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Varying Morphology of *E.coli* - an Experimental Study

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A B S T R A C T

Escherichia coli is a Gram negative facultative anerobe which is rod shaped and found in the gut of warm blooded animals. Since this rod shaped bacteria showed varying morphology in different environmental conditions, an experimental study was conducted to find out the changes in the morphology of the bacteria in different culture media. *E.coli* was seen as typical, uniformly stained long gram negative rods only when it was cultured on MacConkey agar

Introduction

Escherichia coli (*E.coli*) is a coliform bacilli found abundantly in the intestine of warm blooded animals. (Tenaillon *et al.*, 2010) It is a Gram negative bacilli which is about 2 to 3 microns long and 0.25 to 1 micron broad. Optimum growth of E. coli occurs at 37°C. It can be grown in any media that contains Glucose, ammonium phosphate monobasic, sodium chloride, magnesium sulphate potassium phosphate dibasic, and water. (Madigan and Martinko, 2006) It is called a facultative anaerobe which can grow by aerobic and anaerobic respiration using a variety of redox pairs including oxidation of pyruvic acid, amino acids, hydrogen and formic acid. It also cause reduction of substrates like nitrate, oxygen, fumarate, dimethyl sulfoxide and trimethylamine N oxide. Since the bacilli showed varying morphology when isolated from different sources, an experimental study was conducted to find out the factors influencing the morphology of *E.coli*.

Materials and Methods

Standard *E.coli* ATCC 25922 was used for the present study. The bacilli was inoculated on enriched media like - Blood agar and Chocolate agar; MacConkey agar; Nutrient agar; Muller Hinton agar; Liquid media like- Peptone water and Glucose broth; Sugar containing media like - Triple sugar iron agar and Mannitol motility media and anaerobic media like Robertson's cooked meat medium. All the media were incubated at 37^{O}C for 24 hours. The Gram staining of *E.coli* was done from the growth that appeared in all these media.

Results and Discussion

E.coli is a non fastidious organism hence there was abundant growth of the bacilli in all the media studied here.

Enriched media

E.coli grew as greyish white moist colonies on Blood agar and Chocolate agar. The gram stain of the colonies from these media had similar morphology. The bacilli were uniformly stained thin, short and some appeared like gram negative cocci (Figure.1).

MacConkey agar

The morphology of *E.coli* was best appreciated when it was grown on MacConkey agar. The bacilli appeared uniformly stained, long and slender. (Figure 2)

Sugar media

The morphology of *E.coli* grown on the sugar media like Triple sugar iron agar and Mannitol motility agar appeared similar. The gram stain showed thick bacilli of varying lengths. (Figure 3) *E.coli* consume sugars through a process called catabolite repression (Ammar *et al.,* 2018). They usually consume the sugars yielding the highest growth rate first. The growth is maximized by sugars, perhaps that is leading to increase in thickness and length of the bacilli in sugar containing media.

Nutrient agar and Muller Hinton agar

E.coli grown in these media looked similar. They showed pleomorphic bacilli which were thin without much outstanding features.

Liquid media

The *E.coli* grown in peptone water and glucose broth showed bacilli of varying sizes from thin slender, long rods to short, thin bacilli. There were short chains of bacilli. Some bacili showed granules inside. The exact scientific reason for this could not be postulated and has to be investigated further. One of the probable reasons could be the fast growth rate in liquid media. The bacterial cell cycle is divided into three stages. The B period occurs between the completion of cell division and the beginning of DNA replication.

Fig.1 Gram stain of *E.coli* from blood agar

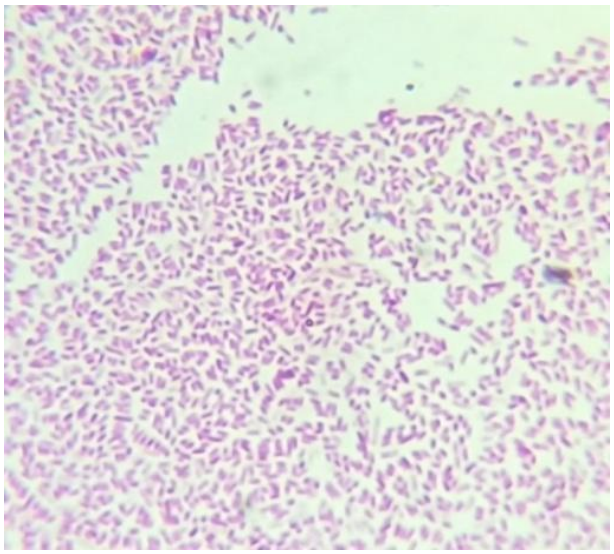


Fig.2 Gram stain of *E.coli* taken from MacConkey agar

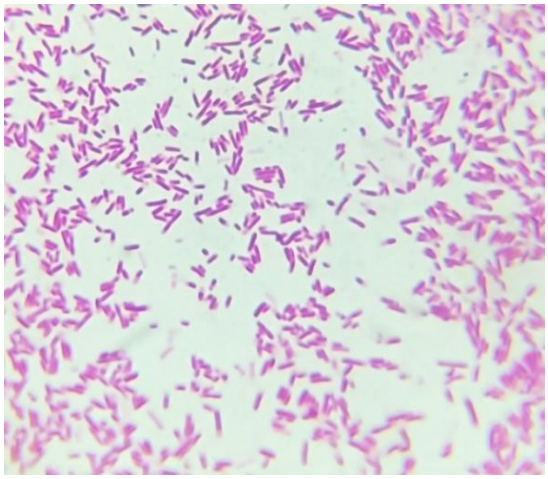
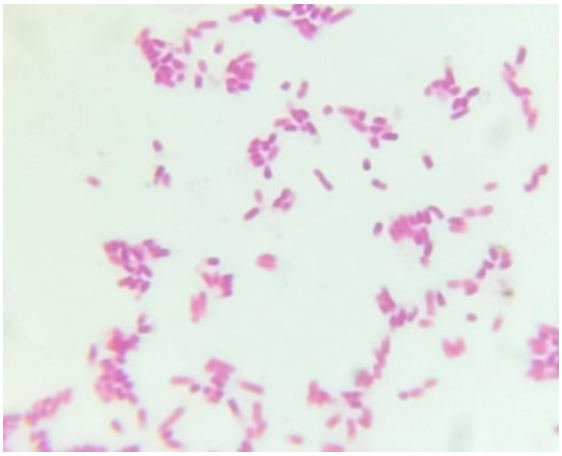


Fig.3 The gram stain of *E.coli* taken from sugar media



The C period encompasses the time it takes to replicate the chromosomal DNA. The D period refers to the stage between the conclusion of DNA replication and the end of cell division.

The doubling rate of *E. coli* is higher when more nutrients are available. However, the length of the C and D periods do not change, even when the doubling time becomes less than the sum of the C and D periods.

At the fastest growth rates, replication begins before the previous round of replication has completed, resulting in multiple replication forks along the DNA and overlapping cell cycles. During these multiple replication forks, maybe cell wall deficiency occurs at multiple sites at the point of binary fission. This might cause an uneven staining of the bacilli, leading to a look of granules inside the bacilli.

Anaerobic media

Robertson's cooked meat media was used. Here the bacilli had good morphological features like uniform staining, long slender bacilli but was not as good as the morphology seen in MacConkey agar.

It was finally seen that *E.coli* had the best morphology when it was taken from MacConkey agar. Perhaps the presence of bile salts were providing an environment similar to that of the gut

of animals and helping the bacilli to grow at it's best natural environment.

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