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Comparative Study of Selective Media for Enumeration of *Pseudomonas aeruginosa* from Water by Membrane Filtration

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In the present study, mPA-D and mPA-E agar, modifications of mPA-C agar that reduce background fecal streptococci that interfere with the differentiation and enumeration of the *Pseudomonas aeruginosa* colonies grown in other mPA media, are proposed for use in analyzing natural water samples. In addition, the efficiencies of several culture media for the recovery of *P. aeruginosa* in water after membrane filtration and multiple-tube techniques are compared. The degree of selectivity, precision, efficiency, and sensitivity achieved with the proposed media exceeded that achieved by current methods. Furthermore, they yielded equal rates of accuracy and specificity. Incubation at 36°C resulted in an improved recovery of stressed *P. aeruginosa*. In conclusion, we propose the use of mPA-D and mPA-E agar, both incubated at 36°C for 24 to 48 h, for analyzing river water and seawater, respectively.

Pseudomonas aeruginosa is considered to be a ubiquitous and easily detectable microorganism in waters and soils, although it is not autochthonous to these environments (16). Among its most important habitats are human and animal fecal wastes, which are the main source of pollution in natural surface waters (3, 10, 16).

The importance of the study of *P. aeruginosa* in natural surface waters used for swimming is based on its potentiality in originating different kinds of infections, especially otitis (5, 20, 25). It has also been considered to be a water quality indicator microorganism (3, 13, 21).

Several different methods, including the multiple-tube technique (1, 9) and membrane filtration (4, 11, 23, 24), have been developed for the enumeration of *P. aeruginosa* in water (10, 17). However, most of them show some disadvantage, and none of them are completely accepted (10). Generally, the counting techniques based on most probable number (MPN) are considered to be less precise and effective than those based on membrane filtration (6, 14).

In 1972, Levin and Cabelli (23) developed the mPA medium for the isolation and enumeration of *P. aeruginosa* from natural waters that was later modified by Dutka and Kwan (11) and by Brodsky and Ciebin (4). Even so, these media do not provide suitable results, because of the growth of background fermentative microorganisms (A. de Vicente, J. J. Borrego, and P. Romero, Abstr. 9th Congr. Nac. Microbiol. 1983, 436, p. 929–930). To eliminate this problem, mPA-D agar, in which sugars present in mPA-C agar (4) are suppressed, and mPA-E agar, with xylose as the unique sugar, are proposed for analyzing river water and seawater samples, respectively.

TABLE 1. Average percent recovery of *P. aeruginosa* strains from different stressed suspensions[a]

Medium	Incubation (°C/h)	% Recovery of strain:					Avg % recovery
		ATCC10145	ATCC14216	J75	T26	N61	
mPA	41.5/48	5.14	21.41	18.41	5.50	4.90	11.07
mPA-B	41.5/72[b]	96.42	84.44	89.55	87.58	90.10	89.62
mPA-C	41.5/24	94.45	74.55	82.06	85.13	98.49	86.94
mPA-D	36/24	87.55	87.10	74.34	80.80	67.47	79.45
	36/48	94.98	88.51	75.01	82.08	72.26	82.57
	41.5/24	85.42	80.92	76.08	81.22	96.86	84.10
	41.5/48	89.87	83.20	77.05	81.38	99.35	86.17
mPA-E	36/24	87.52	83.77	87.28	91.60	72.34	84.75
	36/48	90.61	85.21	88.26	93.09	76.64	86.97
	41.5/24	92.54	76.44	80.21	90.68	92.53	87.08
	41.5/48	94.32	83.33	82.90	95.27	93.47	89.45

[a] Calculated with equation 1. Ten samples of each strain were used to calculate the average percent recovery.
[b] Similar values were obtained at 96 h.

MATERIALS AND METHODS

Field samples. The number and kinds of water samples provided were as follows: 10 samples of polluted river water, 6 samples of seawater, and 9 samples of sewage water, all collected during an 8-week period. The samples were refrigerated at 4°C and processed within 6 h of collection. All samples were collected from superficial layers (up to 30 cm below the water surface).

Microorganisms. The following five strains of *P. aeruginosa* were used for the evaluation of the accuracy of the

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TABLE 2. Effect of exposure to different stressed suspensions on the recovery of *P. aeruginosa* strains[a]

Medium	Incubation (°C/h)	Control[b]	% Recovery of strains from stressed suspensions of:									Avg % recovery
			Seawater stressed for (h):			Freshwater stressed for (h):			Distilled water stressed for (h):			
			0	6	24	0	6	24	0	6	24	
mPA	41.5/48	0.00	34.29	19.60	34.33	7.57	9.85	0.00	2.25	0.70	2.09	11.07
mPA-B	41.5/72[c]	86.85	92.46	105.73	100.42	82.34	85.98	94.88	59.43	93.60	94.45	89.62
mPA-C	41.5/24	74.10	103.80	88.41	99.12	81.86	94.86	73.51	74.91	99.80	79.00	86.94
mPA-D	36/24	84.86	78.38	80.32	85.68	66.06	74.30	77.32	90.05	93.12	64.43	79.45
	36/48	94.69	80.25	81.43	87.29	68.19	78.91	81.51	93.71	94.14	65.55	82.57
	41.5/24	82.87	87.73	94.35	87.36	74.68	90.99	59.84	87.51	97.75	77.92	84.10
	41.5/48	85.89	90.39	95.46	87.96	75.45	91.12	71.07	88.42	97.85	78.07	86.17
mPA-E	36/24	87.53	87.94	89.41	98.80	74.05	71.94	95.57	76.80	98.34	69.95	84.75
	36/48	90.14	90.48	92.08	99.82	76.36	74.06	96.65	80.12	100.14	72.42	86.97
	41.5/24	93.15	93.24	94.44	91.32	74.59	87.66	68.34	88.61	98.92	80.52	87.08
	41.5/48	96.25	95.96	94.85	91.41	75.91	88.32	80.69	90.57	100.04	80.55	89.45

[a] Calculated with equation 1. Five samples of each strain were used to calculate the average percent recovery.
[b] Unstressed cells in BHI.
[c] Similar values were obtained at 96 h.

different methods: ATCC 10145, ATCC 14216, and J75 from seawater; T26 from river water; and N61 from untreated sewage. Twenty-one *Streptococcus* strains, including 4 *S. faecalis*, 7 *S. faecium*, and 10 *S. avium* strains, isolated from samples of natural waters, and *S. faecium* ATCC 10541 were used for the growth assay.

Recovery media. Drake 10 medium (9) and Favero asparagine broth (1) were used as recovery media in the MPN technique and incubated for 48 h at 36°C. All the tubes that showed growth with greenish-blue pigment, fluorescence, or both under UV light were subcultivated in acetamide agar and milk agar (1) for confirmation as *P. aeruginosa*.

The following recovery media, with membrane filtration, were used: nalidixic acid-cetrimide agar (24), mPA agar (23), mPA-B agar (11), mPA-C agar (4), and mPA-D and mPA-E agar. mPA-D and mPA-E agar are modifications of the mPA-C agar developed by us which suppress all of the sugar compounds (mPA-D) or only lactose and sucrose (mPA-E). These two media were prepared by the procedure of Brodsky and Ciebin (4), with suppression antibiotics sulfapyridine and actidione. Both of these media were incubated at 36 and 41.5°C and examined at 24 and 48 h.

Methods. The MPN assays were carried out as described in *Standard Methods* (1). The filtrations were fivefold for each one of the assayed methods with 0.45-μm membrane filters (HAWG 047; Millipore Corp., Bedford, Mass.). Phosphate-buffered saline (23) was used as diluent solution. The membrane filtration technique was used following the specifications described previously (1).

Control media and suspensions. *P. aeruginosa* cultures were prepared by inoculation of strains into brain heart infusion (BHI) (Difco Laboratories, Detroit, Mich.) and incubated for 18 to 20 h at 36°C. Suspensions and culture dilutions were prepared in phosphate-buffered saline blank tubes. The concentrations of suspended microorganisms were determined by the spread plate technique with 0.1-ml portions of tryptic soy agar (Difco), BHI agar (BHIA; Difco), and King's A agar (22). The recounts were determined after 48 h at 36°C with three replicate plates. BHIA, plate count agar (Difco), and mPA-B agar without antibiotics (11) were used to quantify the total number of organisms in natural water samples by membrane filtration.

Fermentative colony verification. The fermentative colonies grown on mPA, mPA-B, and mPA-C agars were isolated on nutritive agar containing 0.1% glucose. The colonies were identified by the following tests: morphology and Gram stain; motility; oxidation-fermentation test; catalase and cytochrome oxidase tests; xylose, lactose, and sucrose fermentation; growth in BHI at 10°C, at 45°C, at pH 9.6, and after 30 min at 60°C; growth in milk–0.1% methylene blue, in broth with 6.5% NaCl, and in 0.04% potassium tellurite; and resistance to 0.01% sodium azide (7, 15). Confirmation was accomplished with the API 20 Strep system (Analytab Products, Plainview, N.Y.).

***Streptococcus* qualitative growth test.** *Streptococcus* cultures obtained from BHI containing 0.1% glucose were streaked on the different assayed mPA and *m* Enterococcus (Difco) media. The growth and fermentation results were recorded at 24, 48, and 72 h.

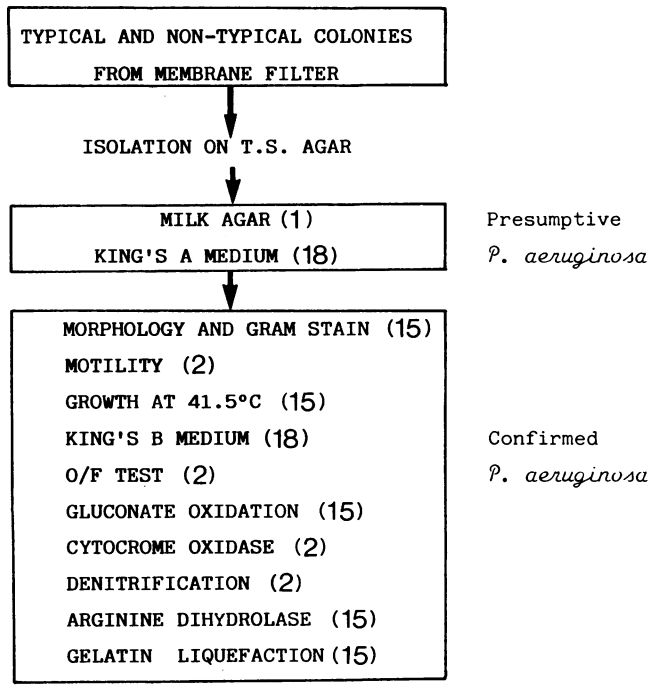

FIG. 1. *P. aeruginosa* identification scheme (numbers in parentheses indicate references in Literature Cited).

TABLE 3. Verification as *P. aeruginosa* of colonies isolated by different methods

Medium	Incubation (°C/h)	No. of colonies examined	Typical colonies		Atypical colonies	
			No. examined	% Verified[a]	No. examined	% Verified[a]
mPA	41.5/48	71	19	84.21	52	1.92
mPA-B	41.5/72	377	162	83.95	215	8.37
mPA-C	41.5/24	263	126	90.48	137	8.76
mPA-D	36/48	443	299	95.65	144	5.55
	41.5/48	101	53	98.11	48	4.16
mPA-E	36/48	287	204	98.04	83	7.23
	41.5/48	114	50	90.00	64	9.37
Nalidixic acid-cetrimide	36/24	283	58	87.93	225	6.67

[a] Verified as *P. aeruginosa*.

RESULTS

Accuracy was determined by comparison of the *P. aeruginosa* recoveries obtained with the mPA media with those obtained with the reference media (BHIA, tryptic soy agar, and King's A agar) by the following equation:

$$\Sigma \frac{\text{number of CFU for each method}}{\text{(number of CFU in BHIA + tryptic soy agar + King's A agar)/3}} \times 100/\text{number of samples} \quad (1)$$

Cultures of the five *P. aeruginosa* strains, with cell densities of 10^9 to 10^{10} CFU per ml in BHI, were suspended in seawater, distilled water, and river water; all water samples had been sterilized by filtration. Appropriate volumes of *P. aeruginosa* cultures were used as inocula to obtain a concentration of ca. 10^7 to 10^8 cells per ml and were stored at 4°C. Accuracy tests of strain cultures and suspensions were realized at 0, 6, and 24 h by the spread plate method. The counts were done in triplicate, and the arithmetical mean was calculated. The results, with an interval average recovery of 79.41 (mPA-D agar, 36°C for 24 h) to 89.45% (mPA-E agar, 41.5°C for 48 h) for the proposed media, are given in Tables 1 and 2. The accuracy of the methods always exceeded 75% recovery, except in mPA.

Specificity of the methods (culture medium and procedure used) was determined by testing seawater, river water, and sewage water samples from different locations. The typical *P. aeruginosa* colony was flat and dry, and its color varied between greenish grey with a dark center or black with or without a greenish rim and dark brown without a rim and more round (0.8- to 2-mm diameter), with irregular edges. Both typical and atypical colonies obtained from the different enumeration media were biochemically identified (Fig. 1). The confirmation percentages of typical colonies such as *P. aeruginosa* in mPA-D agar were 96.88% and of atypical colonies were 4.85%. In mPA-E agar, the confirmation percents were 90 to 98% for typical colonies, depending on incubation conditions, and 8.3% for atypical colonies. It can also be seen that all of the assayed media exceeded 83% confirmed typical colonies and that they never exceeded 10% false-negative results (Table 3).

mPA, mPA-B, mPA-C, mPA-D, mPA-E, and nalidixic acid-cetrimide agar selectivities for quantitative *P. aeruginosa* recovery from natural waters are shown in Table 4. Such selectivities were obtained by observing the degree of reduction of background microbial flora in the assayed methods. Reduction factors were obtained by calculating the rate between the average number of colonies per 100 ml detected in control media (BHIA, plate count agar, and mPA base agar) and the total number of colonies per 100 ml detected in the different selective *P. aeruginosa* media. These factors are highly variable, depending mainly on the media, the incubation conditions, and particularly the nature of the studied sample. In the two proposed media, these factors varied between 801 for mPA-E agar (36°C for 48 h) and 23,500 for mPA-D agar (41.5°C for 24 h) in freshwater samples. The degree of reduction ranged from 1,820 for mPA-D agar (36°C for 48 h) to 14,500 for mPA-E agar (41.5°C for 24 h) in seawater samples. In sewage samples, minimal and maximum reduction factors were obtained on mPA-E agar, and the values were 1.81×10^7 (36°C for 48 h) and 3.87×10^7 (41.5°C for 24 h), respectively. Generally, the average reduction values of the different water samples, obtained with mPA-D and mPA-E agar, were equal or higher than those with other media.

The precision of the different methods was graphically determined from dispersion of Fisher index D^2 values of the assay variability for mPA-B, mPA-C, mPA-D, and mPA-E agar as calculated from the following equation (12, 23):

$$D^2 = | N\Sigma X_i^2 - (\Sigma X_i)^2 | / \Sigma X_i \quad (2)$$

where X_i is the bacterial number obtained from each plate of

TABLE 4. Selectivity of methods for enumeration of *P. aeruginosa* from natural water samples

Medium	Incubation (°C/h)	Mean background reduction factor[a]		
		Freshwater ($\times 10^3$)	Seawater ($\times 10^3$)	Sewage ($\times 10^7$)
mPA	41.5/48	30.70	16.90	27.20
mPA-B	41.5/72[b]	8.36	1.22	5.81
mPA-C	41.5/24	44.40	4.75	21.50
mPA-D	36/24	1.85	2.11	1.95
	36/48	1.03	1.82	2.03
	41.5/24	23.50	14.20	3.41
	41.5/48	10.60	5.40	2.62
mPA-E	36/24	1.69	3.13	1.83
	36/48	0.80	1.92	1.81
	41.5/24	17.10	14.50	3.87
	41.5/48	7.65	5.81	2.37
Nalidixic acid-cetrimide	36/24	0.40	1.16	0.89

[a] Ratio between total colonies recount on control media (plate count agar, BHIA, and mPA-B base agar) and total colonies recount by each method assayed. Values shown are the arithmetical mean of five samples.

[b] Similar values were obtained at 96 h.

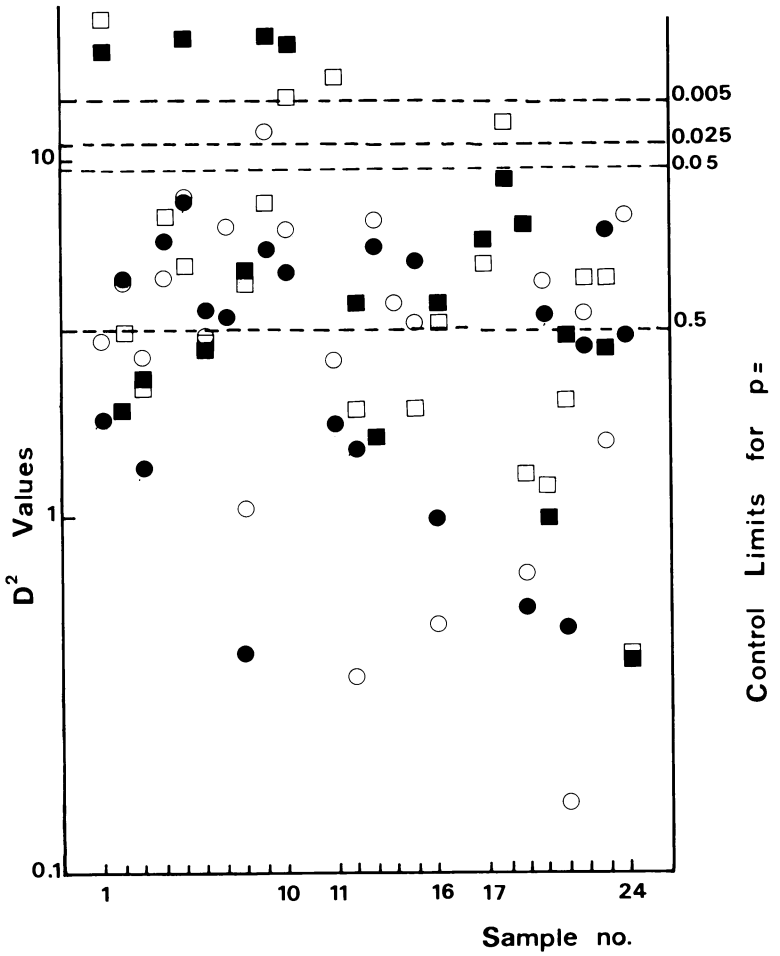

FIG. 2. Precision of mPA-D agar procedures for *P. aeruginosa* as estimated from dispersion of D^2 values (equation 2). Symbols: ●, incubated at 36°C for 24 h; ○, incubated at 36°C for 48 h; ■, incubated at 41.5°C for 24 h; □, incubated at 41.5°C for 48 h; -----, control limits when probability was as stated. Sources of samples: 1 to 10, freshwater; 11 to 16, seawater; 17 to 24, sewage. Data from five replicate plates were used to calculate the D^2 value for each point.

the same portion and sample and N is the number of replicate plates, five for each assayed portion. The D^2 values, determined for the different methods with mPA-D and mPA-E agar and 24 analyzed water samples (river water, numbers 1 to 10; seawater, numbers 11 to 16; sewage water, numbers 17 to 24) and theoretical D^2 values for different probabilities are given in Fig. 2 and 3. D^2 values of mPA-B and mPA-C agar are given in Fig. 4. From mPA-D and mPA-E agar results, it may be deduced that there was a uniform distribution and that, from the recount variability results, there was not a significant effect from plate to plate. On the other hand, mPA-B and mPA-C agar presented an extended dispersion of results because of a more heterogeneous recount of the different replicates.

Twenty-four water samples from different sources were assayed in parallel by the multiple-tube and membrane filtration procedures with the media described previously. The results of each method, expressed as the relative percent recovery with respect to the maximum recount for that sample, given as 100% (equation 3) and as the percentage of samples in which *P. aeruginosa* was detected with respect to the total number of tested samples (equation 4), were compared as follows (12):

$$\Sigma \frac{(P.\ aeruginosa \text{ concentration for assayed method}/P.\ aeruginosa \text{ maximum concentration obtained for that sample})}{\text{number of samples}} \times 100 \quad (3)$$

$$\frac{\text{number of samples in which } P.\ aeruginosa \text{ was detected}}{\text{total number of analyzed samples}} \times 100 \quad (4)$$

Positive *P. aeruginosa* percents recovery in each method (considering that in every sample *P. aeruginosa* was detected by one or more methods) and average *P. aeruginosa* percent levels in each method compared with the maximum level obtained for the same sample are expressed in Table 5. This comparison was also carried out under different culture conditions (incubation temperature and time).

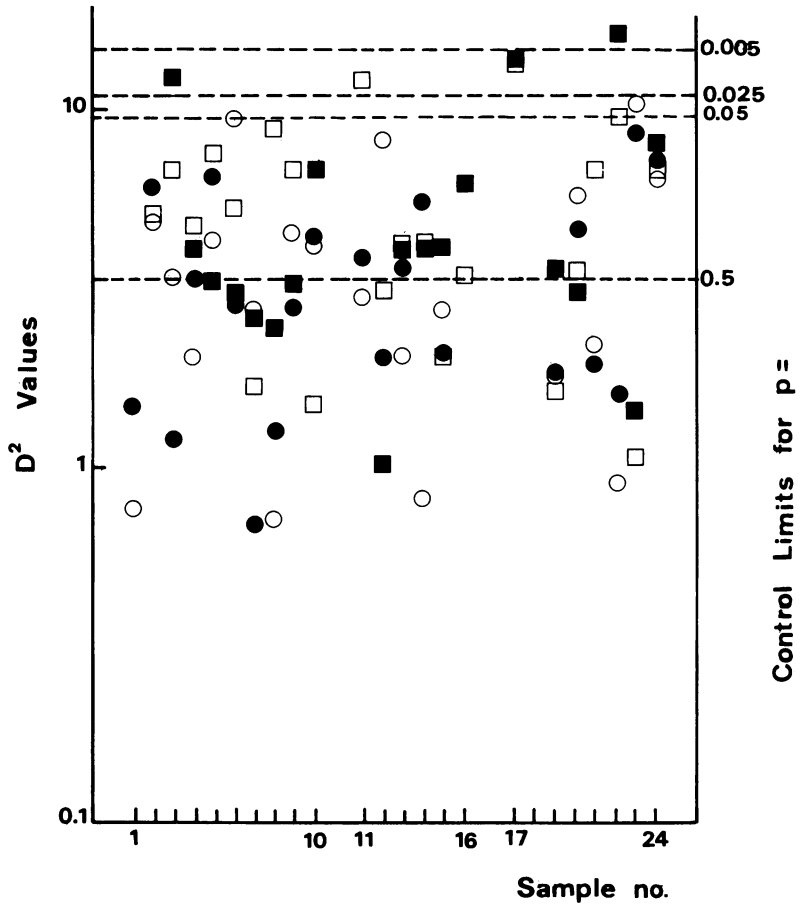

FIG. 3. Precision of mPA-E agar procedures for *P. aeruginosa* as estimated from dispersion of D^2 values (equation 2). Symbols: ●, incubated at 36°C for 24 h; ○, incubated at 36°C for 48 h; ■, incubated at 41.5°C for 24 h; □, incubated at 41.5°C for 48 h; -----, control limits when probability was as stated. Sources of samples: 1 to 10, freshwater; 11 to 16, seawater; 17 to 24, sewage. Data from five replicate plates were used to calculate the D^2 value for each point.

Fermentative colonies that interfered with *P. aeruginosa* recounts appeared after 24 h of incubation on mPA-B and mPA-C agar when different kinds of water were analyzed. When identification tests specified in *Bergey's Manual* (7) were used, these microorganisms appeared to be of the genus *Streptococcus*. The percentages of the isolated microorganisms were as follows: *S. avium*, 35.30%; *S. faecalis*, 23.53%; *S. faecium*, 41.17%. Also, fecal streptococci from the same water samples were investigated with *m* Enterococcus agar; the percentages of fecal streptococci were as follows: *S. avium*, 33.33%; *S. faecalis*, 16.66%; *S. faecium*, 50%. To determine whether these microorganisms were responsible for the interference described above, qualitative growth tests on mPA and *m* Enterococcus agar were carried out. The results show that there was a delay in and inhibition of streptococcal development on the proposed media which improved *P. aeruginosa* colony recount (Table 6).

DISCUSSION

When water samples with high concentrations of bacteria are studied, detection methods for specific organisms must fulfill two basic conditions: high selectivity and optimal recovery efficiency. Several methods have been proposed to detect and quantify *P. aeruginosa* in water samples, but none of them can be considered optimal. The exclusion of sugar by mPA-D agar delayed the growth of *Streptococcus* colonies. Because xylose is metabolized by *P. aeruginosa* but not by *Streptococcus* species, we decided to design mPA-E agar.

mPA-D and mPA-E agar accuracy were evaluated by comparison with media used in other methods (4, 11, 23). Average recoveries with mPA-D agar varied from 79.45 to 86.17% for the different kinds of samples tested, depending on incubation conditions. The range varied from 84.75 to 89.45% for mPA-E agar. These percents are similar to those obtained with mPA-B (89.62%) and mPA-C (86.94%) agar under the same conditions but are much higher than those obtained with mPA agar (11.07%) which also fluctuated, depending on the suspension tested (Table 2). Analyzing the results of the different *P. aeruginosa* strains (Table 1), it can be seen that the recovery interval for mPA-D agar oscillated from 67.47 to 99.35% with the N61 strain. The percents ranged from 72.34% for the N61 strain to 95.27% for the T26 strain when mPA-E agar was used. These values are very similar to those obtained with mPA-B and mPA-C agar but considerably different from those obtained with mPA agar, ranging from 4.90 to 21.14% for N61 and ATCC 14216, respectively, both under culture conditions proposed by

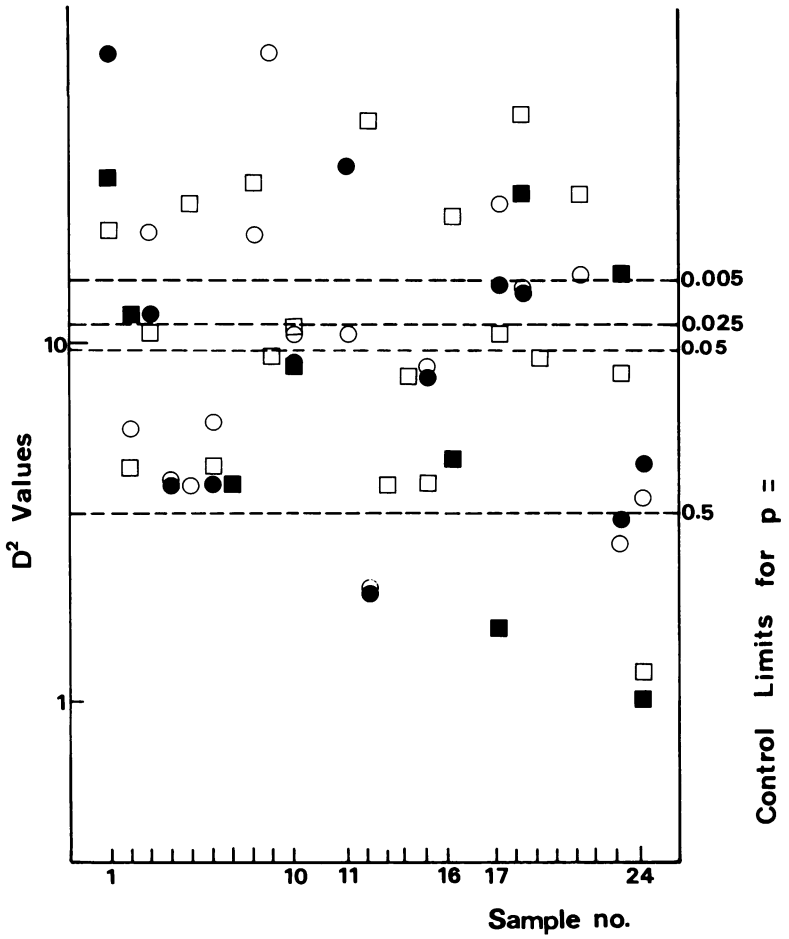

FIG. 4. Precision of mPA-B and mPA-C agar procedures for *P. aeruginosa* as estimated by dispersion of D^2 values (equation 2). Symbols: ●, mPA-B agar incubated at 41.5°C for 48 h; ○, mPA-B agar incubated at 41.5°C for 72 to 96 h; ■, mPA-C agar incubated at 41.5°C for 24 h; □, mPA-C agar incubated at 41.5°C for 48 h; -----, control limits when probability was as stated. Sources of samples: 1 to 10, freshwater; 11 to 16, seawater; 17 to 24, sewage. Data from five replicate plates were used to calculate the D^2 value for each point.

Levin and Cabelli (23). From these results, it can be seen that these average recoveries exceeded the required levels for the correct accuracy of a medium, except in the case of mPA agar. The accuracy results obtained with mPA-B and mPA-C agar agree with the reports of Brodsky and Ciebin (4), but those obtained with mPA agar are in open opposition to those pointed out by Levin and Cabelli (23).

In the most stressful conditions, the *P. aeruginosa* recovery with unselective media was greater than with selective media (Table 2). However, there are instances in which recovery with the selective media assayed increases with time exposed to a stressful environment. These results suggest that injury to the cells occurs during the lag phase and that the percentage of injured cells is lower after a long exposure time to stressful conditions. The decreased percentage of injured cells could be explained by either the repair or the inactivation phenomena subsequent to the cell injury, as previously reported by Hoadley and Cheng and by Hoadley (18, 19). These results occur more frequently when the incubation temperature is not restrictive, as is 36°C.

Specificity of mPA-D and mPA-E agar was high, because the percentage of colonies confirmed as *P. aeruginosa* was above 90% in all culture conditions, and the percentage of false-negative colonies never exceeded 10%, with over 5% of colonies being falsely negative on mPA-D agar and 8% being falsely negative on mPA-E agar. With the remaining methods, the positive verification percentage of typical colonies ranged between 83.95 and 90.48% for mPA-B and mPA-C agar, respectively, and the percentage of atypical colonies identified as *P. aeruginosa* ranged between 1.92 and 8.76% for mPA and mPA-C agar, respectively (Table 3). Even though these results are slightly lower than those obtained by other investigators (4, 11, 23), they have enough reliability to avoid the routine verification of typical colonies. Furthermore, in disagreement with Dutka and Kwan (11), significative changes in colonies cannot be seen when the incubation temperature is modified.

Selectivity criteria of a recount medium state that the background flora of water samples must decrease by at least three orders of magnitude (23). Results of the ratio between the average concentration of microorganisms detected in control media by membrane filtration and the total concentration of microorganisms grown on *P. aeruginosa*-selective media are given in Table 4. These results show that there was a significant decrease of background flora, depending on the level of bacteria in the sample and also on the source of the assayed water. Therefore, recoveries from freshwater and seawater samples were similar and lower than those

TABLE 5. Comparison of the efficiency of different procedures for recovery of *P. aeruginosa* from waters

Medium	Incubation (°C/h)	% Detection or recovery from the following samples:							
		Freshwater (n = 10)		Seawater (n = 5)		Sewage (n = 9)		% Overall (n = 24)	
		Detection[a]	Recovery[b]	Detection[a]	Recovery[b]	Detection[a]	Recovery[b]	Detection[a]	Recovery[b]
mPA	36/48	10.00	5.88	20.00	25.19	55.55	23.98	29.16	21.57
	41.5/48[c]	0.00		0.00		0.00		0.00	
	41.5/72	0.00		0.00		0.00		0.00	
mPA-B	36/48	90.00	46.02	40.00	34.02	100.00	53.39	82.61	47.86
	41.3/48	60.00	10.09	40.00	17.17	55.55	63.72	54.16	31.81
	41.5/72[c]	90.00	12.34	40.00	26.26	66.66	54.45	70.83	28.84
mPA-C	36/24	100.00	74.50	60.00	55.50	87.50	59.02	86.95	66.23
	36/48	100.00	75.46	60.00	65.30	100.00	68.18	91.30	71.24
	41.5/24[c]	50.00	11.66	20.00	4.42	55.55	39.64	45.83	23.72
	41.5/48	90.00	26.68	60.00	6.10	77.77	53.29	79.16	33.13
mPA-D	36/24	100.00	82.16	80.00	50.91	100.00	79.24	95.83	75.58
	36/48	100.00	82.71	100.00	46.81	100.00	82.96	100.00	74.99
	41.5/24	90.00	23.90	60.00	28.85	77.77	40.83	79.16	30.92
	41.5/48	100.00	47.30	60.00	42.46	100.00	57.10	91.66	50.64
mPA-E	36/24	100.00	79.83	100.00	66.36	100.00	81.90	100.00	77.81
	36/48	100.00	77.61	100.00	96.18	100.00	87.85	100.00	85.21
	41.5/24	80.00	51.60	100.00	13.96	88.88	35.76	87.50	36.61
	41.5/48	100.00	65.72	100.00	35.32	100.00	66.32	100.00	59.61
Nalidixic acid-cetrimide	36/24	100.00	43.19	60.00	43.46	88.88	57.29	87.50	48.60
	36/48	90.00	64.43	80.00	43.21	87.50	57.31	86.95	57.70
Favero broth		100.00	32.31	20.00	1.73	100.00	38.22	83.33	33.44
Drake 10 medium		100.00	36.94	0.00		100.00	16.21	79.12	27.12

[a] Percentage of samples in which *P. aeruginosa* was detected (equation 4).
[b] Average relative percent recovery with respect to the maximum recount for each sample, based only on samples positive for *P. aeruginosa* (equation 3).
[c] Originally proposed procedure.

from sewage samples. If selectivities of the different methods are compared, similar behavior is observed, because all of the methods presented high selectivity (decrease above 1,000-fold), except in nalidixic acid-cetrimide and mPA-E agar incubated at 36°C for 48 h, which did not achieve such a decrease in river water samples. In all mPA media incubated at 41.5°C, more than a 1,000-fold reduction of overgrowth was obtained, and in sewage samples this reduction was even higher. But when the incubation temperature was lowered to 36°C, selectivities of mPA-D and mPA-E agar were reduced by one order of magnitude in river water and seawater samples, but in sewage samples this decrease was insignificant. To explain this phenomenon, it can be implied that selectivity is affected by the temperature of the water samples, often over 20°C, and by a high concentration of thermophilic microorganisms (4, 23).

Even though there was not a significant quantitative improvement in selectivity with mPA-D and mPA-E agar, there was a qualitative one, because streptococcal growth that produced fermentative colonies on mPA agar was delayed up to 72 h (Table 6). The *Streptococcus* species isolated from mPA media were the same species that were detected with *m* Enterococcus agar and were obtained from the same samples of polluted natural water. The importance of delaying streptococcal growth on mPA, mPA-B, and mPA-C agar is that these microorganisms present higher concentrations than does *P. aeruginosa* in the same natural polluted water samples (8). Also, by allowing incubation at a temperature of 36°C, detection of stressed *P. aeruginosa* cells, which can be inhibited at 41.5°C, is improved.

Experimental D^2 values in variability assays (Fig. 2 and 3) are approximately equal to the expected estimates, demonstrating the high precision of mPA-D and mPA-E agar. Comparison of D^2 values obtained for mPA-B and mPA-C agar with those obtained for mPA-D and mPA-E agar (Fig. 2 to 4) suggests that mPA-D and mPA-E agar are more precise, especially when incubation is carried out at 36°C. In this study, a higher variability than that obtained by Brodsky and Ciebin (4) can be seen for mPA-B and mPA-C agar. This may be caused by the variation of typical colony morphology (6).

Comparison of mPA-D and mPA-E agar assayed methods with other assayed methods (Table 5) was achieved by means of the efficacy and sensitivity of those methods, designated *P. aeruginosa* recovery efficiency. The techniques based on MPN, with Favero asparagine broth and Drake 10 medium, presented a high detection sensitivity, except with seawater samples, but they showed a poor percent recovery, about 30%. Even so, it must be noted that

TABLE 6. Qualitative growth of *Streptococcus* test strains on mPA media at 36 and 41.5°C[a]

Assayed medium	Time (h)	Growth of test strains[b]			
		S. avium (n = 10)	*S. faecium* (n = 7)	*S. faecalis* (n = 4)	*S. faecium* ATCC 10541
mPA	24	+, F	+, F	+, F	+, F
mPA-B	24	+, F	+, F	+, F	+, F
mPA-C	24	+, F	+, F	+, F	+, F
mPA-D	24	−	−	−	−
	48	−	−	−	−
	72	(+)	(+)	(+)	(+)
mPA-E	24	−	−	−	−
	48	(+)	(+)	(+)	(+)
	72	(+)	(+)	(+)	(+)

[a] Results at 36 and 41.5°C were equal.
[b] +, Growth; F, fermentation; −, no growth; (+), weak growth.

interference which other background flora can produce in the test tubes can produce false results, decreasing the sensitivity of the method. This could also be caused by the individual or global inhibitory effect of processes of amensalism, competition, or toxic substance inhibition of the sample. Another inconvenience of this method is that, because its presumptive test is based on fluorescein production and because for the confirmation test pyocyanine production is required, the existence of apyocyanogenic strains or the nonproduction of fluorescein or both (17) that result in a loss of sensitivity and security of these methods cannot be forgotten.

Efficiency was much higher for membrane filtration methods than for MPN, in agreement with the results reported by other investigators (4, 11, 23). mPA agar, used as described by Levin and Cabelli (23), did not detect *P. aeruginosa* in any of the samples. Incubation at 36°C for 48 h only achieved 29% sensitivity and 21% recovery. Only four assays reached maximum sensitivity detection (100%): mPA-D agar at 36°C for 48 h, mPA-E agar at 36°C for 24 h, mPA-E agar at 36°C for 48 h, and mPA-E agar at 41.5°C for 48 h (Table 5). The highest percent recovery belonged to mPA-E agar at 36°C for 48 h, with an average recovery of 85%; the comparative percent recovery was only above 75% in mPA-D and mPA-E agar incubated at 36°C, so both media in these culture conditions offered higher efficiency in *P. aeruginosa* enumeration from natural water. mPA and mPA-C agar methods presented a low sensitivity and a slight recovery level compared with the methods proposed in this study. Likewise, the mPA-B agar method showed low sensitivity for seawater and a degree of recovery ranging from 12 to 54%, depending on the type of sample. It must be noted that nalidixic acid-cetrimide agar has high sensitivity, about 87%, even though it only gives 50% recovery of *P. aeruginosa*; furthermore, limited definition and concretion of *P. aeruginosa* typical colonies, grown on this medium, must be considered.

Behavior of the media varies with the source of the sample. All methods lose recovery ability or sensitivity or both with seawater samples, except with mPA-E agar incubated at 36°C for 48 h; thus, this medium is recommended for these samples. The method that showed the best results for freshwater samples was mPA-D agar incubated at 36°C for 48 h. Both methods can be used with sewage water.

We must agree with Levin and Cabelli (23) that, with each medium, sensitivity of the method is considerably lower when turbid samples with high contents of particulate matter or with sediments are used. This is because particulate matter produces faulty filtration and disguises the normal morphology and colony color of *P. aeruginosa* in these media.

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