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A STUDY ON BIOCHEMICAL INTERACTION OF *E.coli*, *Lactobacillus delbrueckii* AND *Bacillus subtilis* ON THE BASIS OF STRIKING CHARACTERISTICS

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ABSTRACT

Bacteria are the most abundant of all organisms and are the only ones characterized by prokaryotic cellular organization. Life on earth could not exist without bacteria because bacteria make possible many of the essential functions of ecosystems, including the capture of nitrogen from the atmosphere, decomposition of organic matter, and, in many aquatic communities, photosynthesis. In our study a combination of the three bacteria species viz. *Escherichia coli, Lactobacillus delbrueckii* and *Bacillus subtilis* studied biochemical assay individually and mixed culture. When cultured together *E.coli* and *Lactobacillus delbrueckii* gave same results as pure cultures except being positive for citrate utilization. When *Lactobacillus delbrueckii* and *Bacillus subtilis* were cultured together the motility was decreased considerably along with significant loss of carbohydrate fermentation activity. The mixed cultures of *Bacillus subtilis* and *E.coli* showed inability to ferment sucrose while rest of the tests were same as pure cultures. The mixed culture obtaining all three bacteria gives positive results for all the tests, thus compensating for each other's weakness. Thus a mixture of all three of these may prove beneficial for the internal environment of gut in animals. Our studies conclude that the behaviour of three bacteria species which are considered to be enteric bacteria and usually form the normal flora in the gut of living organism including human.

KEYWORDS: Aquatic Communities, Decomposition, Carbohydrate Fermentation Activity, Enteric Bacteria

Many of the organisms living around, on, and in human beings are too small to be seen without a microscope. They include viruses, bacteria, fungi, and protozoa. The simplest organisms living on earth today are bacteria, and biologists think they closely resemble the first organisms to evolve on earth. Bacteria are too small to see with the unaided eye and bacteria mostly unicellular having a rigid cell wall. *Escherichia coli* are a naturally occurring germ (commensal) in the intestine of birds and warm-blooded mammals. It is likewise an integral part of the intestinal flora of humans. Certain strains of *Escherichia coli* can cause serious diseases in animals and humans. *Escherichia coli* are the predominant non pathogenic facultative flora of the human intestine. (Agarwal *et al.,* 2009).

Lactic acid bacteria are a group of Gram-positive rods and cocci occurring naturally in a variety of niches, including the gastrointestinal tract, plants and fermented foods such as dairy products, meat and alcoholic beverages. *Lactobacilli* are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the lactic acid bacteria, which are used in making starter culture for dairy products (Renuka Goyal, *et.al*., 2012). Lactic acid bacteria are the most important group of microorganism used in food fermentations, they contribute to the fast and texture of fermented products and inhibit food spoilage and pathogenic bacteria by producing antimicrobial substances (lactic acid, hydrogen peroxide, bacteriocin) (Chowdhury *et.al.,* 2012).

In 1872, Ferdinand Cohn, a contemporary of Robert Koch, recognized and named the bacterium *Bacillus subtilis***.** *Bacillus subtilis* is a species of Gram-positive bacteria inhabiting numerous environments including soil, dust, water and plant materials and many foods. (Ghanbari *et.al*. 2009). *Bacillus subtilis* can inhabit the gastrointestinal tract (GIT) of insects and animals. Bacillus subtilis entry into the GIT is an essential part of their virulent life cycle. It is probable, though, that Bacillus spores present in the soil enter the GIT associated with ingested organic matter and this could explain the abundance of spore-formers in soil-dwelling animals. (Hoque Hong *et. al*., 2010)

MATERIALS AND METHODS

Pure, authentic and viable microorganisms were purchased from Institute of Microbial Technology, Chandigarh- India. Microorganisms were used in this studied was *Escherichia coli*

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(MTCC-443), *Lactobacillus delbrueckii* (MTCC-911) and *Bacillus subtilis* (MTCC-121). Bacterial strains were morphologically identified using gram staining reaction and other biochemical tests which include; catalase, methyl red, voges proskauer (MR-VP), nitrate reduction test, starch hydrolysis, gelatin test, starch hydrolysis test, indole, motility, oxidase, urease, triple sugar iron agar (TSI), simmon's citarte, macconkey agar, mannitol salt agar , casine hydrolysis and sugar fermentation as described by Ogbulie JN, 1998. Microorganisms were gram stained by four different reagents in the order listed; crystal violet, (primary stain), iodine solution (mordant), alcohol (decolourizing reagent) and safranin (counter stain).

RESULTS AND DISCUSSION

Present study was performed to assess the behaviour of three bacteria species which are considered to be enteric bacteria and usually form the normal flora in the gut of living organism including human. The three bacteria species viz. *Bacillus subtilis, Escherichia coli* and *Lactobacillus delbrueckii* studied for various standard biochemical tests individually and mixed culture. The results obtained were summarized in the Table No.1. *E.coli ,* belonging to the group enterobacteria is marked with positive Methyl red test, Nitrate reduction, Motility test, Indole production test, Catalase synthesis, MacConkey agar test, Triple sugar iron agar and Carbohydrate fermentation tests, when cultured in pure. *Lactobacillus delbrueckii* showed the similar behaviour in pure culture, except that it had no activity for triple sugar iron agar test and is gram positive bacteria. On the other hand *Bacillus subtilis* gave results similar to *E.coli* except being negative results for nitrate reduction and positive for citrate utilization. The mixed cultures of *Bacillus subtilis* and *E.coli* showed inability to ferment sucrose while rest of the tests were same as pure cultures.

In the present study, *Escherichia coli, Lactobacuills delbrueckii and Bacillus subtilis* were performed by biochemical characterization based upon different standard methods and results obtained confirmed the *E.coli* is a gram negative, non-spore forming rod. Kenneth Todar, 2009 has identified that the organism is a facultative anaerobe and ferments simple sugars such as glucose to form lactic, acetic, and formic acids and *E.coli* is a common inhabitant of the intestinal tract of man and warm-blooded animals. It has been reported by Prasad *et.al.,*2012 that most strains of *E. coli* are harmless and are a part of the normal intestinal microflora these strains serve a useful function in the body by suppressing the growth of harmful bacteria and by synthesizing appreciable amounts of vitamins. In present study showed that *E.coli* and *Lactobacillus delbrueckii* gave same results as pure cultures except being positive for citrate utilization (Table -1). According to Patil, *et.al., 2010* it was identify the *E. coli* and its human host coexist in good health and with mutual benefit for decades. The niche of commensal *E. coli* is the mucous layer of the mammalian colon. The bacterium is a highly successful competitor at this crowded site, comprising the most abundant facultative anaerobe of the human intestinal microflora (Nair *et.al*.,2005). It has earlier been reported by Ducluzeau *et.al.,*1989 Lactobacilli are gram-positive, nonsporulated, and anaerobic bacteria. Several species of this group are non-pathogenic, simple to cultivate and secrete enzymes such as proteases, amylases and cellulose that are useful for a number of industrial applications. Although different microorganisms are claimed today as probiotics, usually they represent two main genera *Lactobacillus* and *Bifidobacterium*.

The largest group of probiotic bacteria in the intestine is lactic acid bacteria (LAB) Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut, and have emerged as a major balancing factor influencing gastrointestinal physiology and function. Vijayalakshmi *et al.*,2012 reported that lactobacillus are normal inhabitants of the oral cavity and the digestive tract in humans. Some *Lactobacillus* strains are used in food fermentations, and typical examples are found in the dairy industry for the production of cheese, yogurt, and other fermented milk products. Lactobacilli represent a significant part of our intestinal microflora, and their friendship with the general state of human health is under serious investigation. The genus *Lactobacillus* is one of the major groups of lactic acid bacteria used in food fermentation and is thus of great economical importance. However, Probiotic microorganisms are often incorporated in food in the form of yoghurt and

yoghurt type fermented food. Recently there are also medical probiotics in the form of capsules and tablets. A study reported by Page *et.al*, 2011 that Probiotics refer to viable microorganisms that promote or support a beneficial balance of the autochthonous microbial population of the gut. It is most likely that the influence of probiotics is dependent on at least partly on the indigenous bacteria which are present in the host.

Table 1: Result of Biochemical Tests

S.N.	Tests	*E.coli (A)*	*L. delbrueckii (B)*	*B. subtilis (C)*	*A+B*	*B+C*	*A+C*	*A+B+C*
1.	Methyl-Red	+++	+++	+++	+++	+++	+++	+++
2.	Voges-Proskauer	---	---	---	---	---	---	---
3.	Nitrate Reduction	+++	+++	---	+++	---	+++	+++
4.	Motality Agar	+++	+++	---	+++	---	+++	+++
5.	Indole Production	+++	+++	+++	+++	+++	+++	+++
6.	Simmon's Citarte	---	---	+++	+++	+++	+++	+++
7.	Catalase Test	+++	+++	+++	+++	+++	+++	+++
8.	MacConkey Agar	+++	+++	+++	+++	+++	+++	+++
9.	Mannitol Salt Agar	---	---	---	---	---	---	---
10.	Urease Agar	---	---	---	---	---	---	---
11.	Casine Hydrolysis	---	---	---	---	---	---	---
12.	Triple Sugar Iron Agar	+++	---	+++	---	+++	+++	+++
13.	Carbohydrate Fermentation Test							
a.	Glucose Test	+++	+++	+++	+++	---	+++	+++
b.	Ribose Test	+++	+++	+++	+++	---	+++	+++
c.	Rehminose Test	+--	+++	+++	+++	---	+++	+++
d.	Lactose Test	++-	+++	+++	+++	---	+++	+++
e.	Sucrose Test	+++	+++	+++	+++	++-	---	+++
14.	Starch Hydrolysis	+++	+++	+++	+++	---	+++	-++
15.	Gram's Staining	"-Ve"	"+Ve"	"+Ve"	"+ & -Ve"	"+ Ve"	"+&- Ve"	"+ & - Ve"

Results reveal that *Lactobacillus delbrueckii* and *Bacillus subtilis* were cultured together the motility was decreased considerably along with significant loss of carbohydrate fermentation activity. *Bacillus subtilis* species are Gram-positive, aerobic, rod-shaped, non motile and endospore forming bacteria commonly found in nature (Logan *et.al*., 2008). Bacteria of the *Bacillus* genus are among the most widespread microorganisms in nature. *Bacillus* bacteria could play a significant role in the gut because of their high metabolic activity. Activity of *Bacillus* is largely determined by their ability to produce antibiotics. There were identified 795 antibiotics from *Bacillus* bacteria. *Bacillus subtilis* spores are being used for oral bacteriotherapy and bacterioprophylaxis of gastrointestinal disorders in both humans and animals.

In some countries probiotics are available for oral bacteriotherapy and bacterioprophylaxis of gastrointestinal disorders in humans (Hoa *et.al.*,2000).). Our data reveal that mixed cultures of *Bacillus subtilis* and *E.coli* showed inability to ferment sucrose while rest of the tests were same as pure cultures. (Table -1). The mixed culture obtaining all three bacteria gives positive results for all the tests, which showed that each bacteria's have compensating their weakness. Thus mixed culture of

all three enteric bacteria may prove beneficial for the internal environment of gut in animals.

CONCLUSIONS

In our study a combination of the three bacteria species viz. *Escherichia coli, Lactobacillus delbrueckii* and *Bacillus subtilis* studied biochemical assay individually and mixed culture. When we performed test in mixed cultured we found that they were full fill their weakness and all test were found positive. So we conclude that mixture of all three of these may prove beneficial for the internal environment of gut in animals. The large group of probiotic bacteria in the intestine is *Lactobacillus* and *Bacillus subtilis*. Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut, and have emerged as a major balancing factor influencing gastrointestinal physiology and function. They are normal inhabitants of the oral cavity and the digestive tract in humans. *Bacillus* bacteria could play a significant role in the gut because of their high metabolic activity. It needs to be stressed that long term studies are needed to prove understand the human health significance of *Escherichia coli, Lactobacuills delbrueckii and Bacillus subtilis* by invivo processed to confirmed the biochemical estimation .

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