APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1977, p. 227-230
Vol. 33, No. 2

Printed in U.S.A.

Improved Enumeration of *Streptomyces* spp. on a Starch Casein Salt Medium

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Received for publication 13 September 1976

Well-formed *Streptomyces* colonies were counted more rapidly when a starch casein medium containing antibiotics was supplemented with either magnesium chloride or additional sodium chloride.

Undesirable tastes and odors in drinking water are often caused by the production of odorous metabolites (such as geosmin or 2-methylisoborneol), by blue-green algae, or by *Streptomyces* spp. (2, 7, 8). Since we received complaints from consumers supplied by the Metropolition Water, Sewerage and Drainage Board, we decided to monitor the presence of *Streptomyces* spp. in the water held in our reservoirs. This led to the development of an improved method for the enumeration of *Streptomyces*.

MATERIALS AND METHODS

Eight enumeration media were initially tested: (i) glucose-yeast extract medium (1); (ii) Benedict agar (Porter, 6); (iii) chitin agar (Lingappa and Lockwood; 10); (iv) chitin agar (metropolitan Water Board, London; 4); (v) arginine glycerol medium (El Nakeeb and Lechevalier; 10); (vi) Küster and Williams agar (10); (vii) rose begal malt extract medium (5); and (viii) starch casein agar (3, 11).

Results obtained in triplicate using polluted river water indicated complete overgrowth by fungi and bacteria on all media except those containing chitin and starch casein. The more satisfactory chitin medium was that of Lingappa and Lockwood (10), but colonies were indistinct and small.

Agate and Bhat (1) stated that the moisture on the freshly poured glucose-yeast extract plate appeared to be the primary factor concerned in the excessive spreading of both molds and bacteria and general overcrowding over the slowly growing actinomycetes. Use of a stereoscopic microscope for counting colonies on chitin agar was recommended (10), and for other media (9) plates could be dried overnight at 37°C. Both of these techniques can be eliminated by adopting the approach given below.

Effect of salt on the colony count. The starch casein medium (11) was the most promising medium, so further work was undertaken to reduce the growth of rapidly growing gram-negative bacteria by adding sodium chloride or magnesium chloride to the medium. The rationale behind this approach was that actinomycetes are known to be comparatively salt tolerant (9). Thus, a second series of tests was run in a more precise manner. The media tested were: (i) glucose-yeast extract medium (1); (ii) arginine glycerol medium (10); (iii) Küster and Williams medium (10); (iv) rose bengal malt extract medium (5); and (v) starch casein agar (3, 11). Different concentrations of sodium chloride were tested in media (i) to (v), whereas magnesium chloride was only tested in medium (v).

Two series were run, the variant being the concentration of penicillin G (see tables 1 and 2). Unchlorinated polluted water from the Lane Cove River, which was low in salt (about 36 mg of chloride per liter), was used for the tests. Each test was performed in triplicate. Water was collected in a large plastic 20-liter container. It was poured into a plastic can, mixed well, and poured into 500-ml stoppered glass bottles. These were heated for 2 h at 44°C to decrease the number of non-sporeforming bacteria, and then 50 ml was filtered under suction through a sterile 5-cm-diameter membrane filter of 0.45-μm pore size (Oxoid). The membrane was placed face upwards on the medium in a petri dish and incubated for 10 days at 28 ± 0.1°C. The number of colonies was determined by using a hand counter.

RESULTS

Glucose-yeast extract medium. The actinomycetes were difficult to count since they were pinpoints, were not discrete entities, or had vague lacey outlines on the ever present yellow, red, and white bacterial background. Generally, the number of colonies increased with increasing salt content, but the counting error could well be 5 to 10%.

Arginine glycerol medium. The overgrowth problem was not solved using arginine glycerol medium.

Küster and Williams medium. At lower concentrations of additive, *Streptomyces* colonies tended to be on the rim of the bacterial growth. As the salt concentration increased, colonies became partially exposed but were atypical, lumpy, and distorted. They appeared as aggregates of grey and white colonies, while the bacteria decreased with an increase in additive.

TABLE 1. *Effect of increasing additive on the number of streptomyces colonies developing on six enumeration media in the presence of penicillin G (1 μg/ml)*

Concn of additive (%)	No. of colonies per plate developing on medium[a]					
	GYE (NaCl)	AG (NaCl)	KW (NaCl)	RBME (NaCl)	SC (NaCl)	SC ($MgCl_2$)
Blank	OG[b]	OG	OG	OG	OG	OG
	OG	OG	OG	OG	OG	OG
	OG	OG	OG	OG	OG	OG
2.00	30	43	256	OG	45	3
	47 (57)[c]	39 (41)	160 (187)	OG	102 (76)	81 (24)
	94	OG	145	OG	81	30
2.64	87	106	214	OG	739	20
	85 (73)	38 (72)	303 (270)	OG	518 (591)	120 (54)
	47	ND[d]	293	OG	517	23
3.32	176	OG	432	OG	983	768
	178 (202)	OG	633 (583)	OG	989 (939)	274 (507)
	254	OG	684	OG	845	480
4.00	227	OG	463	OG	1,222	710
	211 (210)	OG	700 (513)	OG	919 (1,038)	406 (589)
	194	OG	378	OG	973	652
4.60	230	OG	766	OG	1,019	386
	114 (215)	OG	606 (721)	OG	962 (1,003)	371 (255)
	301	OG	792	OG	1,029	767

[a] Additive is given in parentheses. GYE, Glucose-yeast extract; AG, arginine glycerol; KW, Küster and Williams medium; RBME, rose bengal malt extract; SC, starch casein.
[b] OG, Overgrown.
[c] Numbers in parentheses are mean number of colonies/plate.
[d] ND, Not determined.

TABLE 2. *Effect of increasing additive on the number of streptomyces colonies developing on six enumeration media in the presence of penicillin G (3 μg/ml)*

Concn of additive (%)	No. of colonies per plate developing on medium[a]					
	GYE (NaCl)	AG (NaCl)	KW (NaCl)	RBME (NaCl)	SC (NaCl)	SC ($MgCl_2$)
Blank	OG[b]	OG	OG	OG	OG	OG
	OG	OG	OG	OG	OG	OG
	OG	OG	OG	OG	OG	OG
2.00	34	OG	216	OG	146	OG
	34 (48)[c]	3	121 (198)	10	119 (134)	3 (15)
	76	OG	259	OG	138	28
2.66	44	OG	173	15	741	107
	52 (51)	OG	199 (186)	21 (17)	632 (546)	18 (59)
	58	2	ND[d]	14	267	54
3.32	267	OG	279	OG	1000	180
	461 (380)	OG	399 (356)	13 (8)	931 (957)	113 (110)
	412	OG	390	3	942	39
4.00	694	OG	529	OG	975	539
	650 (699)	OG	737 (670)	OG	907 (1037)	823 (681)
	753	51	746	OG	1229	ND
4.60	524	OG	311	OG	907	923
	473 (560)	OG	667 (453)	OG	929 (916)	860 (943)
	684	OG	383	OG	913	1047

[a] Additive is given in parentheses. See Table 1 for abbreviations of media.
[b] OG, Overgrown.
[c] Numbers in parentheses are mean number of colonies/plate.
[d] ND, Not determined.

Rose bengal malt extract medium. All plates were overgrown with red and yellow bacteria to such a degree that counting of *Streptomyces* colonies was impossible.

Starch casein medium (NaCl). As the salt concentration increased to 4.6% the bacteria decreased due to suppression. At 2.0% additive, overgrowth occurred, whereas at 3.3% colonies

were mainly on the rim, the center ones being very small. At 4.0%, some bacteria remained and *Streptomyces* colonies were quite large and unobscured. With a salt level of 4.6%, the membrane was free of bacteria, and the grey colonies were typically well-formed units, which were easy to count. They emitted the characteristic musty odor.

Starch casein medium ($MgCl_2$). As the salt concentration increased from 0 to 4.6%, the number of bacteria decreased. At 2.66% additive, a few *Streptomyces* colonies were on the rim, the rest being unable to compete, and a few white wisps were visible on the surface. At 3.32%, colonies in the center were still seen as white dots, and at 4% they were still small, with larger ones on the rim. At 4.66%, there was little bacterial growth, but the colony size was badly retarded in comparison with the same medium plus NaCl.

Effect of trace metals on the colony count. Trace metals were added because Waksman (9) mentioned their beneficial effect. These were in the form of zinc acetate, copper chloride, and ammonium molybdate. Even with such variables as agar concentration and salt concentration the trend is obvious in each of the four treatments. Only zinc ions enhanced *Streptomyces* colony yields (see Table 3).

It can be readily seen that salt (NaCl) addition is not a panacea for the prevention of bacterial overgrowth on all media. However, it has been found to be outstandingly beneficial when used with the starch casein medium (11). On an uncrowded plate, grey-white *Streptomyces* colonies with a maximum diameter of 5 mm develop and emit in all cases the strong, characteristically musty odors. Only *Streptomyces* spp. were encountered, with typical colonial growth patterns of concentric rings of spores developing as shown in Fig. 1. The complete formula is as follows: starch (Ajax Chemical Co.), 10 g; casein (fat and vitamin free; Hopkins & Williams Ltd.), 0.3 g; potassium nitrate, 2.0 g; sodium chloride, 2.0 g; sodium chloride (additional), 26.6 to 40.0 g; dipotassium hydrogen phosphate, 2.0 g; magnesium sulfate, 0.05 g; calcium carbonate, 0.02 g; ferrous sulfate, 0.01 g; agar (Oxoid no. 1), 18.0 g; zinc acetate, 1.0 mg; distilled water, 1,000 ml.

All ingredients, except starch, were combined, and the pH was adjusted to between 7.0 and 7.2. When the medium began to boil, starch was added as a paste in distilled water. After the medium was sterilized at 15 lb/in^2 for 15 min in an autoclave, the precipitate was resuspended before pouring. Four antibiotics were added to each petri dish (per milliliter of agar) before the molten medium was poured: nystatin (E. R. Squibb & Son), 50 μg; cycloheximide (Acti-Dione, Upjohn), 50 μg; aerosporin (Burroughs Wellcome), 5.0 μg; penicillin G (Glaxo Allenbury), 1 to 3.0 μg. For potable water 2.66% NaCl as additive was satisfactory, whereas up to 4.0% may be needed if the competing bacterial load is greater.

DISCUSSION

As mentioned above, tests need to be performed to find the most suitable concentration of NaCl to be used as additive. The counting error level is not relevant in this study as the number and clarity of *Streptomyces* colonies on medium (v) far surpasses those of any competitor. In cases when high counts were obtained, there may have been an error of about 8% due to crowding, but for lower counts no error occurred. The effect of an altered level of penicil-

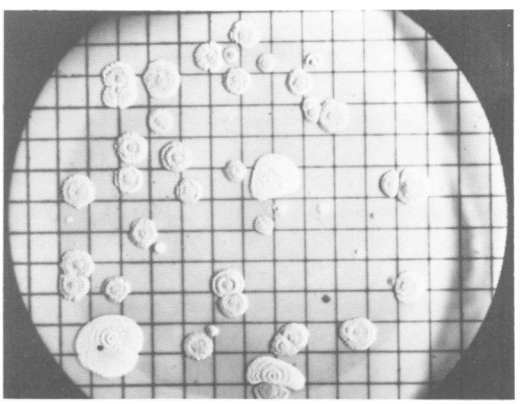

FIG. 1. *Streptomyces colonies growing on a membrane on starch casein agar plus NaCl.*

TABLE 3. *Effect of trace metals on the number of streptomyces colonies on starch casein agar modified with additional NaCl or $MgCl_2$*

Metal (1 μg/ml)	Mean no. of colonies/plate per 100 ml at agar concn of:			
	1.8% (2.64% NaCl)	1.2% (2.64% NaCl)	1.8% (3.3% $MgCl_2$)	1.8% (4.0% $MgCl_2$)
Blank	226	252	320	270
Zinc ions	330	395	420	460
Molybdenum and copper ions	155	160	245	205
Zinc, copper, and molybdenum ions	133	140	200	195

lin G is irrelevant compared with the salt content effect.

ACKNOWLEDGMENTS

I wish to thank the Metropolitan Water, Sewerage and Drainage Board for permission to publish this paper.

LITERATURE CITED

1. **Agate, A. D., and J. V. Bhat.** 1963. A method for the preferential isolation of actinomycetes from soils. Antonie van Leeuwenhoek J. Microbiol. Serol. 29:297-304.
2. **Bays, L. R., N. P. Burman, and W. M. Lewis.** 1970. Taste and odour in water supplies in Great Britain: a survey of the present position and problems for the future. Water Treat. Exam. 19:136-160.
3. **Küster, E., and S. T. Williams.** 1964. Selection of media for isolation of streptomycetes. Nature (London) 22:928-929.
4. **Metropolitan Water Board.** 1966. Metropolitan Water Board 42nd annual report, 1965-1966, p. 23-29. Metropolitan Water Board, London.
5. **Ottow, J. C. G., and H. Glathe.** 1968. Rose bengal malt extract agar, a simple medium for the simultaneous isolation and enumeration of fungi and actinomycetes from soil, Appl. Microbiol. 16:170-171.
6. **Porter, J. N., J. J. Wilhelm, and H. D. Tresner.** 1960. Method for the preferential isolation of actinomycetes from soils. Appl. Microbiol. 8:174-178.
7. **Rosen, A. A., C. I. Mashni, and R. S. Safferman.** 1970. Recent developments in the chemistry of odour in water. The cause of earthy/musty odour. Water Treat. Exam. 19:106-119.
8. **Silvey, J. K., D. E. Henley, and J. T. Wyatt.** 1972. Planktonic blue-green algae. Growth and odour production studies. J. Am. Water Works Assoc. 64:35-39.
9. **Waksman, S. A.** 1961. The actinomycetes. The Williams and Wilkins Co., Baltimore.
10. **Water Research Association.** 1967. Recommended methods for the enumeration of actinomycetes and fungi in waters, p. 67-70. Water Research Association, Technical Enquiry Rep. no. 150, Medenham, England.
11. **Williams, S. T., and F. L. Davies.** 1965. Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. J. Gen. Microbiol. 38:251-261.