

Recovery of stressed Coliforms from seawater samples

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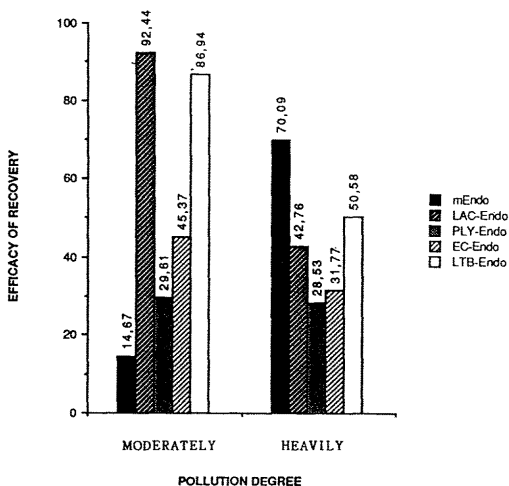
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The 16th edition of Standard Methods for the Examination of Water and Wastewater [1] and the World Health Organization guidelines [8] specified mEndo agar as the choice medium for coliform enumeration. Several authors have pointed out that this medium has several shortcomings, such as: (i) low recoveries of injured and stressed coliforms [4-6]; and (ii) poor differentiation among coliforms and non coliforms [2-3]. For this reason, a comparative study of the standard method for the enumeration of coliforms (MF and mEndo agar) and several resuscitation methods to recover the stressed coliforms from seawater has been the main objective of the present work.

Seawater samples were collected from ten beaches on the coast of Malaga (Spain). The membrane filtration technique was carried out as described by Standard Methods [1]. The filtrations were five-fold replicated for each one of the methods tested with 0.45µm membrane filters (Millipore Ibérica, Madrid, Spain). Phosphate-buffered saline [1] was used as diluent solution. Four resuscitation membrane filtration methods were conducted according to established procedures [4, 6, 7] using Millipore HC-type filters and the following resuscitation media: LAC broth [3]; PLY agar (Difco); EC broth (Difco); and LTB broth (Difco). When the media were liquids, filters were placed onto sterile pads (Millipore) saturated with sterile broth (LAC, EC or LTB), and incubated at 36°C for 2 h. The filters were then transferred to mEndo agar plates and incubated for an additional 22 h. The recovery efficacy of each medium is calculated with respect to the maximal count obtained for one medium and sample, applying the following equation: Relative Percentage of Recovery (Medium A) = (Count on medium A) / (Maximum count on any medium) x 100.

The comparison of the quantitative recovery of coliforms on the different media tested was carried out using 60 seawater samples with different faecal pollution degree (30 moderately polluted and 30 heavily polluted). The efficacy of recovery of each method is represented in Fig. 1. All the methods detected high percentages of coliforms from the samples analysed, except LAC-Endo resuscitation method, which obtained 1% of the coliform recovery from MIS sampling station in comparison with the best method. Statistically significant differences of the efficacy of recovery were obtained for each sample groups, moderately and heavily polluted seawater, and for the media mEndo, LAC-Endo and LTB-Endo. The best methods for the recovery of coliforms from moderately polluted seawater were LAC-Endo and LTB-Endo, with figures of 92.4% and 86.9% of recovery, respectively, in comparison with 14.7% obtained for the standard method (mEndo). On the other hand, for samples with high pollution degree, the best efficacy of recovery was obtained for mEndo agar (70.1%) in comparison with PLY-Endo and EC-Endo methods which achieved only percentages of 28.5 and 31.8%, respectively.

In short, it seems to be that the pollution degree of the samples affect significantly the recovery of stressed and non-stressed coliforms, being advised the use of the resuscitation methods for samples with a low or moderate pollution degree.



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