A NEW CETRIMIDE MEDIUM FOR THE DETECTION OF
PSEUDOMONAS AERUGINOSA

D. A. A. MOSSEL* AND LOURDES INDACOCHEA†
*Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands,
and †San Marcos University, Lima, Peru

ESTIMATES of the numbers of Pseudomonas aeruginosa in food, water supplies and
medicaments may be made either by direct enumeration methods (Selenka, 1960; Drake,
1966; Kielwein, 1969), or by presence-or-absence tests (Favero, Drake and Randall, 1964;
Black et al., 1970). In the former, material is seeded on to a solid diagnostic medium and
the relevant colonies are counted; in the latter, portions are first enriched in a liquid
selective medium and streaks are subsequently made on to a similar solid medium. Cetrimide
agar (Lowbury and Collins, 1955; Brown and Lowbury, 1965) is a useful medium for this
purpose, but it is not completely selective for Ps. aeruginosa (Goto and Enomoto, 1970);
and colonies of this organism on it cannot always be identified with certainty unless con-
firmatory tests are made (Azuma and Witter, 1970).

PRELIMINARY EXPERIMENTS

An attempt was made to increase the diagnostic value of the cetrimide medium by making
use of three fairly constant metabolic attributes of Ps. aeruginosa: (1) rapid growth at
41°–42°C (Selenn and Stark, 1943; Haynes, 1951; Stanier, Palleroni and Doudoroff, 1966);
(2) no formation of acid from polyols (Mossel and Indacochea, unpublished) and (3) rapid
deamination of acetamide (Bühlmann, Vischer and Bruhin, 1961; Kelly and Clark, 1962;
Hedberg, 1969).

Hedberg’s peptone-free acetamide agar was first tried as the basal medium; but, as
shown by Hedberg herself, it readily supported the growth of some other Gram-negative
bacteria. Moreover, we found that approximately 20 per cent. of some 200 strains of
Ps. aeruginosa freshly isolated from clinical material did not deaminate acetamide on
Hedberg’s medium at 42°C.

Next, acetamide was added to Brown and Lowbury’s modified cetrimide agar. Brown
and Lowbury included glycerol in their medium to promote pigment formation. This
was useful for our purpose also, because the enterobacteria that were able to grow on this
medium and to attack glycerol produced sufficient acid to mask any alkalinity resulting
from the deamination of acetamide. However, some of them did not attack glycerol, and
such strains might deaminate acetamide and hence be recorded as Ps. aeruginosa. This
was remedied by replacing half the glycerol by D-mannitol, from which such strains produce
acid; virtually all of the Enterobacteriaceae produce acid either from glycerol or mannitol.
Phenol red was included in the medium as indicator to reveal whether or not the net effect
of polyol dissimilation and acetamide deamination is alkalisation of the medium as in
the case of Ps. aeruginosa. A red zone around the area of growth was therefore taken to
be presumptive evidence for the presence of this organism. The medium thus composed
was called GMAC (glycerol-mannitol-acetamide-cetrimide) agar.

COMPOSITION OF THE MEDIUM

To prepare the medium, 0.2 g peptone, 10 g K2SO4, 1.4 g MgCl2. 6H2O, 0.3 g cetrimide
(AR), 5 ml glycerol (AR), 5 g D-mannitol (AR) and 15 g agar were added to 900 ml distilled
water; the pH was adjusted to 7.0 and the mixture sterilised for 20 min. at 118°–121°C;

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this basal medium was brought to a temperature of 50°C, and 100 ml of a solution containing 10 g acetamide (AR) and 12 mg phenol red, adjusted to pH 7-0 and sterilised by filtration, was added with aseptic precautions. The medium was dispensed, either in thin layers in Kolle flasks (rather than in petri dishes) to allow incubation in a waterbath at 42 ± 0-1°C after surface plating, or as slopes in test-tubes for making subcultures from liquid enrichment media.

The fully prepared medium had a faint yellow-orange colour.

EVALUATION OF THE MEDIUM

For qualitative evaluation of the medium, a total of 450 freshly isolated cultures of Ps. aeruginosa from clinical material was examined. In addition some 100 other bacteria were tested, including three to five cultures each of Acinetobacter, Aeromonas, Alkaligenes, Arizona, Bacillus, Citrobacter, Corynebacterium, Enterobacter, Escherichia, Flavobacterium, Herellea, Klebsiella, Micrococcus, Proteus, Salmonella, Shigella, group-D streptococci, Staphylococcus, Vibrio and Yersinia. Tubes of GMAC-agar were streak-inoculated and then incubated in a waterbath at 42 ± 0-1°C. The colour of the slants was observed after 24 hours’ and approximately 40 hours’ incubation. With six exceptions, all strains of Ps. aeruginosa grew prolifically and changed the colour of the slants to cherry red. Among the other bacteria tested, most did not grow at all on the medium. Some Gram-negative rods grew but, except for one strain of Alkaligenes, did not change the colour of the medium to red.

In addition, the performance of GMAC-agar was tested for the enumeration of Ps. aeruginosa in contaminated materials. Samples of sewage and lake water were spread directly on GMAC-agar contained in Kolle flasks, and incubated at 42 ± 0-1°C; colonies surrounded by red zones were picked. To recognise individual colonies of Ps. aeruginosa when the medium was thus used for direct inoculation it was necessary to restrict the size of the red zones by adding 0-04M phosphate buffer, pH 7-0, to GMAC-agar. Presence-or-absence tests were carried out with the same samples by enriching suitable portions of water at 42 ± 0-1°C for 20 hr in a liquid medium (PCTK) that contained 15 g sugar-free peptone, 1 mg crystal violet, 15 mg kanamycin and 50 mg tylosin per l water. Such enrichment cultures were streaked on to slants of GMAC-agar, incubated at 42 ± 0-1°C. The result was considered positive when the colour of the slants changed to cherry red. In parallel with the above two tests, similar portions of the samples were examined by the standard American method (Favero et al.; Black et al.). The direct plating method yielded almost as many positive results as the standard method, and the enrichment technique yielded slightly more.

The confirmation rate of suspect colonies obtained on GMAC-agar, when it was used as a plating medium after enrichment of samples in PCTK, was over 90 per cent. Occasional strains of Alkaligenes and a rare strain of Ps. stutzeri could be rejected by testing for oxidase, the mode of attack on glucose (Mossel and Martin, 1961), arginine dihydrolase and amylase activity (Stanier et al.; Pickett and Pedersen, 1970).

SUMMARY

A new medium is described for the selection and identification of Pseudomonas aeruginosa. It contains glycerol, mannitol, acetamide, cetrimide, a trace of peptone and phenol red. On this medium incubated at 42°C, nearly all cultures of Ps. aeruginosa, but only very few other bacteria, form colonies or areas of growth surrounded by a red zone. The medium may be used either for the direct enumeration of Ps. aeruginosa or to establish the presence of this organism in liquid enrichment cultures.

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