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## Development of enzyme substrate assay for monitoring E. coli/E. coli O157:H7 in milk

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Introduction: E. coli is used as indicators of faecal contamination of water and food and is of most interest to clinical, food and water microbiologists. Detection of E. coli / E. coli O157:H7 in dairy products still relies on conventional method requiring upto 3-4 days protocol for its identification. In order to meet the emerging demand of dairy industry, an attempt was made to develop a rapid enzyme substrate assay its detection in milk. Initially, commercial media available in the market were screened and Tergitol-7 broth was selected based on better sensitivity and selectivity of target organism.

Material and Methods: Screening of antimicrobials was carried out for their MIC and antibiotics were supplemented for selective inhibition of Gram negative contaminants other than E. coli and growth promoter like KCl was also added to promote the growth of target organism. The modified medium was further developed into a chromogenic medium by addition of selected substrates at optimized concentration and volume. Assay was optimized with pure cells of E. coli /E. coli O157:H7/K-12 and its sensitivity was determined by inoculating different log cells i.e. 1.0 to 8.0 log cfu/ml in novel medium and kept for incubation at 37°C and color change was observed.

Results: E. coli/ E. coli O157: H7/K-12 showed color reaction at 1.0 log cfu/ml while contaminants which include Salmonella, Shigella, Enterobacter, Proteus and Klebsiella failed to give color reaction up to 5.5 log cfu/ml. The growth of B. megaterium and L. monocytogenes could be restricted upto 3.16 log and 3.13 log counts respectively. Further confirmation of E. coli O157:H7 was ascertained based on the fact that it is GUD negative. Negative reaction for GUD enzyme along with GAL positive reaction gives the confirmation of E. coli O157:H7 in the developed test method.

Significance: MT-7 was developed as a medium of choice and can be used for selective enumeration / or detection of E. coli / or E. coli O157: H7 on the basis of marker enzyme-substrate reactions. This assay has shown promising results with milk system and can be used for selective detection of E.coli / or E.coli O157:H7 as one step process within 10±1 h.which will a boon for detection of such pathogens for public health.

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