



# World News of Natural Sciences

An International Scientific Journal

WNOFNS 29(3) (2020) 290-297

EISSN 2543-5426

---

---

## Antibacterial properties of probiotics bacterial isolated from human breast milk

**Ekeoma Nmesoma Blessing<sup>1</sup>, Ihenetu Stanley Chukwuemeka<sup>2,\*</sup>,  
Ukeachu Chigozie David<sup>3</sup>, Ubah Gerald Onuawuchi<sup>4</sup>**

<sup>1</sup>Department of Medicine, Gregory University Uturu, Abia State, Nigeria

<sup>2</sup>Department of Chemistry, Imo State University Owerri, Nigeria

<sup>3</sup>Department of Microbiology, Imo State University, Nigeria

<sup>4</sup>Department of Microbiology, University of Port Harcourt, Nigeria

\*E-mail address: [lihenetustanley@yahoo.com](mailto:lihenetustanley@yahoo.com)

### ABSTRACT

This research work assessed the antibacterial properties of probiotic bacterial which was isolated from breast milk. The breast milk has a distinct amalgamation of minerals, proteins, lipids, carbohydrates and various vitamins that endorse the proper development, growth and immunity of the children. That's the reason behind its consideration to be a comprehensive and inclusive food for newborn babies. Furthermore, it is also abundant in various bioactive compounds which encourage the maturation of the immune system over and above develop body's defense against infections. This research used a standard methodology to isolate the bacterial. In the midst of these bioactive agents, probiotic bacteria were properly isolated from human milk in this research work by means of selective MRS media. Five *Lactobacillus* spp. were isolated from every one of the three breast milk samples and two *Enterococcus species*, were observed as potential probiotics, and identified using morphological and biochemical tests which includes *L. rhamnosus*, *L. plantarum*, *L. casei*, *L. fermentum*, *L. acidophilus*, *En. faecalis* and *En. faecium*. The isolated bacteria were facultative anaerobic, catalase negative, gram positive and non-endospore forming. Sugar fermentation arrangements of equally isolated bacteria also significantly different. The adding up of breast milk probiotics to children formulas possibly will be an innovative substitute to mimic some of the purposeful consequences of human milk in children who are not breastfed.

**Keywords:** probiotic, breast milk, micro-organism, human breast milk, *L. rhamnosus*, *L. plantarum*, *L. casei*, *L. fermentum*, *L. acidophilus*, *Enterococcus faecalis*, *Enterococcus faecium*

## **1. INTRODUCTION**

Probiotics are microorganisms that are believed to provide health benefits when consumed. (Rijkers *et al.*, 2011). The term probiotic is currently used to name ingested microorganisms associated with beneficial effects to humans and animals. (Magdalena *et al.*, 2006) The term came into more common use after 1980. The introduction of the concept is generally attributed to recipient Élie Metchnikoff, who in 1907 suggested that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes". (Magdalena *et al.*, 2006) A meaningful development of the potential market for probiotics has resulted to higher prerequisites for scientific authentication of putative beneficial effects conferred by the microorganisms. (Rijkers *et al.*, 2011; Allen *et al.*, 2010; Gueimonde *et al.*, 2007) Findings on the medical reimbursements of probiotics have yet to expose a cause-effect relationship, and their medical usefulness has yet to be conclusively confirmed for utmost of the studies conducted consequently.

The probiotic contender must be a taxonomically distinct microbe or combination of microbes (genus, strain level and species). It is normally admitted that most outcomes of probiotic are strain-specific and cannot be stretched to other probiotics of the same species or genus. This requests for a precise identification of the strain, i.e. phenotypic and genotypic characterization of the verified microorganism. (Reid *et al.*, 2010; Zacarias *et al.*, 2011) Probiotics must be safe for their proposed use.

Once it comes to the microbiological fact of breast milk, human milk is certainly an important factor in the beginning and development and of progress composition of the neonatal gut microflora given that it comprises basis of microorganisms to the infant gut for numerous weeks after birth. The constitution of the gut microflora is comprehensively prejudiced by the diet of the infant. As a result, the manifestation of a limited predominant Gram-positive species in breast milk might be a cause explaining why microbiota of breast-fed infants is constituted of a slender spectrum of species, and a further dissimilar microbiota develops after deterring (Reid *et al.*, 2010; Daniel *et al.*, 2014).

The findings on the microbiology of human milk are constrained to the identification of possible pathogenic bacteria in medical cases of mastitis or infant infections. However, it is clear that the impediment of infant from contagious diseases owing to the biological flora of human milk. Even though, there is incomplete knowledge in the region of the commensal or probiotic bacteria that breast milk comprehend, bacteria frequently isolated from this biological liquid comprise *staphylococci*, *lactobacilli*, *streptococci*, *enterococci* and *micrococci*. Bacteria from these genera can be effortlessly isolated from fresh milk of different healthy women. So, these groups of bacteria should be considered the natural microbiota of human milk rather than mere contaminant bacteria (Ouwehand *et al.*, 2011, Ayad *et al.*, 2011). The aim of the study is to isolate the probiotic bacteria that can be found on the breast milk.

## **2. EXPERIMENTAL**

### **2. 1. Study area**

Imo State of Nigeria is one of the thirty six states of the federal republic of Nigeria. It is specifically in South Eastern Nigeria. It lies between geographic co-ordinates of latitude 4°45

and 7°15' N and longitude of 6°50' E with an area of around 5,100 sq km (Imo State Government, 2010). The state has a common boundary with Abia state on the East, Anambra state on the North, and Rivers state on the South (Anosike *et al.* 2001).

## **2. 2. Study population**

The study was carried out from May to June 2019. All the samples for this project work were collected from healthy mothers within the age of 22 to 35 years old.

## **2. 3. Sample Collection**

Human breast milk used in this study were obtained from sixty healthy mothers within four months of given birth to healthy babies in federal medical center Owerri. The nipple and mammary areola of the breast were wiped with 70% ethanol and about 5 mL of milk was collected in a sterile test tube using a sterile breast pump. The samples were collected in sterile carriers and stored on ice until delivery to the laboratory.

## **2. 4. Serial Dilution / Culture**

The serial Dilution method was used (pour plate / spread plate technology).

Procedure:

- Test tubes comprising 9 ml peptone water each were marked A-F
  - By means of the sterile peptone water and distinct sterile pipettes or string, serial dilution of the samples everyone were prepared as follows
- A) 1 ml of the sample were preside over into 9ml sterile Peptone Water and homogenized =  $10^1$  dilution.
- B) 1 ml of A into 9 ml sterile Peptone Water of B and homogenized =  $10^2$  dilution
- C) 1ml of the marked B into 9 ml sterile Peptone Water of C and homogenized =  $10^3$  dilution
- D) 1 ml of marked C into 9 ml sterile Peptone Water of D and homogenized =  $10^5$  dilution
- E) 1 ml of marked D into 9 ml sterile Peptone Water of E and homogenized =  $10^6$  dilution
- F) 1 ml of marked E into 9 ml sterile Peptone Water of F and homogenized =  $10^6$  dilution (Fawole and Oso, 1995).

After the serial dilution, 0.5 ml of the serially diluted samples each were inoculated into freshly prepared de Man, Rogosa and Sharpe (MRS) agar medium and incubated for 72 h at 37 °C in anaerobic jars containing gaspack (AnaeroGen, Oxoid, UK) (oxygen level <1%, CO<sub>2</sub> level between 9 and 13%). After the incubation period, colonies were randomly picked from the plates and subcultured three times on fresh MRS agar plates (Martin *et al.*, 2005; Langer, 2009).

## **2. 5. Identification of bacterial**

After the incubation different cultures will be observed on the petri plates. Counting will be done for each plate and different microbes will be identified on the basis of their colour and growth pattern (Ouwehand *et al.*, 2002; Ljungh and Wadstrom, (2009). Biochemical identification was carried out (biochemicl test).

### 3. RESULT

The results below are obtained from the microbiological analysis carried out from twelve samples obtained from healthy mothers within four months of given birth.

Table 1 below shows the bacteria count (cfu/ml). The result shows that the highest bacteria count is seen from the age range of 33-35 years ( $1.9 \times 10^3$ ).

**Table 1.** Total bacteria count

Ages of mothers	Total bacteria count
22 – 25	$3.3 \times 10^2$
26 – 28	$6.8 \times 10^2$
29 – 32	$6.9 \times 10^2$
33 - 35	$3.9 \times 10^3$

**Table 2.** Percentage Prevalence of Microorganisms.

Age	Bacteria isolates				
22-25	<i>En. faecium</i>	<i>L. platarum</i>	<i>L. rhamnosus</i>	<i>En. faecalis</i>	
26-28	<i>En. faecium</i>	<i>L. casie</i>	<i>L. rhamnosus</i>		
29-32	<i>L. acidophilus</i>	<i>L. platarum</i>	<i>L. rhamnosus</i>	<i>En. faecalis</i>	
33-35	<i>En. faecium</i>	<i>L. acidophilus</i>	<i>L. casie</i>	<i>L. platarum</i>	<i>En. faecalis</i>

Bacteria isolated from human breast milk were identified as *Lactobacillus* spp. and enterococcus species by observing their colony morphology, physiological as well as biochemical characteristics

**Table 3.** Identification of probiotic bacteria.

Bacteria strain	Gram	Cat	Sugar Fermentation						Growth at	
			Glu	Suc	Sor	Mel	Rha	Cell	15 °C	45 °C
<i>L. acidophilus</i>	+	-	-	+	+	+	-	+	-	+
<i>L. fermentum</i>	+	-	+	+	ND	+	-	-	-	+

<i>L. platarum</i>	+	-	-	+	+	+	-	+	+	-
<i>L. casie</i>	+	-	-	+	+	-	-	+	+	-
<i>L. rhamnosus</i>	+	-	+	+	+	-	+	+	+	+
<i>En. faecium</i>	+	-	+	+	+	-	+	+	-	+
<i>En. faecalis</i>	+	-	+	+	+	+	-	+	-	+

Key: gram = gram staining, cat = catalase test, glu = glucose, suc = sucrose, sor = sorbitol, mel = melibiose, rha = rhamnose, ND: no data available.

**Table 4.** Minimum inhibitory concentrations (MIC) for antibiotic susceptibility of probiotic bacteria.

Strain	Antibiotic ([MIC ( µg/mL)]						
	Amp	Gen	Kan	Step	Ery	Tet	Chl
<i>L. acidophilus</i>	<0.06	<0.12	<0.12	<0.5	<0.06	<0.04	<0.05
<i>L. fermentum</i>	<0.06	<4	<8	<8	<0.5	<1	<1
<i>L. platarum</i>	<0.15	<8	<6	<4	<0.15	<0.25	<1
<i>L. casie</i>	<0.25	<8	<64	<32	<1	<1	<4
<i>L. rhamnosus</i>	<0.5	<4	<8	<6	<0.25	<2	<1
<i>En. faecium</i>	<0.8	<8	<25	<10	<0.4	<2	<8
<i>En. faecalis</i>	<1	<9	<16	<12	<0.25	<2	<7

Key: Amp = Ampicillin, Gen = Gentamicin, Kan = Kanamycin, Stre = Streptomycin, Ery = Erythromycin, Tet = Tetracycline, Chl = Chloramphenicol. <0.01 = Resistance, 8-15 = Moderate, <16 = Sensitive.

#### 4. DISCUSSION

Form the result obtained in Table 1, shows the bacteria count (cfu/ml). The result showed that the highest bacteria count is seen from the age range of 33-35 years ( $3.9 \times 10^3$ ). On the foundation of biochemical physiognomies (gram positive, catalase negative, endospore absence, non-motile, sugar fermentation pattern, antimicrobial activity), the isolates were classified as *Lactobacillus* spp. and *Enterococcus* species. The colonies of *Lactobacillus* isolates from all the samples are posited to be *Lactobacillus acidophilus* and appeared rough,

dull white, 0.1-0.5 mm in diameter, and demonstrated medium to short rods as earlier studies by Jara *et al.*, 2011 has found similar aforementioned characteristics in isolated *Lactobacilli*.

As of the results obtained in Table 3 above, has revealed that organic acid production increased with the incubation time while pH of the media decreased with the increasing acid production. The capacity of substances to inhibit microbial growth is referred to as antimicrobial activity, from the result in Table 4, the antimicrobial agents used include streptomycin, ampicillin, erythromycin, gentamicin, kanamycin, tetracycline and chloramphenicol. *L. acidophilus* had minimum inhibition in all the antimicrobial agents between 0.04 to 0.6 µg/mL, *L. fermentum* had 0.05 to 8 µg/mL, *L. platarum* had 0.15 to 8 µg/mL, *L. casie* had 0.2 to 4 µg/mL but 32 µg/mL in streptomycin and 64 µg/mL in kanamycin, *L. rhamnosus* had 0.25 to 8 µg/mL. *Enterococcus* species are recorded to be moderate except for *En. faecium* which is sensitive to kanamycin and *En. faecalis* which is sensitive to kanamycin. As the isolated lactic acid bacteria inhibited these pathogenic strains successfully, it may be expected that addition of these human milk probiotics to commercial food products for infants would confer effective protection against infections caused by these pathogens.

All the samples have these probiotic bacteria except for sample one that do not record *L. fermentum* and *En. faecalis*. Human breast milk composes an interesting basis to obtain new precise probiotic stains for neonates pointing at assisting an appropriate development of the gut microbiota and the immune development in infants who, for different reasons, can't be breast-fed. The breast milk collected in this study contained strains of *L. rhamnosus*, *L. plantarum*, *L. casei*, *L. fermentum*, *L. acidophilus*, *En. faecalis* and *En. faecium*. Consequently, breast milk is considering a substantial foundation of lactic acid bacteria that appear to be of endogenous origin. Since breast milk has been suggested as vehicle for potentially probiotic LAB, it could be considered as a natural synbiotic food that is a mixture of probiotics and prebiotics. It has correspondingly been ascertained that breast feeding beneficially can affect infants by improving the survival and implantation of live dietary microorganisms in gastrointestinal tract

## 5. CONCLUSIONS

In conclusion, the breast milk possibly will be a good and safe source for isolation of probiotic bacteria and for improve intestinal microflora of children. This report have establish their use in the development of new pharmaceutical preparations and functional foods that contain milk probiotics for the betterment of health of the public. Lactic acid bacteria were isolated from human milk in pure culture and various properties of isolated bacteria were determined. All of isolates showed tolerance to bile salt, organic acid production and antimicrobial activity against some indicator microorganisms. Phenotypic identification effectively differentiated the isolates especially sugar fermentation patterns. Two different isolate strains were identified and these could be used as potential probiotic strains.

## References

- [1] Anosike, J.C., Nwoke, B.E.B., Okere, A.N., Alozie, J.I., Okoro, U.O., Nwosu, D.C., Ogbulie, J.N. and Amadi, A.N.C. (2001): Endemicity of urinary Schistosomiasis in the

- North Central Zone of Abia State, Nigeria. *International Journal for Environmental Health and Human Development* 2(2): 5-12
- [2] Allen, S., Martinez, E., Gregorio, G., Dans, L., Martinez, G. (2010). Probiotics for treating acute infectious diarrhea. *Cochrane Database Syst Rev* 11 (11): 30-48
- [3] Ayad, E. H. and Shokery, E. S. (2011). Industrial important Lactic acid bacteria isolated from Egyptian Human milk and dairy products. *Egyptian Journal of Dairy Science*, 39: 197-207
- [4] Daniel, J., Merenstein, B., Pot, L., Roberto, B., Harry, J. and Calder, S. (2014): Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology* 11 (8): 506-514
- [5] Élie, M. (2004). The prolongation of life: Optimistic studies, Springer Classics in Longevity and Aging, New York, Springer. P. 89
- [6] Gueimonde, M. K., Laitinea, S., Salminen O. and Isolauri, E. (2007). Breast milk: a source of bifidobacteria for infant gut development and maturation. *Neonatology*, 92: 64-66
- [7] Jara, S., Sanchez, M., Vera, R., Cofre J. and Castro, E. (2011). The inhibitory activity of *Lactobacillus* spp. isolated from breast milk on gastrointestinal pathogenic bacteria of nosocomial origin. *Anaerobe*, 17: 474-477
- [8] Ljungh, A. and Wadstrom, T. (2009). *Lactobacillus* Molecular Biology: From Genomics to Probiotics. Caister Academic Press. Pp 87
- [9] Langer, P. (2009). Differences in the composition of Microbiology, colostrum and milk in Eutherians reflect differences in immunoglobulin transfer. *Journal of Mammalogy*, 90: 332-341.
- [10] Magdalena, A. Catherine, S., Lorenzo, M., Gregor, R., and Maya, P. (2006), Probiotics in food: health and nutritional properties and guidelines for evaluation, Combined Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria, Cordoba, Arentina. Pp 78-90.
- [11] Ouwehand, A., Salminen, S., Isolauri, E., Salminen, I. (2002). Probiotics: an overview of beneficial effects (PDF). *Antonie Van Leeuwenhoek* 82 (1-4): 279-289
- [12] Reid, G., Gaudier, E., Guarner, F., Huffnagle, G., MacKlaim, J., Munoz, A., Martini, M., Ringel-Kulka, T., Sartor, B., Unal, R., Verbeke, K., Walter, J.; Gaudier, G; Huffnagle; MacKlaim; Munoz; Martini; Ringel-Kulka; Sartor; Unal; Verbeke; Walter; International Scientific Association for Probiotics Prebiotics (2010). Responders and non-responders to probiotic interventions: how can we improve the odds. *Gut Microbes* 1 (3): 200-204
- [13] Reid, G., Jass, J., Sebulsky, M., McCormick, J., Jass, S., and McCormick, J. (2003). Potential uses of probiotics in clinical practice. *Clin. Microbiol. Rev.* 16 (4): 658-672.

- [14] Rijkers, G., de Vos, W., Brummer, R., Morelli, L., Corthier, G., Marteau, P. and Corthier, M. (2011). Health benefits and health claims of probiotics: Bridging science and marketing. *British Journal of Nutrition* 106 (9): 1291-1296
- [15] Zacarias, M. F., Binetti, A., Laco, M., Reinheimer, J. and Vinderola, G. (2011). Preliminary technological and potential probiotic characterization of bifidobacteria isolated from breast milk for use in dairy products. *International Dairy Journal*, 21: 548-555
- [16] Martin, R., Olivares, M., Marin, M. L., Fernandez, L., Havenaar, R., Ten Brink, B. and Huis, J. H. J. (2005). Selection of Strains for Probitoic Use. In: of 3 Lactobacilli strains isolated from breast milk. *Journal of Human Lactation*, 21: 8-17