—	—	—	—	O						1
U	+	—	+	—	—	5	1				
W	+	+	+	—	—	5	1		1		
X	+	+	—	—	—	1					
Y	+	—	+	—	F	1	1				
Y1	+	+	+	—	F		1				
Z	+	—	+	—	O	1	2				

Symbols: —, negative; +, positive; O, oxidation; F, fermentation.

a) NP: non-pigmentation; b) OR: orange; c) PP: pale-pink.

non-pigmented bacteria of A1 and A2 types, *Acinetobacter*; yellow pigmented bacteria of those types, *Flavobacterium*; B1 and B2 types, *Aeromonas-Vibrio*; C,G,I and H types, *Pseudomonas*; D and E types, Enterobacteriaceae; F type, *Flavobacterium*; U type, *Bacillus*; and other types, unknown.

being 4.4×10^3 CFU/ml in November at S10. Regarding the relation of water quality variables to CFUs, after log transformation several significant positive correlation coefficients were obtained: $r=0.546$, $p<0.01$ for SS; $r=0.498$, $p<0.02$ for COD; $r=0.479$, $p<0.05$ for TOC; and $r=0.396$, $p<0.1$ for MBAS. These suggest that the overall water quality of stream waters cannot be explained only by bacterial number, because the CFUs were correlated with some of the variables.

Classification of Isolates

Bacteria isolated from stream waters were classified according to colony color on

PYG medium and the results of Gram stain, motility, catalase and oxidase test, and O-F test (5 physiological tests). Table II shows the number of strains belonging to each type (A1–Z). The bacteria were divided into 28 types by the 5 physiological tests and then, based on colony color, into 60 types. In all types, non-pigmented bacteria of C type were the most dominant (13.4%). Other predominant types were: yellow bacteria of A1 type (9.2%), non-pigmented bacteria of I type (7.6%), yellow bacteria of E type (6.0%), yellow bacteria of I type (5.7%) and non-pigmented bacteria of K type (5.5%).

The results of tentative identification of each type by Bergey's Manual^{11b)} are shown in the footnote in Table II.

Relation of Bacterial Type Percentage to Indices of Water Quality

Before analyzing this relationship, correlation coefficients between ratios of pigmented bacteria or positive percentages of 5 physiological tests and indices of water quality in each sample were calculated, and significant correlations are shown in Table III. The results suggest that non-motile or catalase positive or yellow colony-forming bacteria may serve as pollutant indices of aquatic bacteria, though the correlation coefficients were low. The positive percentages of these bacterial tests of isolates in 22 stream waters are shown in Fig. 2. The average percentages of these three tests in all samples were: non-motile strains, 33%; catalase positive strains, 34%; and yellow pigmented strains, 82%. Average percentages of non-motile (39%), catalase positive (91%) and yellow pigmented strains (38%) of polluted sites (S1—S5, refer to Table I) were higher than that of non-polluted sites (S6—S10). But a significant difference was observed with the catalase test ($p < 0.05$). The highest values of non-motile

TABLE III. Correlation Coefficients between Water Quality Variables and Positive Percentage of Bacterial Test of Isolates in Each Stream Water ($n=22$)

Variable	Motility	Catalase	Yellow colony
DO	NS ^{a)}	NS	NS
SS	NS	NS	-0.370*
BOD	-0.363*	0.473**	0.366*
COD	NS	NS	NS
TOC	NS	0.370*	NS
MBAS	NS	0.525***	NS
T-N	-0.444**	0.411**	0.402*
NH ₄ -N	NS	NS	NS
NO ₂ -N	-0.417*	NS	0.608†
NO ₃ -N	-0.451**	0.392*	NS
T-P	-0.393*	0.417*	NS
PO ₄ -P	-0.442**	0.405*	0.419*

a) NS: not significant.

*, **, ***, †: significance of correlation coefficient at $p < 0.1$, 0.05, 0.02 and 0.01, respectively.

and yellow pigmented strains were observed in the non-polluted water, S7 in January, but not in the polluted water.

Correlation coefficients between the percentages of the 60 bacterial types and indices of the water quality in each sample are summarized in Table IV. Significant correlation coefficients were observed for yellow pigmented bacteria of A1 type (YA1), yellow

Fig. 2. Percentage of Non-motile, Catalase Positive- and Yellow Colony Strains in Each Stream Water

Twenty strains were isolated from all water samples except that 14 strains were isolated in July at S9.

□ non-motile strains, ▨ catalase positive strains, ■ yellow pigmented strains.

TABLE IV. Correlation Coefficients between Percentage of Bacterial Types and Water Quality Variables in Each Stream Water ($n=22$)

Variable	Bacterial Type ^{a)}					
	YA1	NPG	YI	NPK	YA1+YI	YA1+YI+NPG
DO	NS	NS	NS	NS	NS	NS
SS	NS	0.544 [†]	NS	NS	NS	NS
BOD	0.504***	0.475**	NS	-0.477**	0.532***	0.711 [†]
COD	0.580 [†]	0.469**	0.414*	-0.421*	0.618 [†]	0.793 [†]
TOC	0.618 [†]	0.361*	0.423**	-0.444**	0.652 [†]	0.783 [†]
MBAS	NS	0.392*	NS	-0.496***	NS	0.410*
T-N	0.684 [†]	0.362*	0.477**	-0.453**	0.725 [†]	0.856 [†]
NH ₄ -N	NS	0.570 [†]	NS	-0.376*	0.401 [†]	0.620 [†]
NO ₂ -N	0.702 [†]	NS	0.440**	-0.395*	0.725 [†]	0.794 [†]
NO ₃ -N	0.684 [†]	NS	0.435*	-0.409*	0.708 [†]	0.624 [†]
T-P	0.697 [†]	NS	0.418*	-0.438**	0.712 [†]	0.840 [†]
PO ₄ -P	0.755 [†]	NS	0.471**	-0.413*	0.779 [†]	0.847 [†]

a) YA1 type, yellow pigmented bacteria of A1 type; NPG type, non-pigmented bacteria of G type; YI type, yellow pigmented bacteria of I type; NPK type, non-pigmented bacteria of K type (refer to Table II).

*, **, ***, †: significance of correlation coefficient at $p < 0.1$, 0.05, 0.02 and 0.01, respectively.

NS: not significant.

pigmented bacteria of I type (YI), non-pigmented bacteria of G type (NPG), and non-pigmented bacteria of K type (NPK). A poor correlation was obtained with C type which was the most dominant in this study (data not shown). For the YA1 type, there were highly significant positive correlations with many water quality variables. For NPG and YI types, positive significant correlations were also obtained with some variables, though the values were a little lower than that of YA1. By determining the relationship of the percentage of YA1+YI type to variables in each sample, this group was significantly correlated with many variables, and correlation coefficients were higher than those for each YA1 and YI type. Moreover, YA1+YI+NPG types highly correlated with most variables (Table IV). These results suggest that these three types of bacteria may be present in polluted waters. Also, the results of relations of bacterial types to water quality were better than those of CFUs to water quality. As shown in the footnotes in Table II, the YA1 type was provisionally identified as *Flavobacterium* sp., and the YI and NPG types as *Pseudomonas* sp.. These three types of bacteria constituted 86 (19.8%) of the total of

434 strains. The yellow pigmented bacteria, YA1 and YI types composed 43.4% (65 strains) of all yellow pigmented bacteria (150 strains), and non-pigmented bacteria, NPG type was 8.0% (21 strains) of all non-pigmented bacteria (263 strains), isolated in this study. These three types being pollutant indices partly satisfied the features of bacteria, that is, yellow pigmented or catalase positive or non-motile bacteria, which may be present in polluted waters cited above.

Fig. 3 shows the percentages of YA1, YI and NPG type strains in each water sample. The highest percentage was observed in November at S1 of the polluted site, and the lowest in January at S7 of the non-polluted site. Most of the highest ratios of each type were obtained in the polluted sites: that of YA1 was 40% at S1 in November; that of YI, 15% at S1 in November, at S2 in February and at S6 in July; and that of NPG type, 20% at S1 in September. The percentages of these types in polluted sites (S1—S5) exceeded 20% and those in unpolluted sites (S6—S10) were less than 20%, though there is one exception of each, at the polluted S5 in January and at the non-polluted S7 in July. A significant difference for a total of the three types was

Fig. 3. Percentage of YA1, YI and NPG Type in Each Stream Water

Twenty strains were isolated from all water samples except that 14 strains were isolated in July at S9. YA1 type, *Flavobacterium* sp.; YI type, *Pseudomonas* sp.; and NPG type, *Pseudomonas* sp.

□ NPG type, ▨ YI type, ■ YA1 type.

obtained between polluted and non-polluted sites ($p < 0.01$), and the significant level for YA1+YI was $p < 0.05$. In November at S1, 92% of all yellow bacteria (Table II) were YA1 and YI type bacteria. At the non-polluted site, S7 in January, YA1 and YI types were not present, although the yellow bacteria showed the highest ratio of all samples (Fig. 2). In October at S9 and in June at S10, YA1 and YI types were not present. From these results, the ratio of the index bacteria in polluted waters was also proved higher than that in non-polluted waters.

The NPK type showed significant negative correlations with many water quality variables, though coefficients were lower than those of the YA1+YI+NPG types (Table IV). Thus, this type may be present in non-polluted waters. The NPK type was not identifiable by the manuals.¹¹⁾

Discussion

Water quality of the 22 stream waters used in this study was determined by analysis of the relationship between bacterial populations and water quality variables.

CFUs on PYG agar plates were significantly correlated with SS. Similar results were obtained by Baker and Farr⁵⁾ and

Goulder.⁹⁾ Goulder stated that CFUs were not correlated with free-living bacteria but with particle-bound bacteria, suggesting that CFUs may therefore constitute a large proportion of particle-bound bacteria. Thus, the positive correlation between CFUs and SS indicates that the proportion of particle-bound bacteria in stream waters may be higher than that of free-living bacteria. COD and TOC were also correlated with CFUs. A similar result on COD was obtained by Nuttall¹⁰⁾ who found permanganate values, essentially the same as those of COD, to be correlated positively with CFUs. There are no reports concerning the relationship between CFUs and TOC, but Geesey and Costerton¹⁸⁾ showed TOC to be correlated significantly with direct counts of bacteria. These facts suggested that CFUs alone were an inadequate pollutant index reflecting water quality, because they correlated with few variables.

Relationships of bacterial types to water quality were better than that of CFUs to water quality. YA1 (*Flavobacterium* sp.), YI (*Pseudomonas* sp.) and NPG (*Pseudomonas* sp.) types were correlated significantly with indices of water quality, and may serve as pollutant indices. Use of yellow bacteria as the index bacteria conflicts with the hypothe-

sis of Guthrie *et al.*,³⁾ who suggested that water is clean if chromogenic bacteria accounted for 25% or more of the heterotrophic bacteria in an aquatic environment; the results of our study are, however, similar to a report on drinking waters made by Reasoner *et al.*⁴⁾ In lakes, on the other hand, Tanaka *et al.*¹⁹⁾ reported the yellow pigmented bacteria, *Flavobacterium* sp. to be dominant at all of four different sites in Lake Biwa: one oligotrophic (54%) and three eutrophic sites (10–38%). Lighthart²⁰⁾ found the surface waters of a little eutrophic lake to be dominated by yellow *Flavobacterium* (80%), and Konda and Tezuka²¹⁾ noted the percentages of non-motile *Flavobacterium* at a 2 m depth in an oligotrophic lake to be 7.1, 74.0 and 82.1%. In these studies, most pigmented bacteria were yellow bacteria, and thus the predominance of yellow bacteria is not necessarily the same in oligotrophic lake waters. Konda and Tezuka²¹⁾ described the physiological characteristics of isolated strains which were identified to genus levels, in which *Flavobacterium* sp. contained both positive and negative bacteria in oxidase test, and also both oxidative and negative bacteria in O-F test, therefore, two or more species were mixed in the genus. Isolates having the feature of the YI and NPG type do not appear in published studies on bacterial flora in river waters; however, these types resembled chromogenic and achromogenic *Pseudomonas* sp., respectively, isolated from an oligotrophic lake by Konda and Tezuka,²¹⁾ who isolated 0.6% of chromogenic *Pseudomonas* sp. and 9.5% of achromogenic *Pseudomonas* sp.. They were not predominant and each *Pseudomonas* sp. contained two or more species. Our results on pollutant index bacteria lead to the inference that YA1, YI and NPG types are similar but not identical to the strains reported by Konda and Tezuka,²¹⁾ because the strains isolated by them were from a non-polluted environment. Yellow pigmented bacteria are widely distributed in aquatic environments.

The indication or dominance of chromogenic bacteria containing yellow bacteria has been discussed by many researchers.^{3,4,19–21)} The pollutant index bacteria shown in this study also contained yellow pigmented bacteria, which accounted for most of the pollutant index bacteria (75.6%). Consequently, the yellow bacteria indicating characteristics of the YA1 and YI types were considered to have the most probability as pollutant index bacteria.

At present, there is no information as to whether chromogenic bacteria including yellow types originate from autochthonous or allochthonous sources. These bacteria are often found in environments affected by human activities, for instance, they were isolated from sink faucets, humidifying units, and drinking fountains.²²⁾ They thus appear to be discharged into rivers or streams through domestic and industrial waste.

On the other hand, the following became clear from many physiological tests on pollution index bacteria: YA1 and YI types were less able to utilize low molecular organic compounds such as organic acids and amino acids, and had higher degradability of high molecular compounds such as gelatin and casein than other type bacteria.²³⁾ We also have isolated several strains similar to YA1 type from a domestic waste water.²³⁾

In studies on bacterial flora in aquatic environments, identification of isolates has generally been conducted to genus levels, as stated, and then the relationship of flora to water quality were analyzed.^{3,5–7,19–21)} In such case, there is the possibility that the genus contains two or more strains showing different characters, and this can influence the interpretation of results. In this study, isolates were classified based on the results of various physiological tests, and correlations between the proportions of classified bacterial types and water quality variables in each water sample were determined. This approach reveals that no information on pollutant index

bacteria can be obtained if the analysis is made after identification of isolates to the genus level.

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