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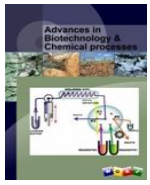

The Probiotic Efficacy of lactobacillus of *Lactobacillus casei* from Human Breast milk against Shigellosis in Albino rats

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Problem statement: The aim of the study was to investigate the probiotic efficacy of *Lactobacillus casei* isolated from human breast milk in the prevention of shigellosis in albino rats infected with clinical strains of *Shigella dysenteriae*. Shigellosis continues to be a major health problem worldwide, causing an estimated 1 million death and 165 million cases annually. Beside these, it was reported that shigella causes approximately 580,000 cases of bacillary dysentery annually among travelers and military personnel from industrialized countries.

Method: Two different concentrations ($1.0x10^7$ CFU/ml and $2.0x10^7$ CFU/ml) of *Lactobacillus casei* isolated from human breast milk and biochemically characterized were administered to two different sets of shigella free albino rats followed by $1.0x10^5$CFU/ml dose of a clinical strain of *Shigella dysentariae*. The cage weights, rectal temperature and clinical signs and symptoms of shigellosis were recorded on daily basis. Liver function test on the serum of the sacrificed rats was also carried out.

Results: The experimental rats infected with *S. dysenteriae* but not treated suffered from shigellosis while the test groups infected and treated with the *Lactobacillus casei* showed no sign of the disease as well as no clinical effect on the liver. This can have clinical implication for individuals and communities prone to shigellosis.

Conclusion: It could be concluded that *Lactobacillus casei* isolated from human breast milk could be used as a prophylactic against gastrointestinal infections by *Shigella dysentariae.*

1. Introduction

Probiotic which originated from a Greek term "probios" meaning "for life" as against "antibiotics" which means "against life" has become a significant concept in biomedical research. It refers to live microorganisms which when administered in adequate amounts confer health benefit on the host [1]. It is also a preparation or product containing viable microorganism in sufficient numbers which can alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in the

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host [2,3]. Probiotic products consist of different enzymes, vitamin capsules or tablets as well as some fermented foods contain microorganisms which have beneficial effects on the health of the host.

The concept, probiotic, is opened to lots of different applications in a large variety of fields relevant to human and animal health. Probiotics have been used for long in food ingredients for human and animals without any side effects as well as provide for the balance of intestinal flora [4]. Data indicate that oral bacteriotherapy and living bacteria feeding in fermented milk foods support the immune system against some pathogens [1]. It is also reported that probiotics affect the immune system in different ways such as producing cytokines, stimulating macrophages and increasing secretory Immunoglobulin A concentrations [5,6].

On the basis of this, this work was designed to investigate the efficacy of *Lactobacillus casei* isolated from human breast milk in the prevention of shigellosis in rats infected with clinical strains of *Shigella dysenteriae* which has the capacity to cause dysentery in animals. Breast milk has been shown to be an excellent and continuous source of commensal and potentially probiotic bacteria to the infant gut [7,8,9] and the colostrums gave rise to several bacterial isolates [10]. *Lactobaccilus casei* was chosen because it met the selection criteria for probiotic Lactic acid bacteria as prescribed [11] and the reported potentials against *Salmonella typhimurium* [1] allergic response, inflammation in inflammatory bowel disease model [12]. Shigellosis is a description of the gastrointestinal infections caused by *Shigella spp*. The disease is characterized by an early non-bloody, voluminous diarrhea when the bacteria are within the small intestinal tract. The invasion of the large intestine results in latter dysentery with blood and pus in stool [13].

2. Materials and Methods

Materials:

Reagents used were of analytical grade. Human breast milk was obtained from two healthy lactating volunteer mothers during the first year after parturition in the University of Jos. Experimental animals were bought from the animal house of the Department of Physiology, University of Jos. Clinical strain of *S. dysentery* was a gift from Mr. Felix of the Department of Microbiology, University of Jos.

Isolation of lactic acid bacteria

The breast milk was collected under aseptic conditions and the samples stored on ice until they were delivered to the laboratory. The pour plate techniques were used to isolate the organism. Briefly, samples were diluted times ten, hundred and thousand using sterile peptone water. 1ml aliquot of the samples and dilutions were plated into Man Rogosa and Sharp (MRS) agar. The plates were incubated at 37^0C for 2days under anaerobic conditions (in anaerobe jar using oxoid anaerogen compact). After incubation, individual colonies were selected and sub cultured in MRS. The colonies were purified using the streak technique.

Gram Staining

The Gram reaction of the isolates was determined by light microscopy after gram staining as described [14]. Lactic acid bacteria are known to be gram positive.

Biochemical Test

The biochemical test take advantage of the ways different bacteria react physiologically due to the presence of certain chemicals. The tests employed were catalase test, oxidase test and sugar fermentation test as earlier described [14]. Nine different broth mixtures were made, each of the nine sugars (manitol, mannose, maltose, glucose, fructose, xylose, galactose, sucrose and lactose) in peptone water and inoculated with colonies of the isolates and incubated at 37^oC for 48hours. Positive results were then observed based on turbidity and gas formation in inverted Durham tubes according to the method described [15]. The procedure is hinged on *L. casei*'s ability to ferment all sugars except xylose as well as fails to produce gas from glucose as against the others.

Animal Experiment

Experimental white wistar rats were organized into four groups and categorized thus: negative control, NC, (rats not administered any of the bacterial spp), positive control, PC, (rats fed with 0.4ml $1x10^5$ CFU/ml *Shigella dysentariae* only); Test 1 (rats dosed with 0.4ml $1x10^5$ CFU/ml *S. dysentria*e followed by 0.4ml 2.0 x 10^{10} CFU/ml of *L. casei*), Test 2 (rats dosed with 0.4ml 1x105 CFU/ml *S. dysentria*e followed by 0.8ml 2.0 x 10^{10} CFU of *L. casei*). The administration of the *L. casei* in the Test groups was after 2days post infection with *S. dysentriae.* The animals were then monitored for the next 7days.

Rectal Temperature

The rectal temperature of the rats was taken in the morning for signs of fever using the thermometer. The thermometer was sterilized and inserted into the

anus. The thermometer was held for 1-2minutes before readings were taken.

Feed consumed

The amount of feed consumed by the rats in each experimental case was obtained by weighing the amount of feed given in the morning and the amount of feed left the following morning. The feed consumed was determined by the difference.

Liver function tests

These tests were done to check any possible effects the test bacteria might have had on the experimental rats. The parameters tested for were albumin, bilirubin, Aspartate amino transferase, Alanine amino transferase, Alkaline phosphatase and total protein from the serum of the sacrificed experimental rats. Each parameter was tested for using the standard procedures of sample preparation and the absorbance read at the respective wave length.

3. Results

Lactic acid bacteria were found to be associated with human breast milk and successfully isolated. The features that distinguished the bacteria from others were their being Gram positive and catalase negative bacilli colonies. Other biochemical features (Table 1) which identified the bacteria were their inability to ferment xylose as well as none gas formation with glucose. These put together brought out the *L. casei.*

The animal experiment revealed the effects of the *S. dysenteriae* on the experimental animals as well as the prophylactic effect of *L. casei* on the infected animals. The health of the animals in the positive group (PC) was affected in terms of rectal temperature, feed consumed, body weight and fecal characteristics as compared with the other groups (negative control, NC, which were not infected and the Test 1 and Test 2, which were infected and treated) (Table 2). The PC group had higher rectal temperature, reduced feed consumed and lower body weight as well as loose feces. The liver function test reveals that the parameters studied for the none infected and infected but treated groups agree (Table 3). The PC tends to be the group with some significant differences in terms of bilirubin, Alkaline phosphatase and Alanine amino transferase levels.

Table1: Biochemical Test on some of the isolates from breast milk

Isolate Code	Catalase	maltose	Manitol	Manose	Glucose	Fructose	Xylose	Galactose	Sucrose	Lactose
C1	-	+	-	+	+	+	+	+	+	+
C2	-	+	-	+	+	+	+	+	+	+
C3	-	+	+	+	-	+	-	+	+	+
C4	-	+	+	+	+	+	+	+	+	+
C5	-	+	+	+	+	+	+	+	+	+
C6	-	+	-	+	+	+	+	+	+	+

Table 2: Summary of evidences for physiological changes in experimental animals

Treatment Group	Rectal Temperature	Body weight	Fecal Characteristics	Feed Consumed (g)	Comments
NC	36.2 ± 1.810	98.12 ± 1.51	Formed	73.50± 0.4	No sign of Shigellosis
PC	40.8 ± 1.91	68.13 ± 1.01	Semi formed	60.09 ± 1.2	Signs of Shigellosis
TEST 1	35.2 ± 1.70	92.20 ± 1.60	Formed	72.16± 1.2	No signs of shigellosis
TEST 2	36.8 ± 2.80	97.18 ± 1.80	Formed	74.25±1.1	No signs of shigellosis

NC means negative control; PC means positive control

Table 3: Liver function tests on experimental animals

Sets of Expt	*Total protein (g/L)*	*Albumin (g/ L)*	*Bilirubin (µmol/L)*	*Alkaline phosphotase (µmol/L)*	*Aspartate Aminotransferase (µmol/L)*	*Alanine amino transferase (µmol/L)*
NC	36.4 ± 0.80	17.9 ± 0.14	2.72 ± 0.14	42.85 ± 0.21	38.0 ± 0.49	12.75 ± 0.19
PC	34.2 ± 0.66	15.3 ± 0.85	8.18 ± 0.75	80.80 ± 1.00	50.5 ± 1.30	09.30 ± 0.65
TEST 1	32.59 ± 0.80	16.7 ± 0.70	3.08 ± 0.07	44.90 ± .30	36.0 ± 0.10	12.25 ± 0.90
TEST 2	36.08 ± 0.60	17.45 ± 0.60	2.72 ± 0.10	43.80 ± 1.10	35.3 ± 0.80	11.45 ± 0.10

4. Discussions

The isolation of Lactobacillus casei from human breast milk is consistent with reports in literature [7,8,9]. The cage weights, rectal temperature and clinical signs and symptoms of shigellosis on the rats were recorded so as to be able to find any clinical manifestations of both *Shigella dysenteriae* infection and the *Lactobacillus casei* on experimental rats. The results obtained showed that the rats fed with *Lactobacillus casei* did not develop shigellosis symptoms unlike the ones induced with only *Shigella dysenteriae*. This probiotic effect could probably be due to the anti shigellosis properties of the *Lactobacillus casei* which had earlier on been reported [16,17,18]. The mechanism of this action may be by competitive inhibition, the generation of a non-conducive acidic environment and or by the production of antibiotic-like substances (bacteriocins) as reported [4]. This may also account for reports that *L. casei* oral supplementation [19] and *L. casei* from breast milk [9] exhibited probiotic effects to varying degrees.

The results of the liver function test showed that there was significant difference in the values of the following liver protein and enzymes: bilirubin, Alkaline phosphatase and Alanine amino transferase of rats not treated with *Lactobacillus casei* but challenged with *S. dysenteriae* compared to those of the negative control (rats not administered any of the organisms). The increase in the value of the enzyme activity of the positive control rats (rat that were administered *S. dysenteriae* but not *Lactobacillus casei*) was attributed to the hepatoxic effects of the *S. dysenteriae* [20,21]. This result implies that the probiotic *Lactobacillus casei* had no attendant toxicological effect on the rats.

Therefore, *Lactobacillus casei* can be of great medical help particularly in individuals, communities and nations prone to rampant shigellosis if the probiotic procedures for isolation and administration are properly developed and standardized for clinical applications.

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