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RESEARCH ARTICLE

Isolation and Characterization of Active Probiotic Organisms in Fermented *Zea Mays, Pentadethra Macrophylla Benth,* and *Ricinus Communis*

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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 (Rijkers et 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 (Rijkers et 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). 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

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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. 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). 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	$1.08x10^6$	$2.40x10^6$

Table 2: - Morphology Characteristics of Isolates.

Plate	Sample name	Media	Morphology
1	Z. mays1	Nutrient	Cream, smooth circular, small, flat, entire, transparent
	Z. mays2	Nutrient Mrs	punctiform
	Z. mays1		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. mays2	Nutrient	Cream, smooth, circular, big, flat, entire transparent white spindle, moderate amber small punctiform
	P. macrophylla benth1	Nutrient	Cream, smooth spindle, punctiform, flat, entire transparent circular.
	P. macrophylla benth2	Mrs	
	P. macrophylla benth1	Mrs	Cream, smooth, small, fat, entire, transparent.
	P. macrophylla benth2	Nutrient	Cream, rough, circular, big, flat, umbonate, transparent, spindle, moderate small punctiform. Cream, smooth, circular, moderate, flat, entire, small punctiform.
	R. communis1	Nutrient	Cream, rough circular, moderate, raised, lobate, transparent irregular small punctiform.
	R. communis2	Mrs	Cream, smooth, circular, small, flat entire transparent irregular small.
	R. communis1	Mrs	Cream, smooth, circular, small, flat, entire transparent spindle punctiform.
	R. communis2		Cream, smooth, circular, small, Raised, entire, transparent.

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	AG	A	AG	AG	AG	-	-	+	-	-	-	+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	Bacillus sp
Zea mays	A	A G	A G	A G	A G	-	-	-	-	-	-	+ve, cocci short chains	Pediococcussp
Zea mays	A G	A	A G	A G	A G	-	-	+	-	-	-	+ve, rod	Lactobacillus sp
P. macrophylla benth	A G	A	A G	A G	A G	-	+	-	+	+	-	+ve, cocci single	Micrococcus sp
Zea mays	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. 68x106.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. The Pediococcus are home fermenters that produce lactic acid. This agrees with the observation of (Ogueke et 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.

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