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Antibacterial Quantification from Lactic Acid Bacteria Isolated from Food Sources and Soil

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Abstract: Indicator bacteria of pathogenic and food spoilage capabilities were isolated from food sources, water and human feaces using MacConkey agar, Deoxycholate citrate agar and selenite F medium. Lactic Acid Bacteria (LAB) were isolated from soil, wine local cheese and corn slurry using MRS medium. Well-in-agar and paper disc methods were emplored for the antagonistic assay. The sensitivity of the indicator bacteria to LAB was indicated by clear zones of inhibition. *Lactobacillus acidophilus* exhibited the greatest inhibitory effect while *Lactobacillus jugurti* and *Lactobacillus casei* could not demonstrate visible inhibitory affinity on all the indicator bacteria. However, the LAB antagonistic activity over the indicator bacteria, displayed more elaborate inhibitory zones in the well -in-agar (5-26 mm) than the paper disc method (3-20 mm). Also the human enteropathogen assay with well-in-agar was between (2-26 mm) over (1-18 mm) with the paper disc method.

Key words: Antibacterial, lactic acid bacteria, food, soil

INTRODUCTION

Food Preservation by lactic acid bacteria fermentation relies mainly on the accumulation of organic acids and the acidification of the substrate[1], furthermore, a great number of strains of Lactic Acid Bacteria (LAB) produce bacteriocins ribosomally synthesized peptides that exhibit antagonistic activity against closely related species[2,3]. These compounds have received increasing attention since they have the potential to inhibit food pathogens[4]. The application of antagonistic compounds by lactobacilli are not limited to food presentation, antimicrobials o f LAB have been employed successfully to present the formation of biogenic amines[5], to inhibit pathogens causing mastitis[6] and to inhibit enteropathogens in the small intestines of animals[7] . Lactobacilli are known to be involved in many locally fermented foods. They have been isolated from fermented cassava products e.g. gari, fufu, lafun; fermented cereal grains and milk curd[8,9].

Some of the inhibitory component produced by lactic acid bacteria have been intensively studies by application in food preservation[10]. *Pediococcus acidolactic* H produces pediocin ACH. The conditions for its production and its mode of action are known[11,12] .

Lactobacillus reuteri LTH 2585 exhibits antimicrobial activity that can be attributed neither to bacteriocin nor to the production of reutin or organic acid[1]. Reutericyclin produced by *Lactobacillus reuteri* have exhibited a broad inhibitory spectrum including *Bacillus subtilis, Bacillus cereus, Enterococcus feacalis, Staphylococcus aureus* and *Listeria innocua*[1]. Elaine *et al.,*[13] have as well isolated lactic acid bacteria from meat, cheese, milk and plant products that inhibited *Staphylococcus aureus, Listeria innocua* and *Pseudomona fragilis.* Therefore, this research is undertaken to examine the efficacy of lactic acid bacteria isolated from food sources and soil to challenge indicator bacteria also isolated from foods, water and humans with a view to assessing their applicability as food-borne pathogenic, food spoilage and human enteropathogenic inhibitor.

MATERIALS AND METHODS

Isolation of food spoilage and food-borne bacteria from foods: Fufu, pounded yam, rice and drinking water in their cold status were purchase from food centers in Akure metropolis in Ondo State. The fufu, rice and pounded yam were homogenized and serially diluted, water samples were as well diluted and plated on nutrient agar, MacConkey agar and deoxychocolate citrate agar. The resultant colonies after incubation at 37°C for 18 h were characterized and identified with the criteria of Holt *et al.*,[14] and other conventional methods.

Isolation of clinical enteropathogens from humans: Stool were obtained from patients with stomach upset and dysentery. The watery stool were diluted and 1mL was pour plated on Mac Conkey agar and selenite F medium. The resultant colonies after incubation were also characterized and identified with the criteria of Holt *et al.*[14].

Isolation of Lactic Acid Bacteria (LAB): The samples

where the lactic acid bacteria were obtained includes: garden soil, wine, local cheese (wara) and corn slurry (Ogi). The materials already in liquids were serially diluted while those in solids or semi-solids, 10 g Samples were blended in a stomacher (IUL instrument masticor) before 1mL was serially diluted to other decimal dilutions. 0.5 mL of each sample was pour plated on MRS medium and the plates were incubated aerobically at 28±1°C for 48 h. Representatives of different colony types were culturally observed, tested for catalase production with 3% hydrogen peroxide for tentative identification. Catalase positive colonies were purified by sub culturing to obtain pure isolates. The pure isolates were characterized to species level.

Antagonistic screening of the bacteria indicators: Both well-in-agar method of Alade and Irobi [15] and filter paper disk assay impregnated with the LAB concentrated broth culture, were used for the screening. In the well-in-agar method, 0.5 mL of the indicator bacteria approximately (10^7-10^8 cells in their log plate) were aseptically spread plated with a glass spreader previously flamed with ethanol on gel nutrient agar plates. The plates were left for about 1½ hours to allow the test organisms fully embedded before wells of equal distant were dug with sterile cork borer (Δ=5 mm). Using sterile glass pipette, 0.2 mL each of the lactic acid bacteria concentrates was added to the wells. The wells were coded with the Lactobacillus specie in them and the plates were incubated at 27±1°C for 24 h. The sensitivity of the bacteria indicators to each of the LAB is indicated by clear zones of inhibition and the wells and diameter of the clear inhibitory zones were taken as an index of the degree of sensitivity.

In the paper disc assay, sterile filter paper cut to size of about 2 mm were impregnated for 24 h in the lactic acid bacteria concentration. The impregnated disc were picked with sterile forceps, allowed to drain and were placed on the indicator bacteria seeded nutrient agar plates and incubated at 27±1°C for 24 h.

Meanwhile, before use, the Lactic acid bacteria were washed off their liquid substrate by centrifugation in sterile peptone water. The washed and concentrated cells in centrifuge tubes were reconstituted with sterile peptone water.

RESULTS AND DISCUSSION

The eight Lactic Acid Bacteria (LAB) identified and used in this study are *Lactobacillus case*i isolated from local cheese (wara), *Lactobacillus acidophilus* and *Lactobacillus lactic* isolated from garden soil, *Lactobacillus brevis* isolated from wine, *Lactobacillus plantarum* and *Lactobacillus delbruckii* isolated from corn slurry (Ogi), *Lactobacillus fermenti* and *Lactobacillus jugurti* isolated from fufu.

The indicator bacteria are *Pseudomonas aeruginosa*, *Streptococcus feacalis* (I) isolated from meat, the food pathogen *Staphylococcus aureus* was isolated from fufu, *Escherichia coli* I was isolated from water, *Listeria monocytogenes* was isolated from kunu (local sorghum beverage), *Sarcina flava* was isolated from pounded yam and *Streptococcus feacalis* (II) and *Escherichia coli* II, *Shigella dysenteriae* and *Klebsiella aerogenes* were isolated from human stool.

Both the well-in-agar and paper disc methods of assay revealed the LAB that were inhibitory to the indicator bacteria. Meanwhile, the LAB with inhibitory affinity over the bacteria indicators exhibited various degree of inhibitory zones (Table 1). Of all the LAB used, *Lactobacillus acidophilus* had the widest inhibitory effect on the indicator bacteria and ranged between 12-36 mm while *Lactobacillus casei* had the least inhibitory effect and ranged between 3-12 mm. There was great difference in the antagonistic activity between the well-in-agar and paper disc method of assay used in this study. Though the impregnated filter paper assay exhibited sensitivity actions, zones of inhibition were not as elaborate as displayed by the well-in-agar method. In either method, the LAB with inhibitory potentials on the indicator bacteria demonstrated their affinity for evaluations (Table 1).

However, *Lactobacillus casei,* though displayed a very low inhibitory potency, its evaluation was of better result to *Lactobacillus jugurti* and *Lactobacillus brevis* that could not display any valuable potency on any of the indicator bacteria used in this study. However, their inabilities to posses inhibitory potentials on the indicator bacteria is never a conclusion to their non-inhibitory effect.

It was observed in the result obtained that the human enteropathogens were of less susceptibility to the LAB than the indicator bacteria isolated from food sources. It was also observed that the inhibitory potentials displayed by the LAB in the well-in-agar and paper disc on the human enteropathogen were of close range in most cases than seen in the indicator bacteria from food sources (Table 2).

The isolation of both the indicator bacteria and Lactic Acid Bacteria (LAB) mainly from food products is to acknowledge the effect of lactic acid bacteria on pathogenic and food spoilage bacteria as a tendency of evaluating their antibacterial potency. Lactic acid bacteria

Table 1: Agar diffusion and paper disc sensitivity test (mm) of LAB on the bacteria indicators from food sources

	S. aureus		*S. flara*		*E. coli I*		*L. monocytogenes*		*S. feacalis I*		*P. aeruginosa*	
	Ad	Pd	Ad	Pd	Ad	Pd	Ad	Pd	Ad	Pd	Ad	Pd
L. plantarum	14	3	13	8	21	5	18	3	15	6	10	3
L. delbruckii	9	5	5	2	13	2	11	6	19	13	15	-
L. fermenti	12	6	11	8	14	11	16	11	21	13	18	4
L. jugurti	-	-	-	-	-	-	-	-	-	-	-	-
L. lactis	15	12	20	8	22	10	20	13	22	12	17	13
L. caei	5	-	6	-	12	-	12	-	10	-	5	-
L. brevis	-	-	-	-	-	-	-	-	-	-	-	-
L. acidophilus	24	20	22	13	26	18	19	18	26	14	24	12

Ad: Agar diffusion, Pd: Paper disc

Table 2: Agar diffusion and paper disc sensitivity test (mm) of LAB on human enteropathogens.

	S. feacalia II		*E. coli* II		*S. dysenteriae*		*K. aerogenes*	
	Ad	Pd	Ad	Pd	Ad	Pd	Ad	Pd
L. plantarum	10	3	15	5	15	4	22	14
L. delbruckii	8	4	6	3	17	11	14	6
L. fermenti	9	4	7	6	19	11	9	3
L. jugurti	-	-	-	-	-	-	-	-
L. lactis	11	9	17	9	20	13	24	15
L. caei	2	1	8	2	8	1	7	2
L. brevis	-	-	-	-	-	-	-	-
L. acidophilus	23	13	19	14	21	11	26	18

Ad: Agar diffusion, Pd: paper disc

have been found to contain inhibitory acids and antibacterial compounds[1,16] thus their great and reliable value as natural preservatives.

However, it was seen in this study that not all the lactic acid bacteria used exhibited antimicrobial activities on the indicator bacteria. This does not imply that they are void of inhibitory compounds when characterized for their antimicrobial components. Michael *et al*,[1] carried out a research on *L. reuteri* whereby indicator bacteria were not inhibited but were inhibited by the compound reuterine characterized and purified from *L. reuteri*. Therefore, the lactric acid bacteria that exhibited their antimicrobial potency in this work could be as a result of the high antimicrobial compounds present and are effectively discharged from the cell wall. It is also prominent that it is not the lactic acid bacteria that are directly involved in the antagonism of other bacteria species but the chemical compounds (antibiotics) contained in them. The medium used for the inhibition assay contained no added fermentable carbohydrate, a factor which reproduce false-positive reaction due to acid production. Most likely, much of the inhibition observed against the indicator bacteria may be due to hydrogen peroxide which is produced majority under aerobic or microaerophilic conditions by lactic acid bacteria. The use of lactic acid bacteria in control of *Staphylococcus aureus* in this work is in accordance with Elaine *et al.,* [13] where they used lactic acid bacteria isolated from milk, meat, cheese and plant products. The work also is similar with Burnet camard *et al,*[7] who also used lactic acid bacteria to inhibit enteropathogenus *E. coli* in the small intestine of animals, as in this work it is on clinical enteropathogens from humans. In this study approximately 10^7-10^8 cells of Lactobacilli species does the inhibition of the indicator bacteria and it however indicates that such number of cell if present in food can exert a probiotic effect. It is understood that mechanical and chemical preservation of foods destroy disease causing organisms, but contradictory report of the efficiency of pasteurization to inactivate *Listeria monocytogenes* have been reported by and Lovet *et al.,* [17]. *L. monocytogenes* has also been reported by Ryser *et al.*[18] to survive the manufacturing of cottage cheese, remained viable for up to 140-436 days in Colby[19], Chedder and Cheese; and increased in population in camembert cheese[20,21], but in this study, *L. monocytogenes* was inhibited by *L. acidophilus, L. lactis* and *L. Plantarum.*

The direct use of these lactic acid bacteria might exhibit other properties from their metabolic activities to make some food undesirable, unacceptable and unpleasant taste if left for too long. It will be of paramount importance for more research to go into the isolation and purification of the antibiotic compounds present in them for effective and more reliable use as preservatives in food industries and antibiotics in clinical sectors. But what is not certain is whether the LAB species present in one source will have the same level of antagonistic affinity with others from different sources and environment.

Meanwhile the more susceptibility observed in the indicator bacteria could be as a result of thermal shock or damage during cooking which some how could make the organism weaker than the human enteropathogens which were less susceptible probably as result of their stability and their virulent state which could not have been altered.

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