

INTESTINAL MICROORGANISMS AFTER PROBIOTIC TREATMENT IN RAT

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ABSTRACT

The study was carried out to know the effect of probiotics (Bio-Top[®], Curd) supplementation on intestinal microorganisms in rats (*Rattus norvegicus*) during the period of 01 March to 20 April 2011. A total of twelve (12), one month old rats were randomly divided into 3 experimental groups: A, B and C, with 04 rats in each group. Group C was fed with commercial rat pellet (20 gm/rat) without supplementation of probiotics (control), group A was fed with commercial ration (20 gm/rat) with addition natural probiotic (Curd) and group B was fed with commercial ration (20 gm/rat) with the addition of commercial probiotic (Bio-Top[®]) feed for 7 weeks. Feed and water were supplied *ad libitum*. The result showed that intestinal bacterial population changed in probiotics treated groups compared to control group. *Bacillus spp.* was isolated from Bio-Top[®], Curd and Probiotic treated rats. Whereas *E. coli* was present in control rats, indicating environmental contamination which is hazardous for public health. The study suggests that probiotics supplementation helps in maintaining normal haematobiochemical values and beneficial gut microbes.

Keywords: Curd, Probiotic, Rat, *Bacillus spp.*, *E. coli*

INTRODUCTION

The intestinal microflora constitutes a metabolically active microbial environment, dominated by a relatively low diversity of genera, which, in the gut of healthy individuals, exist as part of a stable community. Under normal circumstances, these resident gut bacteria cause neither pathogenesis nor inflammation in the host, but instead contribute to health maintenance, by forming a barrier layer against colonization by pathogens and by aiding in nutrient digestion and assimilation (Fuller, 1989). In addition, the resident intestinal microflora plays other important physiological roles in health maintenance: deconjugating potentially damaging oxidative metabolites and toxins in the gut; degrading potentially allergenic food proteins; regulating cholesterol and triglyceride uptake; increasing vitamin biosynthesis; and providing immunosurveillance signals to limit intestinal-tract inflammation. Thus, a stable, properly functioning and active intestinal-tract microflora is essential to the continuance of health. (Lee and Salminen, 1995)

The probiotic approach, therefore, advocates the supplementation of food products with live microbial additions, whilst a prebiotic would be a component of diet that has a selective fermentation by indigenous bacterial species. Both approaches have the common objective of growth promotion through beneficial bacterial microorganisms. One further approach is the combination of probiotic and prebiotic as 'symbiotic', i.e. use of the live microbes in feed additive to a substrate that promote its growth (Singh and Chauhan, 2004)

The residues of antibiotics in milk, egg or meat are the cause of several deleterious effects in man including allergy and resistance of infections. Such problems will get reduced after the use of probiotics particularly in the form of cow curd (Nautiyal, 2002). The aim of this research was to evaluate the effect of probiotics on the intestinal bacterial population.

MATERIALS AND METHODS

Study site and period

The study was conducted in the Bacteriology Laboratory of the Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh. The whole experiment was conducted during the period of 01 March to 20 April 2011.

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Experimental rats and probiotics

A total of 12, one month aged male rats (*Rattus norvegicus*) were used. The rats were purchased from ICDDR'B, Dhaka. They were treated with natural probiotic (Curd) and commercial probiotic Bio-Top® which contains the bacterial strains of *Bacillus subtilis* and *Bacillus licheniformis*. After 7 weeks of treatment, the samples were taken from curd, Bio-Top®, cecum and colon of experimental animal.

Experimental design

The whole experiment is composed of isolation of the bacteria from probiotics and samples collected from cecum and colon of treated rats and identification of *Bacillus spp.* by cultural and morphological characteristics. Carbohydrate fermentation tests were also used to confirm the isolated organisms.

Collection of samples

The samples comprising curd, Bio-Top, content of cecum and colon were collected with proper aseptic measures. Immediately after collection, the samples were put into sterile test tubes and carried to the laboratory of the Department of Microbiology and Hygiene for inoculation into culture media.

Culture of Bacteria

Primary growths of bacteria of each collected sample were performed in nutrient broth (NB). Individual samples were then subjected to Gram's staining to ascertain the different types (morphology) of organism present in the culture. Each incubated broth sample was then streaked onto nutrient agar (NA), blood agar (BA) plates separately as to obtain individual colony (Cheesbrough, 2006).

From individual colony subculture were grown on BA, NA and Eosin methylene blue (EMB) agar media for obtaining pure culture of the isolated organisms. After determining cultural character, these pure cultures of the organisms were subjected to staining and morphological examination for identification of organisms. (Cheesbrough, 2006).

Biochemical tests

Isolated organisms with specific characteristic on NA, BA, and EMB agar were subjected to biochemical tests. Standard methods were followed to conduct this test according to the procedure described by (Cowan, 1985).

Catalase test

This test was used to differentiate bacteria, which produce the enzyme catalase, such as *Bacillus*, from that non-catalase one such as, *Lactobacillus*. To perform this test, a good growth culture of test organism was smeared in a sterile wooden stick or glass rod and was immersed into the H₂O₂ solution. In case bubbles of oxygen are released, the organisms are considered catalase producer (Carter, 1986).

RESULTS AND DISCUSSION

Cultural examinations

Nutrient agar

Nutrient agar plates streaked separately with the samples indicated the growth of bacteria after 24 hours incubation at 37°C aerobically. Production of white colonies indicates *Bacillus spp.* The results of the cultural examination on NA are similar with the report of Gordon (1964), Cheesbrough (2006).

Eosin Methylene Blue (EMB) agar

After overnight incubation there was no metallic sheen on EMB agar, indicate *Bacillus spp* that did not grow on EMB agar. But samples from untreated groups show metallic sheen on EMB agar indicates *E. coli*. The findings are in agreement with the statement of Buxton and Fraser (1977).

Blood agar

After overnight incubation at 37°C on blood agar there was hemolysis indicates presence of *Bacillus spp.* From the sample collected from curd, Bio-Top®, cecum and colon of treated rats. But samples collected from control group did not show any hemolysis which are in agreement of the earlier report of Cheesbrough (2006).

Gram's staining

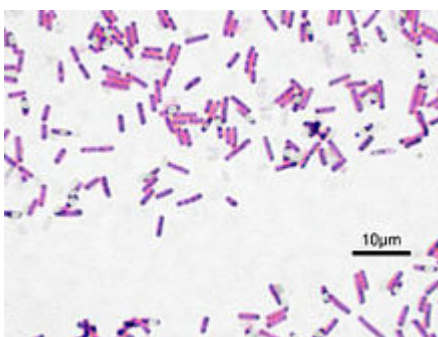
In Gram's staining under microscope, bacterial colony collected from curd, Bio-Top, treated samples appeared as Gram positive, rod shape indicates *Bacillus spp.* On the other hand colony from untreated group appeared as Gram negative, pink color, small rod shaped, arranged in single or paired indicates *E. coli*. These finding are more or less similar with the findings of Freeman (1985) and Honda *et al.* (1982).

Carbohydrate fermentation tests

Acid production was indicated by the color change from reddish to yellow and gas production was noted by the appearance of gas bubbles in the inverted Durham's tubes. Colony inoculated from curd, Bio-Top® and treated samples produce only acid indicates *Bacillus spp.* But colony from untreated samples produce acid and gas indicates *E. coli*.

The results of this investigation indicated that the samples contain both Gram positive and Gram negative bacteria.

The results of this investigation indicated that the samples contained both Gram positive and Gram negative bacteria. Samples collected from curd, Bio- Top®, and probiotic treated groups contained *Bacillus spp.* And samples of control group contained *E. coli*. So from above findings, it can be concluded that bacteria *Bacillus spp.* produces the beneficial effect on rats by improving enzymatic activity, inhibiting growth of pathogenic organisms, enhancing growth and regulating immunity.



Grams staining of *Bacillus spp.* showing Gram positive, rod shaped organisms

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