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D-ARABINOSE INDUCED GROWTH PATTERNS OF SOME GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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ABTRACT

D-Arabinose is usually absent in natural habitat and thus not usually metabolized by bacteria. However, it was found that mutants of bacteria can utilize it as a sole source of carbon as well as energy, particularly when there is mutation of L-fucose pathway. Usually bacteria degrade L-fucose to L-lactaldehyde and dihydroxyacetone phosphate by the enzyme L-fucose permease and some other enzymes like L-fucose isomerase, L-fuculokinase and L-fuculose-1-phosphate aldolase. In mutants there is formation of D-glycoaldehyde instead of L-lactaldehyde. In this study we aimed to find out patterns of growths of some gram positive and gram negative bacteria in different concentrations of D-arabinose in microtitre plates by observing optical density changes at different incubation periods in an ELISA reader at 620 nm. It was found that a concentration of D-arabinose of 2.1 mg/mL appeared to be optimum for almost all types of tested bacteria. Utilization of D-arabinose by *E. coli* was less than the other tested bacteria like *Staphylococcus*, *Salmonella* and *Shigella* spp. There is no significant difference between gram positive and gram negative bacteria for utilization of D-arabinose. The growth was found maximum at 4 hours of incubation at 37^{O}C and in all cases the growth declines with lysis of bacteria in 24 hours. This study thus indicates that the bacteria recognize D-arabinose as inducer for their growth on D-arabinose.

KEYWORDS: D-arabinose, bacteria, L-fucose.

INTRODUCTION

In natural environment D-arabinose is usually not found and thus it is not usually metabolized by bacteria; but when mutatation occurs they may utilize it as a sole source of energy. In bacteria L-fucose pathway commonly active and in this pathway (Fig.1) L-fucose first degrades to L-fuculose by the action of the enzyme L-fucose isomerase; L-fuculose then converted into L-fuculose-1-phosphate by the enzyme L-fuculokinase. Finally L-fuculose-1-phosphate is broken down to L-lactaldehyde and dihydroxyacetone phosphate by the enzyme L-fuculose-1-phosphate aldolase. Thus L-lactaldehyde is normally produced in almost all bacterial cells. Now if by mutation or by any other way L-fucose is not metabolized to L-acetaldehyde then all these enzymes almost equally target D-arabinose which is almost of similar structure (Fig 2) and metabolize it to glycaldehyde. The successive pathway are D-arabinose is converted to D-ribulose by the enzyme L-fucose isomerase; D-ribulose then converted to D-ribulose -1-phosphate by L-fuculokinase and finally D-ribulose-1-phosphate is broken down to glycaldehyde and dihydroxy acetone phosphate by the enzyme L-fuculose-i-phosphate aldolase (Fig 3). The efficacy of the enzymes acting on alternative substrates particularly levo variety to dextro variety is peculiar e.g. L-fucose isomerase enzyme can act on many substrates like L-fucose, D-arabinose, D-altrose, L-galactose etc. A detailed description of all these pathways are available in an important publication by Elsinghorst and Mortlock in 1988. Although the action is almost equal but it may differ bacteria to bacteria and also it may vary on the concentration of the D-arabinose in the medium. As mutations are universal in bacterial population we may expect some utilization of D-arabinose in different bacterial population. There must be also optimum utilization at a defined time period in bacterial growth cycle. To find out probable solutions of this paradox this study has been undertaken.

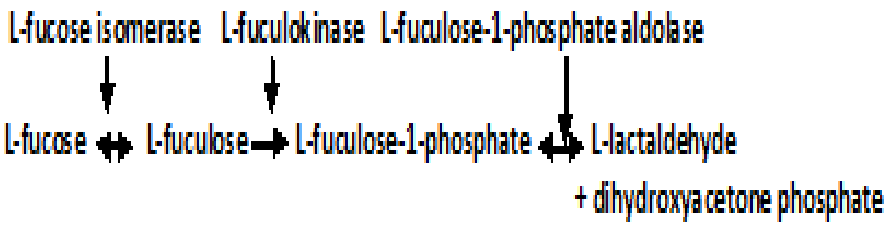

Fig. 1 Metabolism of L-fucose

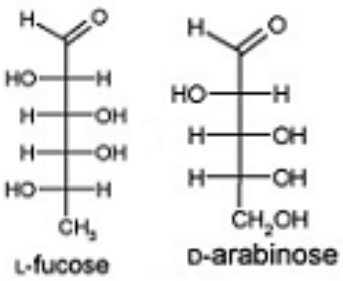

Fig 2. Similarities of the structures of L-fucose and D-arabinose

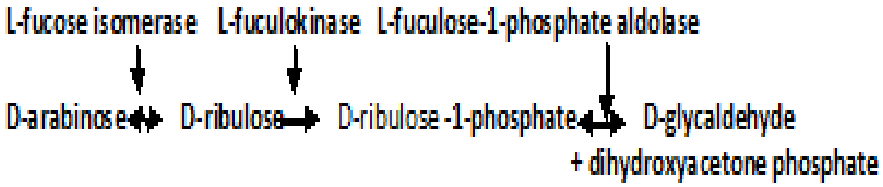

Fig. 3 Metabolism of D-arabinose

MATERIALS AND METHODS

Bacterial strains

Among gram positive bacteria we selected *Staphylococcus aureus*; among gram negative bacteria we selected *Escherichia coli*, *Salmonella* spp. and *Shigella* spp. All these bacteria were clinical isolates and identified by the Vitek system. All cultures were stocked in the laboratory and before the experiment were checked by biochemical and other specific tests. During the experiment 0.5 Macfarland standard opacity inoculums was used in sterile normal saline.

D-arabinose

it was procured from Hi media and stocked in a cool dark place. Different concentrations of D-arabinos were used which was achieved by serial double dilutions of a mother stock solution in sterile normal saline in microtitre plate (Chatterjee *et al*, 2015, Chatterjee *et al*, 2016). Following this 10µL of each bacterial suspensions in sterile normal saline was added in each well containing different concentrations of D-arabinose solutions and then the microtitre plate was incubated at 37^0c in an incubator. Following incubation optical density readings wer taken at 620nm in an ELISA reader at 0 hr, 4 hrs and at 24 hrs. The changes of the optical densities were plotted in graphs and analysed.

RESULTS

All results are given in Fig. 4-8. A concentration of D-arabinose of 2.1 mg/mL appeared to be optimum for almost all types of tested bacteria. Utilization of D-arabinose by *E. coli* was less than the other tested bacteria like *Staphylococcus*, *Salmonella* and *Shigella* spp. There is no significant difference between gram positive and gram negative bacteria for utilization of D-arabinose. The growth was found maximum at 4 hours of incubation at 37^{O}C and in all cases the growth declines with lysis of bacteria in 24 hours.

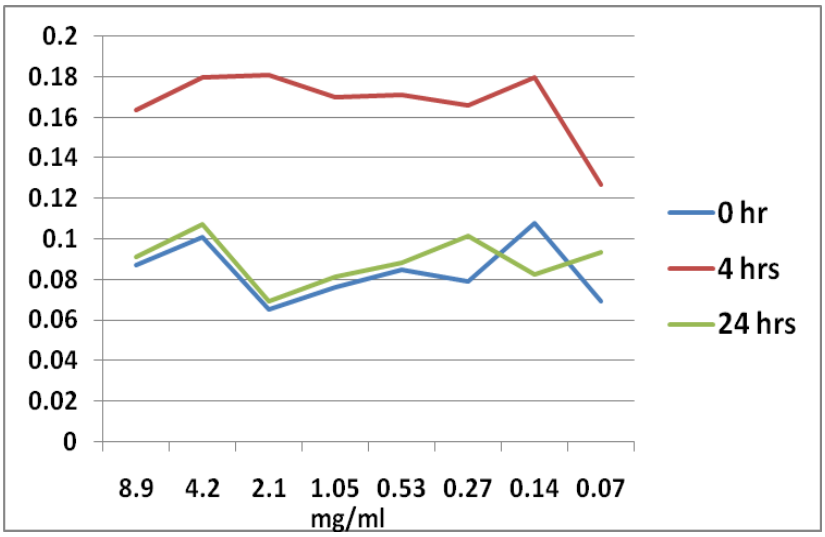

Figure 4: Optical density changes (Y axis) due to *Staphylococcus aureus* growth in different concentrations of d-Arabinose.

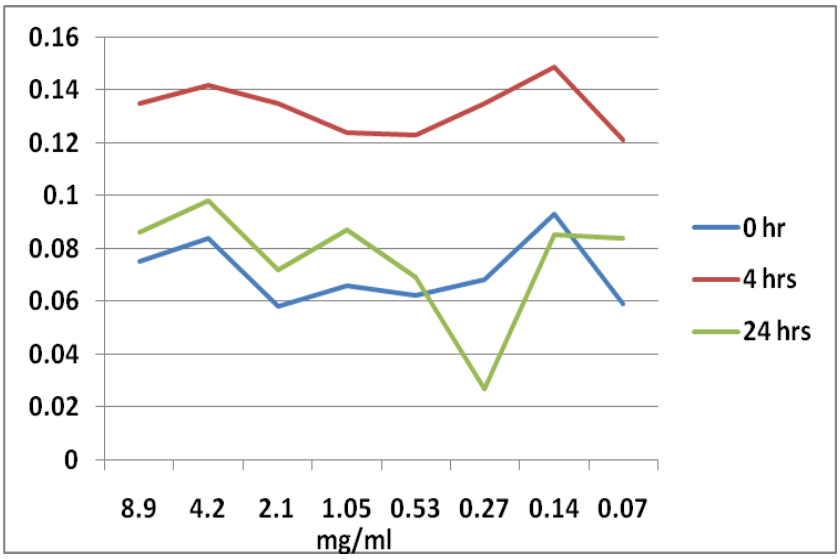

Figure 5: Optical density changes (Y axis) due to *Escherichia coli* growth in different concentrations of d-Arabinose.

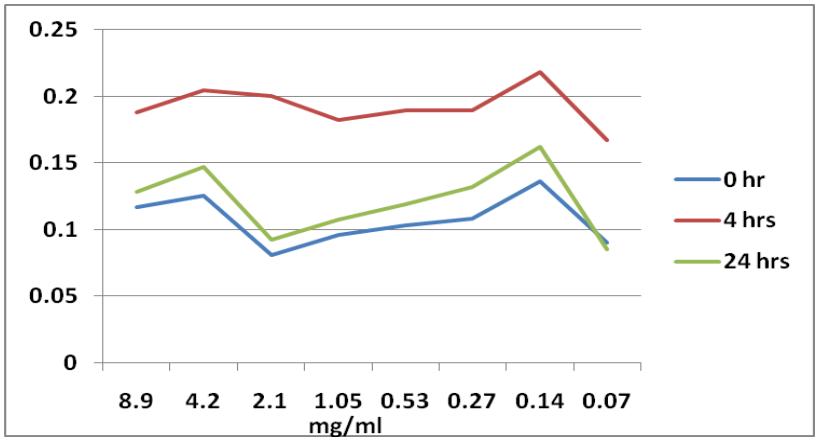

Figure 6: Optical density changes (Y axis) due to *Salmonella spp.* growth in different concentrations of d-Arabinose.

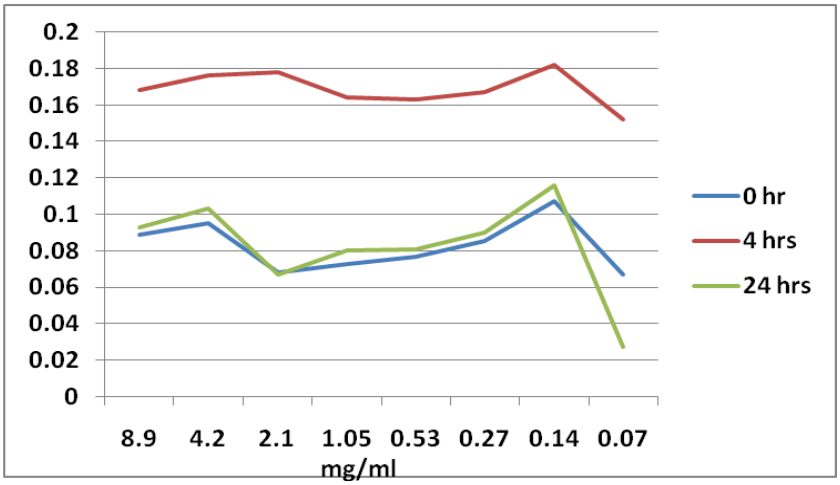

Figure 7: Optical density changes (Y axis) due to *Shigella dysentery* growth in different concentrations of d-Arabinose.

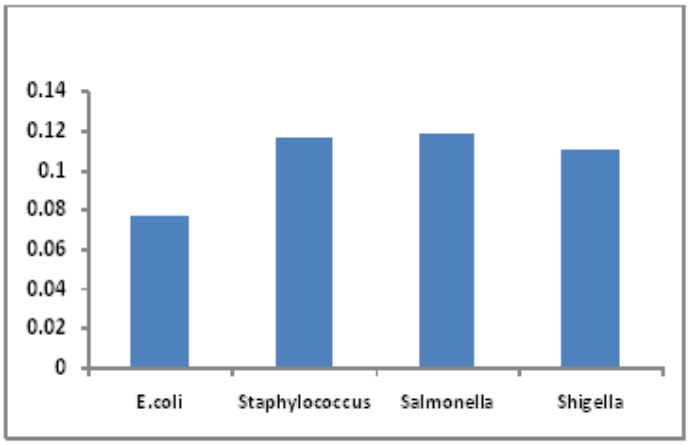

Fig. 8 : Optical density changes due to growth of different bacteria in D-arabinose as a sole source of carbon from zero hour to four hours..

DISCUSSION

It is well known that enzymes in sugar metabolic pathways are expressed after induction by their equivalent sugar or by an intermediate in a down pathway (Mayer and Boos, 2008), but when multiple sugars induce a common pathway then our knowledge regarding this is extremely poor. Similar is the case for D-arabinose which shares a common pathway with L-fucose. In bacteria if inducer arabinose is absent then Ara C binds upstream of PBAD (BAD promoter gene) resulting inhibition of transcription. When arabinose is added there is derepression of the promoter (Guzman *et al*, 1995; Zhang *et al*, 1996).. In alternative pathway metabolizing D-arabinose, pentose phosphate pathway is extremely useful as D-ribulose which is formed from D-arabinose is a natural intermediate of the ribitol pathway. It is important to note that mutant strains can utilize more D-arabinose than the natural strains resulting more rapid growth rate. The presence of ribitol operon in bacteria help utilization of D-arabinose although D-ribulokinase activity is not associated with ribitol operon. (Reiner, 1975). Bacteria recognizes D-arabinose as an inducer for growth on D-arabinose. This experiment clearly showed that in general, bacteria can utilize D-arabinose particularly when given in optimum concentration although it may be contributed by mutants present in clinical isolates and this evolution is somewhat away from the original L-fucose pathway indicating a crucial evolutionary step in nature.

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