Journal of Probiotics & Health

Anzaku and Pedro, J Prob Health 2017, 5:2
DOI: 10.4172/2329-8901.1000171

Research Article Open Access

Antimicrobial Effect of Probiotic *Lactobacilli* on *Candida* Spp. Isolated from Oral Thrush of AIDS Defining Ill Patients

Abbas Abel Anzaku* and Akharenegbe Pedro

Department of Microbiology, Federal University Lafia, Nigeria

Abstract

This study aimed at investigating the growth inhibitory effect of probiotic *Lactobacillus* on *Candida* species isolated from oral thrush of AIDS-defining ill patients. Clinical isolates of *Candida* species were obtained from Dalhatu Araf Specialist Hospital Lafia, Nasarawa State. de Man Rogosa Sharpe agar was used as starter culture for the isolation of *Lactobacilli*. The diameters of the antimicrobial assay were recorded using the following dilution in part per mL (1:10, 1:100, 1:1000, 1:10000 and 1:100000) with the highest inhibition zone of 8 ± 1.414 mm followed by 7 ± 0.000 mm and the lowest dilution recorded as 5 ± 0.000 mm in the highest concentration of 1:10. As the dilution concentration decline, the inhibitions become weaker. The lowest dilution concentration (1:100000) however showed resistance in all the agar wells. Conclusively, *Lactobacillus* species has growth inhibition against yeast organisms *in vitro*. This study therefore recommends further research to identify more probiotic organisms that can potentially inhibit the growth of disease causing pathogens. Researchers should put more mental energy into use as research is the only source of solution to combat most of the emerging diseases around us.

Keywords: Antimicrobial; Probiotic; *Lactobacilli*; *Candida*; Oral thrush; AIDS defining ill patients

Introduction

Currently, a focused attention is given to probiotic *lactobacilli* to be used in treatment of emerging and re-emerging diseases as the world has transited to a molecular level of solving most of her global health challenges. *Lactobacilli* are fermentative bacteria that reside in the gastrointestinal tract of humans and animals that have been identified as one of the beneficial microbial flora of human [1]. Probiotics generally are believed to provide health benefits when consumed and have been recognized to play a significant role in the maintenance of oral health. Because they promote oral health, they are added to different commercial probiotic products. AIDS defining illness also known as AIDS defining clinical condition is the list of diseases published by the Centers for Disease Control and Prevention (CDC) that are associated with AIDS, and are used worldwide as a guideline for AIDS diagnosis in which oral candidaiasis has been identified as one of those diseases *[2]*. Probiotics has been reported to demonstrate the ability to inhibit the growth of some intestinal pathogens such as Escherichia coli, Campylobacter jejuni and *Salmonella enteritidis in vitro* [3]. Zalán et al. [4] reported that *Lactobacilli* universally produce lactic acid that inhibits the metabolic activity of *Candida* sp which has a weak antifungal activity *[5]*. Probiotics generally exert their benefits through several mechanisms such as prevention of colonization, cellular adhesion and invasion by pathogenic organisms, they have direct antimicrobial activity and they modulate the host immune response. The strongest evidence for the clinical effectiveness of probiotics has been in their use for the prevention of symptoms of lactose intolerance, treatment of acute diarrhea, attenuation of antibiotic-associated gastrointestinal side effects and the prevention and treatment of allergy manifestations [6]. For the prevention of intestinal disorders, probiotics can be rendered multidrug resistant to survive in the presence of co-administered antibiotics and can generates the possibility of resistance transfer from the probiotic to human bacterial pathogens, either directly or indirectly via the commensal flora [7]. The effect of Lactobacillus spp. such as L. GG was specific because levels of antibodies to antigens in other vaccines received previously were not increased. The use of probiotics has also been effective in enhancing the mucosal barrier to pathogens and antigen presentation. A recent study reported that known probiotics Lactobacillus strains could stimulate up-regulation of mucous genes in intestinal goblet cells the effect of these probiotics on the activation and secretion of mucus in the intestine is correlated with the inhibition of pathogenic *Escherichia coli* attachment and of damage to the intestinal tract. Various authors have shown that surplus biomass from the fermentation industry, recycled as an additive to cattle, hog and poultry diets improves livestock performance and product quality [8] unlike other bacteria. Antibiotic therapy is well known to destroy the normal bacterial population of the digestive tract, which allows harmful bacteria to colonize and irritate the host gut and cause antibiotic associated diarrhea [9]. For the purpose of this research, *in vitro* inhibitory effect of probiotic lactobaciili on *Candida* species isolated from oral thrush of AIDS-defining ill patients will be evaluated.

Materials and Methods

This study was carried out in Lafia metropolis to assess the inhibitory potentials of probiotic *Lactobacilli* on *Candida* species isolated from oral thrush of AIDS defining ill patients. Clinical isolates were obtained from Dalhatu-Araf Specialist Hospital (DASH) Lafia, while commercial yoghurt was obtained from Lafia Modern Market Lafia, Nasarawa State. Laboratory experiment was carried out in Microbiology Laboratory of Federal University Lafia, Nigeria. Lafia is the capital of Nasarawa state and lies between latitude 8°25'40"N to 8°34'15"N and 8°24'25"E longitude to 8°38'19"E in the guinea savannah region of northern Nigeria. Lafia is the largest town in Nasarawa State with population of 330, 712 [10]. Nasarawa State is one of the Nigerian states situated

***Corresponding author:** Abbas Abel Anzaku, Department of Microbiology, Federal University Lafia, Nigeria, Tel: +2348038141859; E-mail: humbleabel2016@yahoo.com

Received Janaury 18, 2017; **Accepted** April 25, 2017; **Published** May 02, 2017

Citation: Anzaku AA, Pedro A (2017) Antimicrobial Effect of Probiotic *Lactobacilli* on *Candida* Spp. Isolated from Oral Thrush of AIDS Defining Ill Patients. J Prob Health 5: 171. doi: 10.4172/2329-8901.1000171

in the north central that cover an area of 28,735 square kilometres' approximately. It has a population of 1,863,275 (2006 Census figures) with a population density of 65 people per square kilometre. Its population makes up 1.3% of Nigeria's total population. Nasarawa state is bounded on the North by Kaduna State, North-West by the Federal Capital Territory (FCT), Abuja, North-East by Plateau State, and South by Benue State, South-West by Kogi State and on the South-East by Taraba State.

Preparation of inoculums for susceptibility test

Bacterial isolates: *Lactobacilli* from fruit, yoghurt and fermented beverage were used as starter culture in nutrient agar and further sub cultured in selective medium (de Man Rogosa Sharpe Agar) for the isolation of Lactobacillus species. Each distinct colony of lactobacillus was inoculated into 5 ml MRS broth for 24 hours of incubation at 37°C. The supernatant was adjusted to a turbidity of opacity equivalent to McFarland Turbidity Standard No 1. Then 1 ml of the suspension was diluted with 4 ml of sterile saline and used to inoculate the extract-containing media as well as the controls.

Yeast isolates: Pure clinical isolates of yeast isolated from oral thrush of HIV-AIDS defining ill patients were culture in Potato Dextrose Agar (PDA) for 48 hours, pure colony of each organisms were transferred to 2 ml Potato Dextrose Broth and incubated for 24 hours at the temperature range of 37°C. The supernatant was adjusted to a turbidity of opacity equivalent to McFarland Turbidity Standard.

Gram staining: A small portion of a 24 hours presumptive colony of each isolate was emulsified in a drop of water on a clean grease-free glass slide; heat fixed and allowed to air dry. The fixed smear was flooded with crystal violet for 30-60 seconds and rapidly rinsed off with clean water, with the water appropriately tipper off. The smear was then covered with Gram's iodine for 30-60 seconds and rinsed with water. Rapid decolorization of the smear was done for a few seconds using acetone-alcohol and immediately rinsed with clean water. Counter staining was done for 60 seconds using Safranin and subsequently rinsed with clean water. The back of the slide was cleaned and the slide was allowed to air dry. After air drying, the smear was viewed under the microscope using the oil immersion (×100) objective**.**

Biochemical test: Biochemical testing of presumptive organisms was carried out according to the method described by Cheesbrough, Woodland and Food and Agriculture Organization with modifications [11].

Catalase test: This test was carried out after 24 hours incubation at 37°C. To perform this test, 3 ml of 3% hydrogen peroxide (H_2O_2) was dispensed into a clean and sterile test tube. Pure colonies of the presumptive isolates were inoculated into the tube using sterile wooden stick. The test tube was then observed for rapid and immediate bubble formation which signified a positive result.

Coagulase test: The slide test method of detecting the enzyme coagulase was used. A drop of plasma was placed on a slide and a portion of the test organism was added to it. Agglutination of the cells on the slide within one to two minutes indicated the presence of coagulase.

Oxidase test: An oxidase strip was moistened with a drop of sterile water and emulsified with the test organism that was aseptically removed from the Petri dish using sterile applicator stick. Color change from red to purple within 20 seconds signified a positive result.

Motility test: A 24 hours old agar culture of test isolates was inoculated using stab method into test tubes containing motility agar and incubated at room temperature (25°C) for 18-24 hours. Turbidity of the whole medium that radiates from the line of inoculation signified a positive result.

Indole test: To perform this test, 3 ml of sterile tryptone water was dispensed into a sterile bijou bottle, inoculated with the test organisms and incubated for 48 hours at 35°C. After incubation, 0.5 ml of Kovac's reagent was added to the bijou bottle and agitated gently. Formation of red coloration on the surface of the mixture within 10 minutes signified a positive result.

Citrate utilization test: Slants of Simmons citrate agar in test tubes were prepared according to manufacturer's instruction. Using sterile inoculating needle, a saline suspension of the organism was streaked on the slants. Inoculated slants were then incubated for 48 hours at 35°C. Color change from green to blue signified a positive result.

MR-VP (Methyl red Voges-Proskauer) test: A pair of tubes containing 5 ml of sterile MRVP broth was inoculated with presumptive clinical isolates and incubated for 24 hours at 37°C. After incubation, 6 drops of methyl red was added to one of the tubes; 6 drops of Barritt A and 4 drops of Barritt B were added to the other tube. Tubes were then allowed to stand for at least 30 minutes. Formation of red color and pink color ring in the methyl red and Voges-Proskauer tubes indicated a positive result for each test respectively.

Carbohydrate utilization test: The ability of the isolates to utilize carbohydrate was determined in the following sugar (maltose, sucrose, glucose, lactose, D-fructose and xylose). 5 g of each of the sugar was weighed in a weighing balance and dispensed into three different 250 ml conical flask containing 75 ml of distilled water and was shaken thoroughly to homogenize. Five milliliters (5 ml) of each of the homogenous solution were dispensed into 60 test tubes (20 test tubes for each solution) and were labeled as (Maltose, Sucrose and D-fructose AA to AJ and SA to SJ) accordingly. Organisms of each plate were further inoculated into the test tube containing each of the solution and methyl red was drop into the solution. Control tubes were also prepared for comparism. The tubes were incubated for 24 hours for fermentation reaction. A colour change from red to yellow indicates positive result.

Urease test: Urease test was carried out to determine the ability of the isolates to utilize urea. This test was done using urea broth and distilled water. After 24 hours incubation, there was colour change from red to pink which indicate positive result.

Antimicrobial sensitivity assay: Serial dilution was carried out in tenfold step. Undiluted suspension corresponding to approximately 10^3, 10^5, 10^7, and 10^9 were selected for the inhibition experiment. Each one ml of *Lactobacilli* suspension was set and the plates were anaerobically. The plates were allowed to dry for 2 hours at room temperature. Broth cultures of *Candida* species grown for 18-24 hours in Potato Dextrose broth were diluted in the same medium and were adjusted to turbidity opacity equivalent to McFarland turbidity standard (Table 1). Agar-well diffusion method as described by Bonev [12] was adopted for the determination of inhibitory assessment. Exponential cultures of the test micro-organisms were diluted to a suitable turbidity and used to inoculate a melted spreading the cell suspension with a sterile cotton swab. Wells, 6 mm in diameter, were punched in the agar plates and 20 µL of CFU/ml were added to the wells. After incubation overnight at 37°C, the zones of inhibition were observed around the wells. The diameters of the antimicrobial assay were recorded accordingly. Control was stamped onto agar plates without *lactobacilli* within the agar layer hence were recorded as 0 ± 0.000.

Results and Discussion

Lactobacillus species were isolated from commercially produced yoghurt. The colony features, microscopy, biochemical reactions, urease utilization, indole test, citrate utilization test, and Methyl Red voges proskauer (MR-VP) test carbohydrate utilization by the isolates were determined. *Lactobacilli* genera were not further identified to species level. The result of yeast isolated from the specimen is presented in Table 2 below. This shows the characteristics, nature of colony, reverse side, texture, and nature of growth. The presumptive yeast organism was *Candida* species.

Antimicrobial Susceptibility Assay using Bonev-agar well diffusion method is presented in Table 3 below. The sensitivity assay was completed using the following dilution in part per mL (1:10, 1:100, 1:1000, 1:10000 and 1:100000) respectively. Probiotic isolates however showed significant zones of inhibition against yeast strains.

Based on this study to ascertain the antimicrobial effect of lactobacillus species on *Candida* species isolated from oral thrush of AIDS defining ill patients, each distinct colony identified were used for the antimicrobial assay. Isolates were identified by their biochemical characteristics and their ability to utilize certain sugar. In Table 1 above, the bacterial isolates fermented most of the carbohydrate showing characteristics of *Lactobacilli* by their biochemical reactions and morphological characteristics as most colonies demonstrated very similar biochemical characteristics. At different dilution in part per mL (1:10, 1:100, 1:1000, 1:10000 and 1:100000), some isolates showed zones of inhibition against yeast with the highest inhibition zone of 8 ± 1.414 mm in plate 1 followed by 7 ±.000 mm and the lowest was 5 ±.000 mm in concentration of 1:10. The highest dilution concentration 1:10000 showed the lowest zones of inhibition with the highest as 1 ± 0.000 mm while other agar wells demonstrated resistance at the same concentration. The highest dilution concentration 1:100000 however showed resistance in all the agar wells. *Lactobacilli* strains reduced the growth of yeast but some strains were generally weaker than some while others strains were resistant depending on the concentration. This result in this respect conforms to the findings of Jacobsen et al. [13], that *Lactobacilli* and bifidobacteria has *in vitro* antimicrobial effect against transient microorganisms. Comparable reported was made by Ratitsa et al. [14], who tested the antimicrobial activities of *Bifidobacterium* and *Lactobacillus fermentum* intended to be used as starter and probiotic culture. The result showed a promising antimicrobial activities as zones of inhibition were observed. Probiotics has been described by Shira and Sherwood [6], to exert their benefits through several mechanisms such as prevention of colonization, cellular adhesion and invasion by pathogenic organisms; they have direct antimicrobial activity and they modulate the host immune response. The strongest evidence for the clinical effectiveness of probiotics has been in their use for the prevention of symptoms of lactose intolerance, treatment of acute diarrhea, attenuation of antibiotic-associated gastrointestinal side effects and the prevention and treatment of allergy manifestations. Lactic acid bacteria (LAB) in general usually produce antimicrobial substances like bacteriocin which have broad spectrum of antagonist effect against closely related Gram positive and Gram negative pathogens and produces biosurfactant which showed that the wide range of antimicrobial activity against bacterial pathogen as well as its antiadhesive properties reduces the adhesion of pathogens into gastric wall membrane; furthermore, LAB strains have also been reported for production of antioxidants which are ability to scavenge the free radicals such as superoxide anions and hydroxyl radicals. Paulraj et al. [15], Reported that probiotic strains tested using plate assays offered some inhibition of each of the pathogenic strains, *E. coli, S. enteritidis* and *C. jejuni.* The extent of inhibition was dependent on the probiotic

Microscopy	**Biochemical Raction and Cabohydrate Utilization**	
Colony Features	Cream raised, dull colonies, smooth, long and slender, convex, non pigmented and opaque on de Man Rogosa Agar.	
Microscopy	Biochemical Reaction	Carbohydrate Utilization
Cell arrangement: Gram positive rods in pairs and in single.	Catalase -	Glucose +
Spore +	Oxiodase -	Sucrose +
Motility -	Coagulase -	Lactose +
Capsule +	Iondole -	Maltose +
	Methyl Red -	D-fructose -
	VP -	Xylose -
	H2S -	
	Citrate +	
	Urease -	
	Mannitol +	

Keys: +=Positive; -=Negative; VP=Voges Proskauer.

Table 1: Characterization and identification of probiotic Lactobacilli isolated from Yoghurt.

Microscopy	**Macroscopy/Colony Morphology**				
Characteristics	**Nature of Colony**	**Reverse Side**	**Texture**	**Nature of Growth**	**Organism**
Septate pseudohyphae with clusters of round blastospore at the septa and large thick-walled terminal clamydospores, Germ-tube test +	Cream-colour, pasty and smooth	-	-	Rapid	Yeast

Keys: +=Positive, -=Negative.

Table 2: Characterization and identification of yeast organism isolated from oral thrush.

Dilution in Part Per mL	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Control
1:10	8 ± 1.414	8 ± 0.0000	7 ± 1.414	7 ± 0.000	5 ± 0.000	0 ± 0.000
1:100	6 ± 0.000	6 ± 1.414	5 ± 1.414	5 ± 0.000	4 ± 1.414	0 ± 0.000
1:1000	2 ± 1.414	3 ± 0.000	3 ± 0.000	2 ± 0.000	3 ± 0.000	0 ± 0.000
1:10000	R	1 ± 0.000	R	R R	0 ± 0.000	
1:100000	R	R	R	R R	0 ± 0.000	

Keys: R=Signify resistance.

Table 3: Antimicrobial susceptibility assay of Lactobacillus species on yeast species using Agar well diffusion assay.

strain, such that *L. plantarum* 0407 and *B. bifidum* Bb12 tended to inhibit pathogen growth to a greater extent than that observed for the other strains included, particularly with *E. coli*.

Conclusion and Recommendation

This research showed a significant inhibitory effect of some *lactobacillus* strains against *Candida* species *in vitro* though there were cases of resistance in some agar wells. This however has drawn to a rational conclusion that *lactobacilli* have *in vitro* antimicrobial effect and can inhibit the growth of yeast organisms. Based on the fact established by this research on the antimicrobial effect of *lactobacillus* species on *Candida* isolated from oral thrush of AID defining ill patients, the findings recommends more research to be carried out in order to identify more probiotic organisms both bacterial and yeast organisms that can potentially inhibit the growth of disease causing pathogens. Researchers all over the world should put more mental energy into use as research is the only source of solution to combat most of the emerging and re-emerging diseases around us.

Acknowledgment

Warm gratitude goes to the reviewers of this paper for exhaustively making possible corrections to ensure the authenticity of the whole work.

Limitation of the Work

This work was limited genera as there were no funds to identify it to species level. Funds, time and equipped laboratory were the greatest challenges encountered as the research was not funded by any organization.

References

1. Yan S, Baojiang Z, Lingling S (2013) CD4 Detected from Lactobacillus helps understand the Interaction between Lactobacillus and HIV. Journal Microbiological Research 168: 273-277.
2. Lee N (2008) Penicilliosis: an AIDS-defining disease in Asia. Hong Kong Medical Journal 14: 88-89.
3. Viswanathan S, Chinnammal SR, Amuthan M, Rajesh R, Suba P, et al. (2015) Effect of Probiotics, Antibiotics and Herbal Extracts on Aeromonassp and Vibriospisolated from a Marketed Marine Fish. International Clinical Pathology Journal 1: 5.
4. Zalán Z, Hudáček J, Štětina J, Chumchalová J, Halász A (2010) Production of organic acids by Lactobacillus strains in three different media. Eur Food Res Technol 230: 395-404.
5. Köhler GA, Assefa S, Reid G (2012) Probiotic interference of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 with the opportunistic fungal pathogen Candida albican. Jouronal Infectious Disease and Obterics Gynecology 118: 1180-1190.
6. Shira D, Sherwood LG (2014) Probiotics: their role in the treatment and prevention of diseases. Expert Review of Anti-Infective Therapy, London, pp: 261-275.
7. Patrice D (2006) Antibiotic resistance: the pros and cons of probiotics. Journal of International Congress 3: 261-265.
8. Dawson KA, Newman KE, Boling JA (1990) Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. Journal of Animal Science 68: 3392-3398.
9. Coté GA, Buchman AL (2006) Antibiotic-associated diarrhoea. Expert Opin Drug Saf 5: 361-372.
10. National Population Commission (2006) National and State Population and Housing Table. Census Report: Nigeria.
11. FAO/WHO (2001) Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Report of a Joint FAO/WHO Expert Consultation.
12. Bonev C (2008) Principles of Assessing Bacterial Susceptibility to Antibiotic using the Agar well Diffusion Method. Journal of Antimicrobial Chemotherapy 61: 1295-1301.
13. Jacobsen CN, Rosenfeldt NV, Hayford AE, Møller PL, Michaelsen KF, et al. (1999) Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Applied Environmental Microbiology Journal 65: 4949-4956.
14. Ratitsa G, Lyubomira Y, Lilia T, Galina Z, Nina S, et al. (2014) Antimicrobial Activity and Antibiotic Susceptibility of Lactobacillus and Bifidobacterium spp. For use as Starter and Probiotic Culture. Journal of Biotechnology and Biotechnological Equipment 10: 84-91.
15. Paulraj K, Satish KR, Yuvaraj N, Paari KA, Pattukumar V, et al. (2012) Probiotics and its Functionally Valuable Products - A Review. Journal of Food Science and Nutrition 53: 641-658.