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Media for Enterobacteriaceae

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The 'rag-bag character of 'coli-aerogenes' bacteria used originally as markers for process failure has prompted their substitution by the taxonomically accurately delineated group of Enterobacteriaceae. Media previously used for the detection or enumeration of the coli-aerogenes group can be adapted to monitoring Enterobacteriaceae simply by replacing lactose by glucose. Test strains including fastidious as well as more robust Enterobacteriaceae are used for the evaluation of media considered appropriate. They should be recovered within 0.5 $\log_{10}$ of cfu-numbers obtained on non-selective media whereas spore-formers and cocci should lead to less than 5 $\log_{10}$ cycles of the recovery on or in control media.

Key words: Enterobacteriaceae; Coli-aerogenes bacteria; Selectivity of media for enumeration; Productivity of media for enumeration

A short ecological justification of the use of Enterobacteriaceae as marker organisms

At the end of the 19th century Schardinger (1892) introduced the detection of fermentative Gram-negative bacteria, taxonomically and ecologically related to *Salmonella typhi*, as a test for assessing the safety of drinking water, particularly with regard to the typhoid bacterium. Initially this group of bacteria was not and probably could not be, accurately defined taxonomically. It included *Escherichia, Enterobacter aerogenes, Klebsiella pneumoniae, Citrobacter freundii* and a few related types later to be called coli-aerogenes bacteria. A few years later Eijkman (1904, 1913) suggested making the test more specific and precise by raising the incubation temperature to 46°C and hence limiting it to *E. coli*, a more constant inhabitant of the intestinal tract.

In the era 1925–1935 the coli-aerogenes group was also used to assess the efficacy of milk pasteurization (Swenarton, 1927). Sir Graham Wilson (1935) admirably defined the ecological essentials of such a test. Somewhat later examining for coli-aerogenes bacteria was also extended to foods. The 'rag-bag' character of organisms included in this group, depending on minor details of the methods used, now began to interfere seriously with accuracy and precision of these methods of examination: differences of several log-cycles between laboratories examining samples of the same food by different, though equally valid (or rather invalid!) methods were not unusual (Silliker et al., 1979).

This shameful situation called for professional, bacteriological intervention. It came from two masters of the trade independently and almost at the same time. Seeliger in Germany (1952) and Henricksen in Norway (1955) suggested replacing the taxonomically ill-defined coli-aerogenes group by the accurately delineated Enterobacteriaceae. Their excellent suggestion was soon heeded in many countries (Mossel, 1967) and eliminated the inaccuracies in methods and interpretation that had previously marred the use of the coli-aerogenes group as marker organisms.

Nonetheless, even Enterobacteriaceae are not universal marker organisms. They can only be used in the sense of Wilson (1935), i.e. to reveal process failure in foods treated, e.g. by heat, for decontamination purposes. In that instance their presence in numbers exceeding predetermined levels indicates one or more of the following deficiencies: (i) inadequate processing for safety; (ii) post-process recontamination; (iii) temperature abuse during storage and/or distribution. In all other instances more precise tests are absolutely required (Mossel, 1982).

Recommended media

However dramatic the substitution of coli-aerogenes bacteria by Enterobacteriaceae as markers might have been with respect to consistency of results, the change it required in medium composition was trifling. All that was involved was replacing lactose by glucose and, when interpreting results, also including genera that would dissimilate glucose by a homofermentative ('anaerogenic') pathway. Consequently the use of the time-honoured selective media could in essence be continued, albeit with the slight adaptation just indicated.

The classical solid medium for this purpose is MacConkey's (1905) crystal violet bile salts neutral red agar. The liquid medium to be used is a glucose variant of Jordan's (1927) brilliant green bile salts broth, somewhat more strongly buffered than initially suggested (Mossel et al., 1963; Moussa et al., 1973).

Obviously these media should never be used immediately, because the bacteria they aim to detect have as a rule incurred sublethal lesions, resulting in an inability to develop in or on media containing surface active agents or triphenylmethane dyes (Ray, 1979; Mossel and van Netten, 1984). Resuscitation steps will, therefore, always have to precede the ultimate enumeration phase. Depending on the character and intensity of sublethal injury, resuscitation can be attained by a brief liquid medium repair (Mossel et al., 1980) or, when longer repair times are required (Mossel and Vincentie, 1969; Mossel and Harrewijn, 1972), by recovery on a suitable solid medium (Mossel and van Netten, 1984).

Whichever selective medium is used, it should always be decontaminated by pasteurization and never by sterilization. The latter already slightly harms the original lactose containing selective media. It renders the glucose variants almost useless, because the tendency to produce inhibitory Maillard-complexes (Ingram et al., 1955; Jemmali, 1969; Einarsson et al., 1983; Vadehra and Bellamy, 1983) is very much stronger with glucose than with lactose. There is, incidentally, also no need to sterilize media for Enterobacteriaceae; the only organisms that might survive heating

for about 30 min at 80–100°C are spore-bearing bacilli and clostridia which will be unable to develop in media containing bile salts as well as a triphenylmethane dye (Mossel et al., 1974).

Monitoring procedures

Solid media for Enterobacteriaceae can be inoculated by an ecometric procedure (Mossel et al., 1983; Lück and Lategau, 1983), spiral-plating (Donelly et al., 1976; Gilchrist et al., 1977; Jarvis et al., 1977), the spread-drop procedure or the elegant Miles-Misra multi-drop technique (Corry, 1982). Liquid media are usually tested by the dilution-to-extinction method (Richard, 1982).

Strains to be used for challenging should of course include Enterobacteriaceae of various degrees of robustness. In addition a collection of bacteria should be used that, ideally, should not grow on or in media supposed to be selective for Enterobacteriaceae. These should equally possess varying resistance to the inhibitors used in such media.

We found the following ten test strains most useful: (i) *Shigella flexneri, Yersinia enterocolitica, E. coli, Salm. gallinarum, Hafnia alvei;* (ii) *Pseudomonas aeruginosa, Streptococcus faecalis, Micrococcus* sp., *Staphylococcus aureus* and *Bacillus cereus* (Mossel et al., 1983). For collaborative assays carefully selected and maintained test strains should be used. These are already commercially available; it could be made the task of the IUMS Working Party on Culture Media to encourage their general availability (Peterz and Norberg, 1983).

Various suggestions have been made for interpreting the results of testing solid and liquid media for the selective enumeration of Enterobacteriaceae. The target values found attainable when colony counting or MPN-procedures are used are as follows:

- recovery of all Enterobacteriaceae within 0.5 $\log_{10}$ of the recovery on/in the non selective control medium, when tested after 18–24 h incubation at 30°C;
- recovery of non-Enterobacteriaceae (also incubated for 18–24 h at 30°C) with the exception of *Ps. aeruginosa*, less than 5 $\log_{10}$ cycles of the recovery on/in the control medium.

The choice of the control medium obviously has to be made very carefully, as non-selective media can also vary tremendously in productivity for a given strain (Orla Jensen, 1898; Pflug et al., 1979; Abdou and Stöckel, 1980; Pflug et al., 1981). Ideally the control medium should have the same nutrient, buffer etc. composition as the selective one, but of course the selective agents used in the medium under test must be omitted. When it has been demonstrated experimentally that a simple general purpose agar, e.g. tryptone soya agar, recovers all test strains to the same extent as the ideal control medium, then the former can be used, from then on.

In ecometric monitoring Enterobacteriaceae can invariably attain an absolute growth index (AGI, cf. Fig. 1) of at least 4, whereas the AGI of background strains, once more with the exception of *Ps. aeruginosa* will not exceed 2. The same

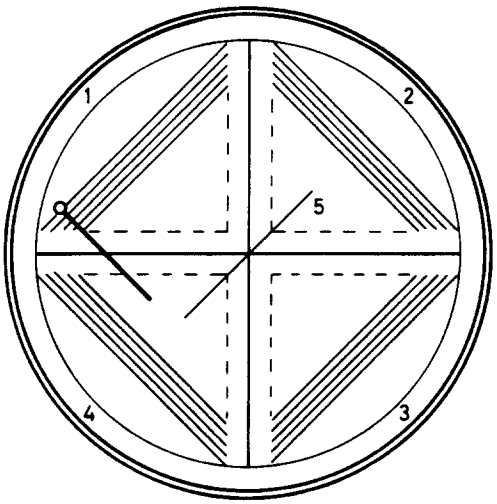

Fig. 1. Network serving as a guide for inoculation and interpretation in ecometric monitoring of culture media. When almost all streaks in quadrant number n and below show full growth while there is virtually no growth on any of the streaks in the quadrant numbers $n+1$ or more, the absolute growth index (AGI) equals n. When approximately half of the streaks in quadrant number n show growth while quadrant number $n+1$ displays virtually no growth and quadrant number $n-1$ shows full, or almost full growth, then the AGI equals $n-\frac{1}{2}$

provisions for the control medium as emphasized for colony counts and P-A-titres (Richard, 1982) clearly apply when ecometric evaluation is used.

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