Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/13627
DOI URL: http://dx.doi.org/10.21474/IJAR01/13627

RESEARCH ARTICLE

Active Probiotic Analysis of Fermented Cereal and Legumes Commonly Consumed in Nigeria

Moses O. Omale
Department of Biology, Kogi State College of Education, Ankpa.

Manuscript Info

Manuscript History
Received: 31 August 2021
Final Accepted: 30 September 2021
Published: October 2021

Key words: -
Probiotics, Zea mays, Pentadethra Macrophylla, Benth, Ricinus Communis

Abstract

Active probiotic organisms are good bacteria considered to be live micro-organisms that are obtained from fermented foods. Evidence has shown that probiotics are essential in human health. This study's primary objective is to isolate and characterize the active probiotic organisms present in certain fermented food samples. Maize, African oil bean, and castor oil were subjected to analysis. The result found the presence of active probiotic organisms such as Pedioccocus, Lactobacillus, Micrococcus, and Bacillus species. The study concluded that these organisms are responsible for the fermentation of carbohydrates and protein-rich seeds.

Introduction:-

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) reports on the scientific evidence indicating the potential for probiotic foods to provide health benefits, and specific strains that are safe for human consumption (FAO/WHO, 2002) probiotics are live micro-organisms which when administer in adequate amount, confer a health benefit on the host (Schlundt, 2001). Probiotics refer to essential micro-organisms that are beneficial to human health (Rijkerset al., 2010). Probiotics denote ingested micro-organisms that are associated with health benefits (Araya et al., 2006).

A significant expansion of probiotics' potential market has led to a higher requirement for scientific substantiation of the putative benefit conferred by the micro-organisms (Rijkerset al., 2000). Active probiotic organisms are good bacteria considered live micro-organisms obtained from fermented foods and promote a healthy digestive tract and a healthy immune system. Research in the probiotic area has progressed considerably over the years (Anukam& Reid, 2007; Barba-Vidal et al., 2019; Bron et al., 2013; Coman et al., 2019; de Melo Pereira et al., 2018; Dowarah et al., 2018; Gueimonde& Salminen, 2006; Holvoet et al., 2013; Shinde, 2012; Shokryazdan et al., 2017). Additionally, significant advances have been made in the characterization of specific probiotics and validation of health benefits associated with the consumption of probiotics (Akter et al., 2020; Amin et al., 2020; Beena Divya et al., 2012; Bharti et al., 2020; Fernandez & Marette, 2017; Hewadmal&Jangra, 2019; Holvoet et al., 2013; Kleerebezem et al., 2019; Park et al., 2014; Shah, 2000).

Cereals are the major carbohydrate food resources for humans and animals. The grains serve as source for energy and nutrients in the form of protein, fat, fiber, and minerals along with vitamins (Beverly, 2014).Cereal crops, including wheat, maize, rice, barley and sorghum provide the essential nutrients and energy primarily required in human diet through direct human consumption.

Corresponding Author:- Moses O. Omale
Address**:-** Department of Biology, Kogi State College of Education, Ankpa.

However, the current study intends to determine active probiotic organisms in fermented maize, African oil bean, and castor oil. Nevertheless, the selected foods for this study have been widely discussed in the literature (Abdoulaye et al., 2018; Adekoya, Njobeh, et al., 2017; Adekoya, Obadina, et al., 2017; Adiaha, 2017; Akande et al., 2012; Olasupo et al., 2016)

Fermentation is the process of microbial breakdown of carbohydrates and other substances to release alcohol, carbon dioxide, and energy (Hiu et al., 2004). Fermentation process plays essential roles in food technology industries. In the traditional fermentation process, natural micro-organisms are employed to prepare and preserve different types of food. The processes add to the nutritive value of foods and enhance flavor and other desirable qualities associated with digestibility and edibility. Fermentationprovides bacteriological safety of food and ensures enormous quantities of food in a wide variety of flavors, aromas, and textures, which enrich the human diet (Kwon et al., 2014).Fermented foods are those foods that have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food (Singh et al., 2012).

Cereal-fermented foods involve a lactic acid fermentation step in most cases, which can be associated with an alcoholic fermentation step depending on the process used (e.g., brewing). It is now commonly known that lactic acid fermentation contributes to human welfare through the preservation of foods, the development of organoleptic characteristics and nutritional improvement of foods, and the health-promoting effects of lactic acid bacteria (probiotics) (Nout and Motarjemi, 1997; Charalampopoulos et al., 2002; Kohajdova and Karovicova, 2007). Lactic acid cereal-fermented foods and beverages are made from a great variety of cereals involving different processing methods.

The commonly fermented food in Nigeria includes cereals (Maize), legumes (oil bean, castor oil seed), beverages (palm wine), and tubers (cassava products). This study is aimed to isolate and characterize the active probiotic organisms present in fermented foods widely consumed in Nigeria, such as Maize (Zea mays), African oil bean (Pentadethra macrophylla benth), and oil bean (Ricinus communis).

Materials and Method:-

Glassware and other materials were sterilized correctly and dried. The traditional fermented products of maize, oil bean, and castor oil seed were collected from different sources and were subjected to laboratory analysis. According to the standard procedures outline in Da Silva et al., (2013), all media were prepared.Furthermore, each of the fermented food samples' grams was mashed with laboratory pestle and mortar and mixed with distilled water as a diluent in a sterile sample bottle. However, characterization and identification of isolates, Gram staining of the isolates, biochemical test for identification of bacteria, catalase test, indole test, citrate test, motility test, methyl red test, plaiting, and sugar fermentation were appropriately employed.

Results:-

Table 1:- Viable colony count of bacteria isolated from the fermented food samples.
Total colony count on media 10^{-3}

Plates	Samples	Nutrient Agar	De Man Rogosa Sharp (MRS)
1	Zea mays	$1.6x10^5$	$2.2x10^6$
2	Zea mays	$4.0x10^5$	$1.12x10^6$
3	Pentadethra macrophylla benth	$1.6x10^5$	$1.04x10^6$
4	Pentadethra macrophylla benth	$1.8x10^6$	$2.2x10^6$
5	Ricinus communis	$1.4x10^6$	$2.68x10^6$
6	Ricinus communis	$1.08x10^6$	$2.40x10^6$

Plate	Sample name	Media	Morphology
1	Z. mays 1	Nutrient	Cream, smooth circular, small, flat, entire, transparent
	Z. mays 2	Nutrient Mrs	punctiform
	Z. mays 1		Cream, smooth, circular, moderate, flat, unulate, transparent filamentous small.
		Mrs	Cream, smooth spindle, big raised curled, transparent white circular moderate amber irregular small irregular punctiform
	Z. mays 2	Nutrient	Cream, smooth, circular, big, flat, entire transparent white spindle, moderate amber small punctiform
	P. macrophylla benth 1	Nutrient	Cream, smooth spindle, punctiform, flat, entire transparent circular.
	P. macrophylla benth 2	Mrs	

	P. macrophylla benth 1	M r s	C r e a m , s m o o t h , s m a l l , f a t , e n t i r e , t r a n s p a r e n t .
	P. macrophylla benth2	N u t r i e n t	Cream, rough, circular, big, flat, unbonate, transparent, spindle, moderate small punctiform. Cream, smooth, circular, moderate, flat, entire, small punctiform.
	R. communis 1	N u t r i e n t	Cream, rough circular, moderate, raised, lobate, transparent irregular small punctiform.
	R. communis 2	M r s	Cream, smooth, circular, small, flat entire transparent irregular small.
	R. communis 1	M r s	Cream, smooth, circular, small, flat, entire transparent spindle punctiform.
	R. communis2		C r e a m , s m o o t h , c i r c u l a r , s m a l l , R a i s e d , e n t i r e , t r a n s p a r e n t .

Table 2:- Morphology Characteristics of Isolates.

Table 3:- Biochemical Test on the Isolate.

Sample name	Glu	Lau	Suc	Fru	Mann	Indole	Catalase	Citrate	Motility	Oxidase	Methyl red	Gram reaction	Presumptive isolated organisms
Z . m , R . c	A G	A	A G	A G	A G	-	-	+	-	-	-	+ve, rods	Lactobacillus sp
R. communis	A G	A	A G	A G	A G	-	+	-	-	-	-	+ve, cocci	Micrococcus sp
P. macrophylla bent, R.c	A	A	A	A	A	-	+	+	+	-	-	+ve, long rod	B a c i l l u s s p
Z e a m a y s	A	A G	A G	A G	A G	-	-	-	-	-	-	+ve, cocci short chains	P e d i o c o c c u s s p
Z e a m a y s	A G	A	A G	A G	A G	-	-	+	-	-	-	+ v e , r o d	Lactobacillus sp
P. macrophylla benth	A G	A	A G	A G	A G	-	+	-	+	+	-	+ve, cocci single	Micrococcus sp
Z e a m a y s	A	A G	A G	A G	A G	-	-	+	-	-	-	+ve, rod in single and cluster	Lactobacillus sp

Note: + = positive, - = Negative, AG = Acid gas, A = Acid, G = Gas, Glu = Glucose, Lac = Lactose, Fru = Fructose, Suc = Sucrose, Mann = Mannitol, Z. m = Z. mays, R. c = Ricinus communis.

Discussion:-

This research shows the micro-organisms isolated from the fermented foods as the bacteria grow on the nutrient agar and de Man Rogosa Sharpe (MRS)agar. The viable colony count of bacteria isolated from the fermented foods, maize, oil bean, and castor oil are shown in table 1. This agrees with the standard plate count of colony range of 30-300cfu on a petri dish. The organisms isolated from maize ranges from $1.6x10^5$ to $2.28x10^6$, oil bean, from $1.6x10^5$ to $2.2x10^{6,}$ while castor oil ranges from $1.4x10^6$ to $2.68x10^6$. The microbiological characteristics of the bacteria colonies were identified through visual counting from the plates containing the aliquot dilute samples of each fermented food, as shown in table 2.

Table 3 shows the probiotic organisms that were isolated and characterized from maize, oil bean, and castor oil through the biochemical test. The active probiotic organisms isolated were Pedioccocus, Lactobacillus, Micrococcus, and Bacillus species, and these organisms are responsible for fermentation and are capable of utilizing constituents of the fermented foods.

This finding is consistent with Aworh (2008), who reported that fermentation improves the texture and flavor of foods imparting a pleasant sour taste. It also improves the value of food materials giving it higher quality, better preservation, and detoxification.

The Lactobacillus produces acid, which further inhibits the growth of non-desirable organisms. ThePedioccocus are home fermenters that produce lactic acid. This agrees with the observation of Oguekeet al. (2005) that organisms isolated from fermented maize seed, soil bean seed, and castor oil seed have proteolytic, aminolytic and lipolytic ability breakdown protein, carbohydrates, and lipids.

The Bacillus, Lactobacillus, Pedioccocus, and Micrococcus isolated from the three fermented foods are gram-positive and produce lactic acid and acetic acid, making them be identified as probiotics.

Conclusion:-

Lactic acid bacteria are among the most critical groups of micro-organisms used in food fermentation. Probiotics are not pathogenic organisms in foods that can positively influence the host's health and modulate the gastrointestinal tract. The study results show that the following organisms were isolated from the respective fermented foods: Maize:

Lactobacillus and Pedioccocus species, Oil bean: Bacillus and Micrococcus species, castor oil: Lactobacillus and Bacillus species. Lactobacillus and Bacillus species are found to be common. It fermented the foods very well and gave it the desired texture, flavor, and taste. Therefore, it could be concluded that these organisms are responsible for the fermentation of carbohydrates and protein-rich seeds to give them desired fermented products (Maize, Oil bean, and Castor oil). However, certain micro-organisms were detected and isolated from the sample. Perhaps, the presence of these micro-organisms could be attributed to the poor hygienic condition. Therefore, the study recommends that proper hygienic conditions should be observed while preparing food fermentation. Also, foods that contain active probiotics are recommended to enhance immune system responses, prevent infection and reduce inflammation.

References:-

1. Abdoulaye, T., Wossen, T., &Awotide, B. (2018). Impacts of improved maize varieties in Nigeria: ex-post assessment of productivity and welfare outcomes. Food Security, 10(2). https://doi.org/10.1007/s12571-018-0772-9
2. Adekoya, I., Njobeh, P., Obadina, A., Chilaka, C., Okoth, S., de Boevre, M., & de Saeger, S. (2017). Awareness and prevalence of mycotoxin contamination in selected Nigerian fermented foods. Toxins, 9(11). https://doi.org/10.3390/toxins9110363
3. Adekoya, I., Obadina, A., Phoku, J., Nwinyi, O., &Njobeh, P. (2017). Contamination of fermented foods in Nigeria with fungi. LWT - Food Science and Technology, 86. https://doi.org/10.1016/j.lwt.2017.07.044
4. Adiaha, M. S. (2017). Economic value of Maize (Zea mays L.) in Nigeria and its impacts on the global food production. International Journal of Scientific World, 6(1). https://doi.org/10.14419/ijsw.v6i1.8771
5. Akande, T. O., Odunsi, A. A., Olabode, O. S., &Ojediran, T. K. (2012). Physical and Nutrient Characterization of Raw and Processed Castor (Ricinus communis L.) Seeds in Nigeria. World Journal of Agricultural Sciences, 8(1).
6. Akter, S., Park, J. H., & Jung, H. K. (2020). Potential Health-promoting benefits of Para probiotics, inactivated probiotic cells. In Journal of Microbiology and Biotechnology (Vol. 30, Issue 4). https://doi.org/10.4014/JMB.1911.11019
7. Amin, T., Thakur, M., & Jain, S. (2020). Microencapsulation-the future of probiotic cultures.Journal of Microbiology, Biotechnology and Food Sciences, 2020(vol. 10).
8. Anukam, K. C., & Reid, G. (2007). Probiotics: 100 years (1907-2007) after Elie Metchnikoff ’s observation. Communicating Current Research and Educational Topics and Trends in Applied Microbiology.
9. Araya, M., Stanton, C., Morelli, L., Reid, G., and Pineiro, M., (2006) "Probiotics in Food: Health and
10. Nutritional Properties and Guidelines for Evaluation" Combine Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Bacteria, Cordoba Publisher, Journal of Dairy Science, pp. 9-11.
11. Barba-Vidal, E., Martín-Orúe, S. M., & Castillejos, L. (2019). Practical aspects of the use of probiotics in pig production: A review. In Livestock Science (Vol. 223). https://doi.org/10.1016/j.livsci.2019.02.017
12. Beena Divya, J., Kulangara Varsha, K., Madhavan Nampoothiri, K., Ismail, B., & Pandey, A. (2012). Probiotic fermented foods for health benefits. In Engineering in Life Sciences (Vol. 12, Issue 4). https://doi.org/10.1002/elsc.201100179
13. Bharti, N., Kaur, R., & Kaur, S. (2020). Health benefits of probiotic bacteria as nutraceuticals. European Journal of Molecular and Clinical Medicine, 7(7).
14. Bron, P. A., Tomita, S., Mercenier, A., &Kleerebezem, M. (2013). Cell surface-associated compounds of probiotic lactobacilli sustain the strain-specificity dogma. In Current Opinion in Microbiology (Vol. 16, Issue 3). https://doi.org/10.1016/j.mib.2013.06.001
15. Coman, M. M., Verdenelli, M. C., Cecchini, C., Belà, B., Gramenzi, A., Orpianesi, C., Cresci, A., & Silvi, S. (2019). Probiotic characterization of Lactobacillus isolates from canine faeces. Journal of Applied Microbiology, 126(4). https://doi.org/10.1111/jam.14197
16. de Melo Pereira, G. V., de Oliveira Coelho, B., Magalhães Júnior, A. I., Thomaz-Soccol, V., & Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. In Biotechnology Advances (Vol. 36, Issue 8). https://doi.org/10.1016/j.biotechadv.2018.09.003
17. Dowarah, R., Verma, A. K., Agarwal, N., Singh, P., & Singh, B. R. (2018). Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. PLoS ONE, 13(3). https://doi.org/10.1371/journal.pone.0192978
18. Fernandez, M. A., & Marette, A. (2017). Potential health benefits of combining yogurt and fruits based on their probiotic and prebiotic properties. Advances in Nutrition, 8(1). https://doi.org/10.3945/an.115.011114

19. Food and Agriculture Organization of the United Nations; and World Health Organization, (2001).
20. Regulatory and Clinical aspects of dairy Probiotics Food and Agriculture Organization of UN and WHO
21. Expert Consultation Report.
22. Gueimonde, M., & Salminen, S. (2006). New methods for selecting and evaluating probiotics. Digestive and Liver Disease, 38(SUPPL. 2). https://doi.org/10.1016/S1590-8658(07)60003-6
23. Hewadmal, N., &Jangra, S. (2019). A Review on Probiotic and Health Benefits of Probiotics. International Journal of Current Microbiology and Applied Sciences, 8(05). https://doi.org/10.20546/ijcmas.2019.805.218
24. Holvoet, S., Zuercher, A. W., Julien-Javaux, F., Perrot, M., &Mercenier, A. (2013). Characterization of candidate anti-allergic probiotic strains in a model of Th2-skewed human peripheral blood mononuclear cells. International Archives of Allergy and Immunology, 161(2). https://doi.org/10.1159/000343703
25. Hui, Y.H, Meunier-Goddik, L., Josephsen, J, Nip, W.K., and Stand field P.S. (2004). Handbook of Food and Beverage Fermentation Technology, CRC Press. Pp 27
26. Kleerebezem, M., Binda, S., Bron, P. A., Gross, G., Hill, C., van HylckamaVlieg, J. E., Lebeer, S., Satokari, R., & Ouwehand, A. C. (2019). Understanding mode of action can drive the translational pipeline towards more reliable health benefits for probiotics. In Current Opinion in Biotechnology (Vol. 56). https://doi.org/10.1016/j.copbio.2018.09.007
27. Ogueke, C.C. and Aririatu, L.E. (2004). Microbial and Organoleptic Changed Associated with
28. Ugba stored and Ambient Temperature. Nigerian Food Journal of Springer Publisher; 22 pp. 133-140.
29. Olasupo, N. A., Okorie, C. P., &Oguntoyinbo, F. A. (2016). The biotechnology of Ugba, a Nigerian traditional fermented food condiment. In Frontiers in Microbiology (Vol. 7, Issue AUG). https://doi.org/10.3389/fmicb.2016.01153
30. Park, K. Y., Jeong, J. K., Lee, Y. E., & Daily, J. W. (2014). Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. In Journal of Medicinal Food (Vol. 17, Issue 1). https://doi.org/10.1089/jmf.2013.3083
31. Shah, N. P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. Journal of Dairy Science, 83(4). https://doi.org/10.3168/jds.S0022-0302(00)74953-8
32. Shinde, P. B. (2012). Probiotic: An overview for selection and evaluation. In International Journal of Pharmacy and Pharmaceutical Sciences (Vol. 4, Issue 2).
33. Shokryazdan, P., Faseleh Jahromi, M., Liang, J. B., & Ho, Y. W. (2017). Probiotics: From Isolation to Application. In Journal of the American College of Nutrition (Vol. 36, Issue 8). https://doi.org/10.1080/07315724.2017.1337529
34. Singh, V.P, Pathak, V., Akhilesh, K. and Verma (2012). Fermented Meat Products; Organoleptic
35. Qualities and Biogenic Amines – A Review, American Journal of Food Technology, 7: 278-288.