A Study on Inhibitory Effects of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as Probiotics on Some Clinical Pathogens.

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Abstract: A study was carried out on inhibitory effects of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* on growth of some pathogens isolated from clinical specimens of pathogens selected for this study were *E. coli*, some species of *Klebsiella*, *Pseudomonas*, *Proteus*, *Salmonella* and *Shigella* isolated from stool and urine samples of patients attending State Specialist hospital, Yola. Results of agar spot revealed that *Lactobacillus bulgaricus* produced highest antimicrobial activity against *Klebsiella* spp. (3.5 mm) followed by *E. coli* (2.4 mm), *Proteus spp.* (1.9 mm) *Pseudomonas sp.* (1.5 mm) and *Shigella sp.* (1.0 mm) while *Streptococcus thermophilus* produced highest antimicrobial activity against *Klebsiella* spp. (4.2 mm) followed by *E. coli* (4.0 mm), *Proteus spp.* (2.3 mm) *Pseudomonas sp.* (1.8 mm) and *Shigella sp.* (1.6 mm). Both organisms (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) may be used as probiotics when preceded by further studies.
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Key words: Pathogens, Probiotcs, *Lactobacillus bulgaricus* and *Streptococcus thermophilus.*

1. Introduction

Large intestines of humans contain a very complex and balanced microbiota. These microorganisms normally prevent infection and have positive effect on nutrition. Any abrupt change in diet, stress or antibiotics therapy can upset this microbial balance, making the host susceptible to disease and decreasing the efficiency of food use. Probiotics which promote health and growth has the potential to reestablish the natural balance and return the host normal health and nutrition. Probiotics microorganisms are host specific and strains selected for or as probiotics must exert a beneficial effect on the host (Sanders, 2000). There are several possible explanations to how probiotics microorganisms displace pathogens and enhance the development and stability of the microbial balance in large intestines (Bradley et al, 2000). It is well known that the presence of *lactobacilli* is important for the maintenance of the intestinal microbial ecosystem' they have shown to possess inhibitory activity towards the growth of pathogenic bacteria. This inhibition could be due to the production of inhibitory compounds such as organic acids, hydrogen peroxide, bacteriocins or reuterin or competitive adhesion to the epithelium (Onwehand et al, 2002). In order to survive in and colonize the gastrointestinal tract, probiotics should express high tolerance to acid, bile and ability to adhere to intestinal surfaces.

Lactobacillus is an adhesive probiotic strain with good survival in the gastrointestinal tract and in functional foods. Typical colony morphology of the strain gives a practical tool to its isolation sin stool samples and to analyze daily doses needed in the diet. Administration in milk has been shown to enhance the intestinal viability of the strain so that with milk based products, the lowest colonizing daily dose was 100 million living bacteria. But in dry pharmaceutical preparations, a daily dose needed was 1o billion colony forming units (Johansson et al, 1997). The most widely used is yogurt which is fermented with *Lactobacillus bulgaricus* and *streptococcus thermophilus.* These bacterial species are not of human origin and do not belong to the normal gut flora. They are sensitive to acid conditions and are easily destroyed in the stomach.

The objective of this study was to isolate *Lactobacillus bulgaricus* and *Streptococcus thermophilus* from yogurt, isolate clinical pathogens (*E. coli*, *Salmonella sp.*, *Shigella sp.*, *Klebsiella sp.*, *Pseudomonas* and *Proteus*) from clinical samples and determine the inhibitory effects of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* on these pathogenic microorganisms.

2. Material and Methods

Lyophilized cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were isolated from Nagge yogurt, Yola, Nigeria. The cultures was grown on MRS agar plates (Oxoid, 2004)

Cell morphology was performed according to Ridge (1982) and Cheesbrough, (2000), catalase test according to Schieri and Blazevic (1981), physiological test was as described by Oxoid (2004) and biochemical test according to methods of Harrigan and McCance (1993) and Tserovska et al, (2002).

Clinical isolates were obtained from stool and urine of patients attending State Specialist Hospital, Yola. The isolates were identified using methods of Cheesbrough (2000), Collins and Patricia (1984), Schieri and Blazevic (1981).

Standard method of agar spot test was used for the antimicrobial activity assay of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

3. Results and Discussion

The result of characteristics of the isolates of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is as shown in table 1. All the colonies of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* isolated are gram positive, showed positive growth on MRS agar medium at pH 6.3 and 5.4 except for *Streptococcus thermophilus* which showed a negative growth at pH 5.4, and negative catalase activity. *Lactobacillus bulgaricus* isolates showed characteristic rod shape, creamy grey colonies; circular and irregular shaped. While the *Streptococcus thermophilus* isolates showed characteristic cocci shape, big creamy white colonies, circular and irregular shaped. The isolates showed positive growth on MRS agar, the selective media for lactic acid bacteria (Oxoid, 2004) at pH 6.3, at incubation temperature of 42°C and pH 5.4, and incubation temperature of 30°C revealed big sized, circular, irregular, creamy grey colonies is indicative of *Streptococcus themophillus* and *Lactobacillus bulgaricus* bacteria (Sudi et al, 2008) respectively consistent with the work of Sudi (2006). The morphological and biochemical characteristics of the clinical isolates are as shown in table 2. All the isolates are gram negative. Plate A1 showed smooth, pink, circular colonies, negative to coagulase reaction and positive to catalase reaction. Plate A2 showed large mucoid, grey white irregular colonies and are positive to coagulase and catalase reactions. Plate A3 showed swimming, irregular, non lactose fermenting colonies, positive to coagulase reaction and negative to catalase reaction. Plate A4 showed large flat and circular colonies. Plate A5 showed grey white, irregular and circular colonies. Plate A6 showed pink circular colonies. Plate A4, A5 and are negative to coagulase and catalase reactions. All the clinical isolates; A1, A2, A3, A4, A5 and A6 demonstrate characteristics of *E. coli*, *Klebsiella sp.*, *Pseudomonas sp.*, *Proteus sp.*, *Salmonella sp.*, *and Shigella sp.* respectively.

The result of measurement of antimicrobial activity (zone of inhibition) of *lactobacillus bulgaricus* and *Streptococcus thermophilus* against the clinical isolates is as shown in table 3. Out of the six clinical isolates *Klebsiella sp.* showed the highest zone of inhibition (3.50 mm) against *Lactobacillus bulgaricus* , followed by *E. coli* (2.40 mm), *Pseudomonas sp.* (1.90 mm), *Proteus sp.* (1.50 mm), *Shigella sp.* (1.00 mm) and the least by *Salmonella sp.* (0.00 mm).

Growth inhibition is highest with *Streptococcus thermophilus* against *Klebsiella sp.* (4.20 mm), followed by *E. coli* (4.00 mm), *Proteus sp.* (2.30 mm), *Shigella sp.* (1.80 mm), *Pseudomonas sp.* (1.60 mm) and no or least zone of inhibition was shown by *Salmonella spp* (0.00mm).

Table 1: Characteristics of the Isolates of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

Characteristics of isolates	*Lactobacillus bulgaricus*	*Streptococcus thermophilus*
Growth on MRS agar at ph 6.3	+	+
Growth on MRS agar at pH 5.4	+	-
Incubation temperature	30°C	42°C
Catalase activity	-	-
Gram reaction	+	+
Cell morphology	Rods	Cocci
Colony size	Small	Big
Colony shape	Circular, irregular	Circular
Colony colour	Creamy grey	Irregular Creamy white

Key: + = positive, **-** = negative

Table 2: Morphological and Biochemical Characteristics of the Isolates.

Plate No.	Colony morphology	Cell size	Gram reaction	Coagulase test	Catalase activity	Possible organism
A1	Smooth, pink	Circular	-	-	+	*Escherichia. Coli*
A2	Large, grey white, mucoid	Irregular	-	+	+	*Klebsiella sp.*
A3	Swimming, non lactose fermenting	Irregular	-	+	-	*Pseudomonas sp.*
A4	Large, flat colonies	Circular	-	=	-	*Proteus sp.*
A5	Grey white	Irregular, circular	-	-	-	*Salmonella sp.*
A6	Pink colonies	Circular	-	-	-	*Shigella sp.*

Key: + = positive, **-** = negative

Table 3: Measurement of Antimicrobial Activity (zone of inhibition in mm) of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* Against the Clinical isolates.

Indicator organisms	*Lactobacillus bulgaricus*	*Streptococcus thermophilus*
Escherichia. Coli	2.4	4.0
Klebsiella	3.5	4.2
Pseudomonas	1.9	1.6
Proteus	1.5	2.3
Salmonella	0.0	0.0
Shigella	1.0	1.8

In vivo studies on lactic acid bacteria have been used to evaluate various characteristics of potential probiotics. The characteristics of the isolates of lactic acid bacteria used for this study are typical of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as described by Oxoid (2004), Ridge (1982), Schieri and Blazevic (1981), Cheesbrough, (2000) and Sudi (2006). Growth of isolate on MRS agar, the selective media for lactic acid bacteria (Oxoid, 2004) at pH 6.3, incubation temperature of 42°C and pH 5.4, incubation temperature of 30°C which revealed big sized, circular, irregular, creamy grey colonies is indicative of *Streptococcus themophillus* and *Lactobacillus bulgaricus* bacteria respectively (Buchanan and Gibbons, 1974; Tserovska et al, 2002). These isolates were then used to study their inhibitory effects on the pathogens isolated from clinical samples such as *E. coli, Klebsiella sp., Pseudomonas sp., Proteus sp., Salmonella sp., and Shigella sp.*

The morphological and biochemical characteristics of the pathogens isolated from clinical samples according to Cheesbrough (2000), indicates that samples A1: *Escherichia. Coli;* A2: *Klebsiella spp;* A3: *Pseudomonas spp;* A4: *Proteus sp.;* A5: *Salmonella sp.* and A6: *Shigella sp.*

The antimicrobial activities of *Lactobacillus bulgaricus* isolated were variable while *Streptococcus thermophilus* inhibited the growth of the pathogenic organisms more especially of *E. coli* and *Klebsiella sp.* However, both strains were unable to inhibit *Salmonella sp.* and *Lactobacillus bulgaricus* unable to inhibit the proliferation of *Shigella sp.* tremendously. The production of reuterin by *Lactobacillus bulgaricus* could possibly account for its inhibitory activity as reported by Drago et al, (1997).

4. Conclusions

This work showed that, these yogurt probiotics indeed possess inhibitory effects on some of the selected intestinal pathogenic organisms. Hence these probiotics (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) can be used to prevent or ameliorate some diseases. Their administration in healthy and extreme conditions may be recommended preceded by further studies. This study supports the use of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as possible candidates in the prevention and treatment of gastrointestinal tract infection.

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