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Tryptophan-Lactose-Bile Medium for the Rapid Detection of Coli-Bacteria in Ice-Cream

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Summary. A new medium for the detection of *E. coli* in milk and milk products has been developed. The medium contains tryptophan, lactose, and bile salts, and is based on the ability of *E. coli* to produce gas from lactose and indol from tryptophan. 142 *E. coli* test strains and 47 ice-cream samples were examined. The medium proved to be efficient in detecting the *E. coli*.

Zusammenfassung. Ein neues Medium für den Nachweis von *E. coli* in Milch und Milchprodukten ist entwickelt worden. Das Medium enthält Tryptophan, Lactose und Gallensalz und basiert auf der Fähigkeit von *E. coli*, Gas aus Lactose und Indol aus Tryptophan zu produzieren. 142 *E. coli*-Versuchsstämme und 47 Eisproben wurden geprüft. Das Medium erwies sich wirksam beim Nachweis von *E. coli*.

Introduction

The presence of coli-bacteria in milk and milk products is detected by using fluid media, such as brilliant green, gentian violet or triphenyltetrazoliumchloride solutions. The detection of coli-bacteria in these media is based on their ability to ferment lactose and to produce gas from it. As other bacteria can do the same, at our Institute we always inoculate an Endo-plate from all tubes with gas production and identify the grown organisms biochemically.

In this paper we present a new prepared medium for the rapid detection of coli-bacteria. This medium is also based on gas production from lactose, but in addition and simultaneously it enables the detection of indol.

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Experimental Part

1. *Media*: The following media were prepared:

a) Tryptophan-lactose-bile solution (TLB) composed of 0.7 g $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 1.0 g KH_2PO_4 , 1.0 g K_2HPO_4 , 1.0 g NaCl, 1.0 g DL-tryptophan, 10.0 casiton, 3.0 g lactose, 0.2 bile salts, and 850.0 ml dest. water.

b) Tryptophan-lactose-bile solution + 0.0133 g/L brilliant green.

2. *Bacteria*: 142 different *E. coli* serotypes were used in this test. The test strains were obtained from the International Escherichia Center (Dr. F. Ørskov, Kopenhagen).

3. *Ice-Cream*: 87 samples of different kinds of ice-cream.

The TLB solution, or the TLB solution + brilliant green, was distributed in 5 ml quantities in test tubes with inverted Durham's tubes and then sterilized by autoclave for 5 min at 110° C (0.9 atu).

In order to test the efficiency of the media, both solutions were inoculated with all known serotypes of *E. coli* (142 strains). The inoculated tubes were incubated for 24 hrs. at 37° C and then examined for gas production in the Durham's tubes. A few drops of indol reagent were added to those tubes showing gas production. In positive cases, i.e. presence of indol, a pink ring formed on the surface of the solution.

In order to demonstrate the usefulness of these media when examining foods for the presence of *E. coli*, we tested 87 ice-cream samples. These samples were obtained from the Food Control Laboratory in our Institute, and 47 of these samples were *E. coli* negative and 40 samples contained *E. coli*. (In our Food Control Laboratory the samples are inoculated on gentian-violet medium, from tubes with gas a selective medium is inoculated and the organisms are identified biochemically.) The ice-cream samples were diluted in NaCl solution to 1:10, 1:20, 1:100, and 1:1000. From each dilution one ml was added to TLB solution and TLB + brilliant green.

Results

TLB solution: of the 142 *E. coli* serotypes, 134 serotypes produced gas in Durham's tubes and were indol positive. Six strains produced gas but were indol negative. Two strains did not produce gas but were indol positive. The indol negative strains were the *E. coli* serotypes 039, 040, 062, 095, and 0142. The *E. coli* serotypes 086 and 0144 produced no gas.

TLB solution + brilliant green: The addition of brilliant green to the TLB medium affected the gas and indol formation. On this medium, gas was produced by only 117 strains of which 102 were indol positive. Fifteen strains produced no gas and were indol negative (Table 1). 62 *E. coli* serotypes decolorized the brilliant green.

At the same time all 142 *E. coli* serotypes were tested for lactose fermentation and production of gas by using 1% lactose in peptone water. 139 serotypes produced acid and gas after 24 hrs. incubation at 37° C. The serotypes 014, 0124, and 0130 were positive after 48–72 hrs. incubation.

Table 1. Examination of 142 *E. coli* serotypes on TLB and TLB + brilliant green for gas and indol formation

Test	TLB solution	TLB solution + brilliant green
gas + indol +	134 serotypes	102
gas + indol —	6 serotypes	16
gas — indol +	2 serotypes	9
gas — indol —	— serotypes	15
Total nos. of strains	142	142

Examination of ice-cream: The results obtained with the TLB solution agreed with those of our conventional method used in our Food Control Laboratory. 50 samples produced gas, of which only 40 were indol positive. 37 samples were negative for gas and indol. On the TLB-solution + brilliant green the gas and indol formation were positive mostly in dilutions of 1:10 and 1:20 and maximally in dilution of 1:100, whereas on TLB medium without brilliant green, positive gas and indol could be seen in most of the positive samples in dilution 1:1000.

Discussion

The TLB medium prepared by us is recommended for the detection of *E. coli* contamination of milk and milk-products. It is based on the ability of *E. coli* strains to produce gas from lactose and indol from tryptophan. It is known that indol formation is inhibited by the presence of sugars [1, 2], therefore, we added only 3 g lactose/l. This amount of lactose is sufficient to detect gas production and does not affect indol formation.

From the results obtained it is clear that the TLB medium prepared by us is very efficient for the detection of *E. coli* in foodstuff (e.g. ice-cream) on the basis of gas and indol production. Because brilliant-green is widely used in fluid media, we added brilliant green to our TLB medium in the usual concentration (0.0133 g/l). We have found that brilliant green has an inhibitory effect on gas and indol production and, therefore, we recommend the preparation of TLB medium without brilliant green for the examination of milk and milk products for *E. coli* contamination. Especially in laboratories who receive daily a large number of samples, the TLB medium helps to save time, material, and work in comparison with the conventional method.

Although the TLB ingredients should be dissolved in 1000 ml dest. water, for the examination of ice-cream we dissolved them in only 850 ml dest. water because the medium diluted when inoculated with 1 ml ice-cream suspended in NaCl.

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