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Further applications of Tryptophan-Lactose-Bile-Medium (TLBM) for the rapid detection of Coli bacteria in milk and milk products

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Introduction

The Tryptophan-Lactose-Bile-Medium (TLBM) is a new medium prepared by REFAI and ROHDE (1972) for the rapid detection of Coli bacteria in ice-cream. This medium is composed of 0.7 g $MgSO_4 \cdot 7H_2O$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g NaCl, 1.0 g Tryptophan, 10.0 g casiton, 3.0 g lactose, 0.2 g bile salts and 850.0 ml dest. water. It is based on the ability of Coli bacteria to produce gas from lactose and indol from tryptophan; both can be tested in the same tube after incubation for 24—48 hours at 37 °C. The medium proved to be efficient in detecting Coli bacteria in ice-cream.

In the present work the medium was used for examination of milk, cheese, yoghourt and water for the presence of Coli bacteria.

Material and methods

The medium was prepared by dissolving the above mentioned ingredients in 850 ml dest. water and after adding bromthymol blue indicator the medium was distributed in 5 ml amounts in tubes containing inverted Durham tubes, and sterilized at 110 °C for 5 minutes.

Samples of milk, cheese and yoghourt, collected from markets, were diluted in NaCl solution to 1 : 10, 1 : 100 and 1 : 1000; from each dilution 1 ml was added to the TLBM. Incubation was made for 24—48 hours at 37 °C.

For examination of water, single and double strength TLBM were prepared by dissolving the above mentioned ingredients in 1000.0 ml dest. water (single strength) and the double amount also in 1000.0 ml dest. water (double strength). The single strength medium was distributed in 5 ml amounts and the double strength medium was distributed in 10 and 50 ml amounts. From the water sample to be examined 50 ml were added to 50 ml TLBM, 10 ml were added to each of 5 tubes of 10 ml TLBM and 1 ml was added to each of 5 tubes of 5 ml TLBM.

After 24—48 hours incubation at 37 °C indol reagent was added and in positive cases a pink ring was formed on the surface of the solution.

Results

All 20 milk samples were negative. The result was confirmed by inoculation of MacConkey and blood agar. On the other hand, in 18 out of the 20 samples of cheese examined acid was produced, of which only 16 produced gas and showed pink ring when indol reagent was added.

From these 16 samples Escherichia coli was isolated on MacConkey's medium. Most of these samples were positive in dilutions up to 1 : 1000.

Yoghourt was less contaminated. Only 11 out of 20 samples were positive, i.e. acid, gas and indol were produced.

Water samples collected from tap water (10 samples) were all free from Coli bacteria and the medium showed no change, also on MacConkey's medium there was no growth. In 10 samples of water collected from wells 1–7 organisms were found in 100.0 ml water according the probability tables of McCRADY cited by CRUICKSHANK (1972).

It is worthwhile to mention that tubes of TLBM inoculated with a big inoculum of E. coli and incubated at 37 °C and tested for indol production every 1/2 hour were found to give positive pink ring when the indol reagent was added 2 1/2 hours after incubation.

Discussion

From the results obtained in the present work as well as those obtained by REFAI and ROHDE (1972) it is clear that the TLBM is an efficient one for the detection of E. coli in milk and milk products as well as in water. This medium has the advantage, that in one tube 3 important criteria of E. coli, namely, acid, gas and indol production can be determined.

Trials to test the ability of E. coli to produce indol at 44 °C in TLBM were not successful and this point needs further study. The detection of indol production by E. coli after 2 1/2 hours incubation is interesting. This may help also in rapid differentiation of indol positive from indol negative organisms and accordingly the number of biochemical tests needed for further identification can be reduced. According to EDWARDS and EWING (1972) indol is produced only by E. coli, some Shigella serotypes, some Proteus and Providence as well as some Klebsiella, however, all of these, with the exception of E. coli and Klebsiella, are non-lactose fermenters.

Zusammenfassung

Das Tryptophan-Laktose-Galle Medium wurde zunächst von REFAI und ROHDE für den raschen Nachweis von Coli-Bakterien in Eiskrem entwickelt.

In der vorliegenden Arbeit wurde die Verwendung dieser Methode beim Nachweis von Coli-Bakterien in Milch, Käse, Joghurt und Wasser geprüft.

Alle 20 Milchproben waren negativ, dagegen erwiesen sich 18 von 20 Käseproben als positiv für Säurebildung, 16 bildeten Gas und einen roten Ring nach Zusatz von Indol-Reagenz; aus diesen 16 Käseproben wurde auf MacConkey's Medium Escherichia coli isoliert.

11 von 20 Joghurtproben waren positiv, die 20 Wasserproben dagegen sämtlich negativ.

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