DEVELOPMENT OF E. COLI ON LEVINE CULTURE MEDIUM

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RESEARCH ARTICLE

Abstract

E. coli is a short, gram-negative bacillus with rounded ends, non-sporulating, non-capsulated, generally motile. It is aerobic, facultatively anaerobic. It is part of the normal flora of the intestine in humans and animals, representing approximately 80% of the resident, aerobic flora of the colon. It plays an important role in the synthesis of certain B and K group vitamins and in maintaining balance in the intestinal biocenosis. They are aerobic, facultatively anaerobic, and nutritionally non-demanding bacteria. They grow on both usual and selective lactose-containing media, forming lactose-positive colonies. The colonies are of the S type, and pseudocapsulated strains form colonies with a mucous appearance. They exhibit common biochemical characteristics of enterobacteria. It is noteworthy that out of 100 strains of E. coli, only 95 ferment lactose. Biochemical characteristics are investigated using a set of biochemical tests that allow for the identification of the genus.

Keywords: Germen, aerobes, Gram-negative

INTRODUCTION

The antigenic structure of E. coli is complex, with numerous O, H, and K antigens described. Based on O antigens, the bacilli are divided into serogroups, and based on H antigens, the groups are divided into serotypes. There are 165 O antigenic groups of E. coli identified, 103 K antigens, and 54 H antigens.

They are conditionally pathogenic bacteria, being the most frequently isolated bacteria in the bacteriology laboratory.

Enterotoxigenic E. coli (ETEC) secretes thermolabile or thermostable enterotoxins encoded plasmidically. A strain of ETEC produces one or both toxins. In addition to toxin production, the ability to colonize the small intestine through adhesion pili also plays a role. ETEC produces mild forms of enteritis or a cholera-like diarrheal syndrome.

Enteropathogenic E. coli (EPEC) is the main etiological agent of diarrheal syndrome in young children, leading to early immunization. Therefore, illnesses caused by EPEC in children older than 2 years are rarely reported. The pathogenic factors are adherence pili, encoded plasmidically, and a Shiga-like toxin produced through lysogenic conversion. This results in the destruction of enterocytes in the small intestine. Enterohemorrhagic E. coli (EHEC) produces two Shiga-like toxins, called verotoxins, because they exhibit a cytopathic effect on Vero cell line. Initially, watery diarrhea occurs, which in a few days becomes bloody, and the mucosa of the rectum and sigmoid colon becomes friable and bleeds. Fever is low or absent. Hemorrhagic colitis frequently complicates into hemolytic uremic syndrome. The disease predominantly occurs in the warm season, in children under 5 years old, through consumption of inadequately cooked beef and unpasteurized milk. Approximately half of EHEC belongs to serotype O157: H7. Enteroaggregative E. coli (EAEC) exhibits the peculiarity of "aggregatively" binding to enterocytes. Diffusely adherent E. coli (DAEC) has a controversial role in diarrheagenesis, as the only known virulence factor in this pathotype is the diffuse adherence to HeLa cells and intestinal epithelial cells in cultures. Diffuse adherence and cellular invasion are believed to be the cause of the diarrheal syndrome produced by this pathotype.

The infective doses of diarrheagenic E. coli pathotypes are on the order of 10^8 ingested bacteria. Transmission occurs primarily through the consumption of food in which E. coli has multiplied (foodborne toxoinfections) or through the consumption of water with intense fecal contamination. The reservoir of infection for strains of EIEC, ETEC, and EPEC is human, while for those of EHEC, it is bovine.

MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the medical analysis laboratory, S.C. Diaser, Oradea. To conduct the study, I also accessed the archive, recorded in the laboratory's computer program in S.C. Diaser, Oradea, as well as the computerized database of the unit.

Materials required for the examination:

- A collection container (a collection container with a spoon) with transport medium
- Wooden spatulas
- Latex gloves

For a stool culture, a fecal sample of 5-10g should be collected and placed in the collection container with a transport medium. If the stool is liquid, 5ml should be collected. It is recommended to choose a liquid, mucous, or bloody portion, if available. Do not collect quantities greater than 10g, as they may reduce the chances of isolating pathogenic bacteria.

• The sample is inoculated onto two culture media, one weakly selective (MacConkey) and one moderately selective (Hektoen), and incubated for 24 hours at 35-37°C. The cultures are observed at 24 and 48 hours for the appearance of characteristic colonies. For the genus Vibrio, the recommended selective medium is BSA (bile salts agar), and for yeasts, Sabouraud medium with Chloramphenicol is used.

• To increase the chances of isolation, the sample is subcultured on enrichment media that promote the multiplication of pathogens (selenite sodium acid broth for Salmonella spp., alkaline peptone water or broth with taurocholate and peptone at pH 8.0-9.0 for Vibrio, from which after incubation, smears and cultures can be performed from the upper part of the medium). Incubation is done for 24 hours at 35-37°C, followed by transfers to culture media.

RESULTS AND DISCUSSIONS

Colonies of E. coli can grow on either solid or liquid growth media under laboratory conditions. It can be cultivated in a minimal medium that includes glucose as a carbon and energy source, ammonium salts as a nitrogen source, other salts, and trace elements. Because E. coli has simple nutritional requirements, it can be easily cultured on common media such as nutrient agar, MacConkey agar, and EMB agar.

MacConkey agar is a selective and differential medium as it inhibits the growth of Gram-positive bacteria and allows the growth of Gram-negative ones, such as E. coli. It also allows differentiation between bacteria that ferment lactose and those that do not.

Eosin-Methylene Blue agar (EMB), similar to MacConkey agar, is both selective and differential for Gram-negative bacteria. E. coli can form characteristic metallic green colonies on this medium.

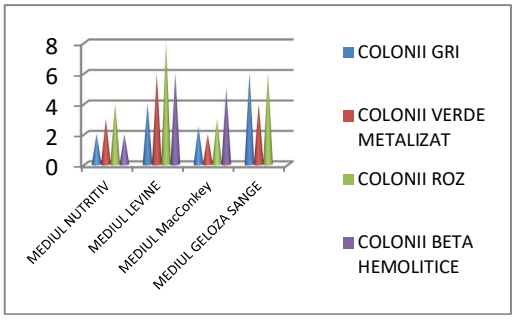
Salmonella-Shigella agar (SS) is a selective medium used for isolating Salmonella and Shigella but also allows the growth of E. coli. E. coli bacteria can form colorless or pink colonies on this medium.

Hektoen Enteric agar (HE) is another selective and differential medium used for isolating Salmonella and Shigella but can also allow the growth of E. coli. E. coli colonies can appear pink on this medium.

Luria-Bertani agar (LB) is a nutrient-rich culture medium commonly used in research laboratories. It is excellent for the rapid growth of E. coli and is often used in genetic and microbiological experiments.

E. coli thrives on culture media optimized at a temperature of 37°C. On simple media, it grows promptly within 24 hours and forms either smooth (S) or rough (R) colonies. On blood agar, some strains of E. coli cause incomplete hemolysis.

On media containing inhibitors, E. coli differentiates both through tolerance to various inhibitors and its ability to ferment lactose or other sugars. E. coli forms characteristic lactose-positive colonies. Consequently, it grows well on weakly selective media, experiences partial inhibition on most moderately selective media, and does not grow on highly selective media. It is partially inhibited on enrichment media. E. coli produces gas from glucose in moderate amounts and does not produce hydrogen sulfide. It acidifies the slant portion of the TSI medium.



On Nutrient Agar culture medium, large, convex, grayish-white, moist, smooth, opaque, or translucent colonies are formed. Smooth forms, termed S-type colonies, seen in fresh isolation, are easily emulsifiable in saline solution. Rough colonies, termed R-type, observed in older cultures, have wrinkled surfaces, often self-agglutinate in saline solution. The variation

between S and R colonies arises due to repeated subculturing and is associated with the loss of surface antigens and typically virulence.

Some strains exhibit beta hemolysis, especially those isolated from pathological states, while those isolated from asymptomatic individuals may or may not exhibit hemolysis on blood agar.

Colonies appear pink due to lactose fermentation, which is important for distinguishing E. coli from other bacteria in specimens, especially Gram-positive bacteria and Salmonella species, which are non-lactose fermenters and produce colorless colonies on MacConkey agar.

E. coli colonies grow with a metallic green sheen, attributable to the metachromatic property of the dyes and the lactose fermentation property of E. coli, which shifts the medium pH towards acidic.

In the study "Growth and maintenance of laboratory strains of Escherichia coli," E. coli, a member of the Enterobacteriaceae family, grows optimally at 37°C under aerobic conditions, although it is facultatively anaerobic and therefore can grow under anaerobic conditions. Additionally, it has been previously reported that some strains of E. coli have been known to grow at temperatures up to 53°C, although this is not typical nor recommended for commonly used laboratory strains. E. coli is a relatively robust bacterium and can survive at 4°C for extended periods (up to 3 months) on solid media, although prolonged storage times at low temperatures can lead to decreased viability. E. coli can also grow over a wide range of pH levels; typical growth and maintenance occur at a neutral pH of 7.0. Considering all optimal growth conditions (i.e., 37°C, aeration, pH of 7.0), a doubling time of approximately 20 minutes should occur when E. coli is cultivated in a rich liquid medium such as Luria-Bertani broth, and it will reach an overnight cell density of >109 cfu/ml (colony forming units per milliliter) of culture.

CONCLUSIONS

E. coli is known for its rapid growth rate in chemically defined media and its adaptability, making it easy to handle. It grows optimally at 37°C under aerobic conditions, although it is facultatively anaerobic and can therefore grow under anaerobic conditions. Additionally, it has been previously reported that some strains of E. coli have been known to grow at temperatures up to 53°C. Some strains exhibit beta hemolysis, especially those isolated from pathological states, while those isolated from asymptomatic individuals may or may not exhibit hemolysis on blood agar.

Widely used in research, E. coli serves as a model organism for investigating protein engineering for biological processing, genetic research, and biotechnology. Its versatility continues to pave the way for future investigations.

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