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Antibacterial Activities of *Micrococcus lactis* Strain Isolated from Nigerian Fermented Cheese Whey Against Diarrhea-Causing Organisms

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Abstract: Microorganisms associated with fermented cheese whey were isolated and identified. Four different bacteria; *Bacillus brevis*, *Lactobacillus acidophilus*, *Micrococcus lactis* and *Pediococcus cerevisiae* were isolated. These bacteria were then tested against diarrhea -causing organisms; *Bacillus cereus, Enterobacter faecium*, *Escherichia coli* NCTC 10418, *E. coli* ETEC, *Salmonella enteritidis*, *Salmonella paratyphi A*, *Salmonella typhi*, *Shigella dysenteriae* and *Staphylococcus aureus*. *Micrococcus lactis* inhibited all the test organisms with zones of inhibition from 7.0-24.0mm diameter, unlike the other isolates which inhibited few of the test organisms. The inhibition mediated by *M. lactis* was also superior to most of the antibiotics used except cephorex and oflomed which ranged from 8.0-27.0 mm and 1.0-36.0 mm, respectively. It is therefore being suggested that in the absence of antibiotics, fermented cheese whey can be used to treat diarrhea especially in rural areas where there may not be quick access to convectional antibiotics.

Key words: *Micrococcus lactis*, diarrheic bacteria, biotherapeutic agents, cephorex, oflomed

INTRODUCTION

Diarrhea, a condition in which there is abnormal frequency in stooling which is watery and in some studies containing mucus and blood is of great concern in the developing countries. This is so because diarrhea is a common cause of morbidity of all ages especially among pre-school children[1]. Up till today, it is very difficult to treat diarrhea because most of the antibiotics that are normally been used also may induce diarrhea which is known as antibiotic induced diarrhea. Not only that, most of the organisms causing infection have become resistant to most of these antibiotics.

The concept of improving intestinal health using biotherapeutic agents is presently one of the avenues being exploited for possible treatment of diarrhea especially antibiotic induced diarrhea. Research findings have revealed that the use of biotherapeutic agents such as *Lactobacillus* sp., *Streptococcus thermophilus*, *Bifidobacterium bifidum* etc has promising effects[2].

Whey, a by-product of cheese- making is considered a waste product until recently particularly in advanced countries when its importance as a nutrient- rich protein source was discovered[3,4]. Whey contains excellent quality protein, carbohydrate and lipids with specific health benefits. Not only that, whey is also a very rich source of lactic acid bacteria. In an earlier work done by Adebolu and Ademulegun[5], they observed that Nigerian cheese whey has inhibitory effects against certain diarrheic bacteria. This present research is furthering on this previous findings and is investigating the microflora in cheese whey that are producing the antibacterial substances which are capable of exhibiting inhibition on a wider range of diarrhea-causing bacteria.

MATERIALS AND METHODS

Source of materials: Freshly prepared cheese whey was obtained from cattle rearers at cattle market, Akure, Ondo State, Nigeria. The sample was stored in sterile tight fitting container and taken immediately to the laboratory.

Test organisms used: *Bacillus cereus*, *E. faecium*, *E. coli* NCTC 10418 *E. coli* ETEC, *Salmonella enteritidis*, *S. paratyphi A*, *S. typhi*, *Shigella dysenteriae* and *Staphylococcus aureus* were obtained from microbiology laboratory of Federal Institute of Industrial Research (FIIRO) Oshodi, Lagos and Nigerian Institute of Medical Research (NIMER) Yaba, Lagos. The isolates were maintained throughout the period of study aseptically by subculturing them into freshly prepared nutrient agar medium.

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Isolation and identification of fermented whey microflora: The sample was left to ferment for 72 h at room temperature. Standard methods of isolation and enumeration of bacteria were employed. The whey sample was cultured on Nutrient agar, Man de Rogosa and Sharpe agar (MRS agar) and MacConkey agar. Pure cultures of the isolates were then subjected to Gram's reaction and biochemical tests to characterize them. These isolates were characterized according to the criteria documented by Holt[6-8].

Detection of antimicrobial activity: The four bacterial isolates obtained from the whey were grown overnight in nutrient broth at 37°C. The cells were then removed by centrifuging the cultures at 3300 rpm for 20 min, the supernatants sterilized by filtration (0.65 μm-pore size cellulose acetate filter, Millipore) and used immediately against the test organisms following the well diffusion method of Jin[9]. Solidified nutrient agar (20 mL) was inoculated with 0.05 mL of an overnight broth culture of each of the diarrheic organisms, separate plate for different organism. Five wells, four at the periphery and one at the centre were made into the agar with 6mm cork borer and 50 μL of supernatant from the isolates were introduced into separate wells and the centre well was filled with distilled water to serve as control. The plates were incubated aerobically for 24 h at 37°C after which they were examined for zones of inhibition. Antibiotics assay was also conducted on the test organisms. Diameter of zones of inhibition were measured after the period of incubation and compared with the zones of inhibition obtained from the antibiotics assay.

RESULTS AND DISCUSSION

From the fermented cheese whey, four different types of bacteria were isolated and identified. These were *Bacillus brevis*, *Lactobacillus acidophilus*, *Micrococcus lactis* and *Pediococcus cerevisiae* (Table 1). This result showed relevance to results documented by Savadogo[8] in which they observed that *Lactobacillus fermentum Pediococcus* sp., *Leuconostoc mesenteroides subsp. mesenteroides* and *Lactococcus* sp. were present in fermented milk.

Of all the isolates, *Micrococcus lactis* inhibited all the test organisms with diameter of zones of inhibition ranging from 7.0 to 24.0 mm. *L. acidophilus* inhibited only four of the test organisms, which were, *S. aureus* (9.0 mm), *E. coli* NCTC 10418 (12.0 mm), *Sh. dysenteriae* (14.0 mm) and *B. cereus* (29.0 mm). *B. brevis* inhibited seven of the test organisms viz; *Staphylococcus aureus*, *Enterococcus feacium*, *Shigella dysenteriae*, *E. coli* NCTC 10418, *E. coli* ETEC, *S. enteritidis* and *B. cereus* with zones of inhibition ranging from 1.0-33.0 mm (Table 2). *P. cerevisiae* also inhibited seven of the test organisms as shown in the Table.

The result of the antibiotic assay showed that two out of the antibiotics used were highly potent. Cephorex and Oflomed were effective against all the test organisms with zones of inhibition ranging from 8.0 to 31.0 mm and 1.0 to 36.0 mm, respectively. Antibiotic like Augmentin did not inhibit any of the test organisms and antibiotics like Colistin, Nitrofurantoin and Cotrimoxazole only had minimal inhibitory effect ranging from 1.0-3.0 mm on the few organisms they exerted their effect on (Table 3). When the results of the antibiotic assay was compared to that of the cheese whey, it was observed that, the diameter of zones of inhibition obtained with the isolated *M. lactis* was higher than that of all the antibiotics with the exception of cephorex and oflomed. The inhibition obtained from this isolate may be attributed to presence of metabolites such as organic acids, hydrogen peroxide and bacteriocin produced by lactic acid bacteria of which this organism is one.

The antibacterial effect of lactic acid bacteria through its production of organic acid, hydrogen peroxide and bacteriocin has been well documented[10,8]. These substances inhibit growths of pathogenic bacteria and also alter the ecological balance of enteric commensals. The fermented whey showed a decline in pH value (6.10-3.92) as fermentation progressed, showing that organic acids were actually produced in the whey.

This study has been able to show that *Micrococcus lactis* isolated from fermented cheese whey had inhibitory effect on the entire test organisms that cause diarrhea used in this study and that this inhibition was superior to that of most of the antibiotics used in this work except for cephorex and oflomed. Although the actual substance responsible for the inhibition is yet to be determined, it is conceivable that when the substance is identified, it could be exploited in getting a new drug for the treatment of bacterial diarrhea. However in the interim, cheese whey especially fermented cheese whey can be administered to people having diarrhea especially in rural communities where access to convectional medicine is absent.

Table 1: Morphological and biochemical characteristics of fermented whey isolates

Whey isolates	Grain rxn	Morphology	Motility	Acid-fast	spore	Oxidase	Catalase	Glucose	Arabinose	Salicin	Sorbitol	Sucrose	Trechalose
1 S 1	Long rod	-	-	-	-	+	-	+	-	+	+	+	+
1S2	Cocci	-	-	-	-	+	+	-	-	-	+	-	-
1 S 3	Short rod	-	+	-	+	+	-	-	-	-	-	-	-
1S4	Ovoid	-	-	-	-	-	+	+	+	+	+	+	-

Continued

Maninitol	Maltose	Galactose	Cellobiose	Lactose	Mellibiose	Raffinose	Xylose	H2S	Indole	Urease	Growth in 6.5%	Nitrate redution	Arginine hydroplysis	Identified organism
-	+	+	+	+	+	+	-	-	-	-	-	-	-	Lactobacillus acidophilus
+	+	-	-	+	-	+	+	-	-	+	-	+	-	Micrococcus lactis
+	+	-	-	-	+	+	-	-	-	-	-	+	-	Bacillusbrevis
+	+	+	-	+	-	+	-	-	-	-	-	-	-	Pediococcus cerevisiae

+ = Positive, - = Negative, IS = Isolate

Table 2: Diameter of zones of inhibition (mm) of whey isolates against diarrhea-causing organisms

Diarrhea-causing organisms	Isolates			
	L. acidophilus	*M. lactis*	*B. brevis*	*P. cerevisiae*
S. aureus	9.0	12.0	3.0	9.0
Enterobacter faecium	-	10.0	11.0	11.0
Sh. dysenteriae	14.0	7.0	1.0	11.0
E. coli NCTC 10418	12.0	10.0	9.0	12.0
E. coli ETEC	-	13.0	13.0	-
S. paratyphi A	-	11.0	-	15.0
S .typhi	-	10.0	-	-
S. enteritidis	-	11.0	13.0	9.0
B. cereus	29.0	24.0	33.0	32.0

Table 3: Diameter of some of inhibition of commercial antibiotics against the tested organisms

Test organism	AMX	COL	CPX	GEN	AUG	OFL	NAL	TET	NIT	COT
S. aureus	6.0	2.0	23.0	0.0	0.0	21.0	10.0	7.0	0.0	0.0
Enterobacter faecium	1.0	0.0	16.0	4.0	0.0	25.0	1.0	0.0	2.0	1.0
Sh. dysenteriae	0.0	0.0	17.0	3.0	0.0	19.0	0.0	3.0	2.0	0.0
E. coli NCTC 10418	7.0	0.0	27.0	6.0	0.0	35.0	5.0	6.0	1.0	0.0
E. coli ETEC	10.0	1.0	31.0	9.0	0.0	36.0	6.0	7.0	2.0	0.0
S. paratyphi A	1.0	0.0	8.0	3.0	0.0	1.0	2.0	0.0	1.0	1.0
S. typhi	1.0	0.0	10.0	9.0	0.0	2.0	1.0	0.0	1.0	1.0
S. enteritidis	0.0	0.0	17.0	5.0	0.0	19.0	0.0	4.0	3.0	0.0
B. cereus	0.0	0.0	15.0	6.0	0.0	20.0	0.0	0.0	0.0	0.0

AMX = Ampiclox, COL = Colistin, CPX = Cephorex, GEN = Gentamicin, AUG = Augmentin, OFL = Oflomed, NAL = Nalidixic acid, TET = Tetracycline, NIT = Nitrofurantoin, COT = Cotrimoxazole

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