RESEARCH PAPER | Microbiology | Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

Attempts to Study Naturally Prevailing Lactic Acid Bacteria in Fermentation of Steeped Sorghum Grains

KEYWORDS: Varhad-khandesh region, sorghum, Pediococcus acidilactici, Lactic acid bacteria

Pruthviraj Jagannath Khade
P. G. Department of Microbiology, Sant Gadge Baba Amravati University, Amravati-444602, India

*** Niteen Vinay Phirke**
P. G. Department of Microbiology, Sant Gadge Baba Amravati University, Amravati-444602, India
* Corresponding author

ABSTRACT *In Varhad-Khandesh region, several seasonal food items are made by steeping and fermenting sorghum grains especially during summer. To check the prevalence of different microflora from the procedure of steeping and fermentation, attempts were made to isolate fermenting microorganisms. The sorghum grains were soaked in water in 1:2 proportions and allowed to ferment. From this, the bacteria especially lactic acid bacteria were tried for its isolation morphological and biochemical characterization and compared with a reference standard strain of lactic acid bacteria. The total 9 lactic acid bacterial species from fermentation batter were isolated. Out of nine, four resembled with Pediococcus acidilactici strains, two resembled like Streptococcus species, two resembled like Lactobacillus species and one resembled like Leuconostoc mesenteroides. Although, the confirmation needs to be concluded on the basis of 16s RNA base sequencing, at this preliminary study, it can be atleast seen clearly that Pediococcus acidilactici prevails dominantly during fermentation of steeped sorghum grains.*

INTRODUCTION

Sorghum is a tropical plant belonging to the family of Poaceae is one of the important crops of Africa, Asia and Latin America (Anglani, 1998). Sorghum is one of the major crops in India because of its adaptation to hard environment and drought tolerance (Doggett, 1988). The Varhad-Khandesh region is situated at the central regions of India, its environment which belong to the assured rainfall, dry weather and typically rainfed to minutely irrigated areas, majorly dependent on agriculture, economically producing various cereals including sorghum, wheat and rice. Among these, the sorghum is cultivated as a cereal for human consumption and fodder for livestock. Most of the part of Sorghum is grown directly for human consumption. The remaining is used primarily for animal feed, alcohol production and industrial products (FAO, 1995).

In Varhad-Khandesh region, several seasonal food items are made by steeping and fermenting sorghum grains especially during summer. Some of the important fermented foods of India are Aambil (Sorghum product), Bhatura (White wheat flour product), Dhokla (Bengal gram product), Dosa (Rice and black gram product), Idli (Rice and black gram), Papdum (Sorghum, black gramand rice), Vadai (black gram product) etc (Sanjeev and Sandhu, 1990).

The group that includes the lactic acid bacteria is known as one of the most diverse group of bacteria. Lactic acid bacteria are generally known as safe for use in food and food products. Food fermentation is regarded as one of the oldest ways of food processing and preservation more than anything else, man has known the use of microbes for preparation of food products for thousands of years and all over the world a wide range of fermented foods and beverages contributed significantly to the diets of many people (Achi, 1992). The purpose of this study to check the prevalence of different microflora from the procedure of steeping and fermentation of sorghum grains.

MATERIALS AND METHODS

Materials

MRS agar (deMan, Rogosa and Sharpe), MRS broth and Hi-media rapid detection kit KB009. Composition of MRS media (Kunene,et al,2000) is as follows.

Table 1: Composition of MRS media	
Ingredients	g/L
Protease	10.00
Beef extract	10.00
Yeast extract	5.00
Dextrose	20.00
Polysorbate 80	1.00
Ammonium citrate	2.00
Sodium citrate	5.00
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium phosphate	2.00
pH (at 25 °C)	6.5 $\pm$ 0.2

Laboratory preparation and set up of sorghum fermentation

The Sorghum was first cleaned by winnowing to remove chaffs and other light contaminants. It is then poured in a bowl of water so that the bad seed can float and be skimmed off. Then it washed by sterile distilled water 2 to 3 times. Then this sorghum was mixed with sterile distilled water in ratio of 1:2 (dry w/v). Then this mixture was incubated at 30^0C temperature for seven days in sterile covered flask. During fermentation samples were aseptically withdrawn for its physicochemical and microbiological analysis. Then isolation of bacteria or microbes on preferable cultivated media from fermented batter followed by characterization of these bacteria. Isolated bacteria/microbes were preserved.

Isolation and biochemical characterization

The isolation was made by inoculating the culture from fermentation set on solid MRS agar plate. The well isolated and morphologically distinct colonies from the plate were

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

selected and stock cultures were prepared for further analysis. All these isolates were further characterized by standard biochemical test according to Bergey's manual of systematic bacteriology

RESULTS AND DISCUSSION

Nine bacterial spp. were isolated from the fermentation sets on solid MRS agar plate. Out of nine, four resembled with Pediococcus acidilactici strains (K1), two resembled like Streptococcus species, (K2) two resembled like Lactobacillus species (K3) and one resembled like Leuconostoc mesenteroides (K4). The isolates K1, K2, K3 and K4 were Gram positive cocci in tetrads or pairs, Gram positive cocci in chain, Gram positive short rod and Gram positive cocci in chains or pairs respectively. The isolates species were different in their cultural and biochemical characteristics (Table 2). The biochemical characteristics of all isolate were also done by the commercially available Hi-media rapid detection kit KB009. Catalase and oxidase test for all bacterial isolates were negative. Oyewole (1995) showed that naturally fermented cereal- based African foods are dominated by L. plantarum, Lactobacillus fermentum, Lactobacillus reuteri, L. mesenteroides, P. pentosaceus, and L. lactis strains. Kunene,et al,(2000) were recovered, members of several genera of lactic acid bacteria from sorghum powder and corresponding fermented and cooked fermented porridge. The dominant groups found in the fermented porridge samples were L. plantarum and L. mesenteroides.

Table 2: Morphological, Cultural and Biochemical Characterization

No. of Isolates	Test	K3(4)	K2(2)	K1(2)	K4(1)
Cultural Characteristics	Colour of colony	White	Pale yellow	White	Pale yellow
	Colony shape	Circular	Circular	Circular	Circular
	Margin	Entire	Entire	Irregular	Entire
	Elevation	Convex	Convex	Convex	Convex
	Density	Opaque	Opaque	Opaque	Opaque
Morphological characteristics	Cell shape	Cocci in tetrads	Cocci in chain	Short Rod	Cocci in chain or pairs
	Gram reaction	+	+	+	+
	Motility	NM	NM	NM	NM
	Endospore formation	NS	NS	NS	NS
Biochemical characteristics	Catalase	-	-	-	-
	Oxidase	-	-	-	-
Growth at	15^0C	+	-	-	+
	45^0C	+	+	+	+
	pH 3.9	+	-	+	+
Growth in Nacl	2%	+	+	+	+
	5%	-	+	-	+
	10%	-	-	-	-
Sugar fermentation	Lactose	+	+	+	+
	Xylose	+	+	-	+
	Maltose	+	+	+	-
	Fructose	+	+	+	+
	Dextrose	+	+	-+	+
	Galactose	+	+	+	-
	Raffinose	+	+	+	+
	Trehalose	+	-	+	-
	Melibiose	+	+	+	+
	Sucrose	+	+	+	+
	L-Arabinose	+	+	+	-
	Mannose	+	+	+	-
	Insulin	+	+	+	-
	Sodium gluconate	+	+	+	+
	Glycerol	+	+	+	+
	Salicin	+	+	+	+
	Dulcitol	+	+	+	+
	Inositol	+	+	+	+
	Sorbitol	+	+	+	+
	Mannitol	+	+	+	+
	Adonitol	+	+	+	+
	Arabitol	-	+	+	+
	Erythritol	+	+	-	+
	Alph-Methyl-D-glucoside	+	+	+	+
	Rhamnose	+	+	+	+
	Cellobiose	+	-	+	+
	Melezitose	+	+	+	+
	Alph-Methyl-D-mannoside	+	+	+	+
	Xylitol	+	+	+	+
	ONPG	-	-	-	-
	Esculin hydrolysis	-	+-	-	+
	D-Arabinose	+	+	+	+
	Citrate utilization	-	-	-	+
	Malonate utilization	-	-	-	-
	Sorbose	+	+	+	+
	Probable Microorganism	*Lactobacillus* spp.	Streptococcus spp.	Pediococcus spp.	Leuconostoc mesenteroides

Where NM = Non Motile, NS = Non Sporulating, (-) = Negative, (+) = Positive.

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

Nout, (1991) isolated the lactic acid bacteria from fermentation of mixed sorghum-cowpea and sorghum-milk-powder; Olsen, et al., (1995) has been also isolated Lactobacillus and Leuconostoc as a dominant species from a fermentation of sorghum- based infant-weaning foods. The genus Pediococcus have often been found at low frequencies together with leuconostocs and lactobacilli on plant material and in various foods. They are also widely used as starter cultures in the fermentation of sausages and have been used to control food pathogens in vegetables (Vescovo et al., 1996). Pediococci have previously been isolated from fermented cereal gruels (Kingamkono et al., 1995). In present study the isolates Pediococcus acidilactici showed dominant prevalence in fermentation batter at laboratory scale and optimum conditions were provided for the study. The study also showed that sorghum fermentation set during zero day of fermentation also harbours diverse microflora especially enterobacteriaceae but as the time increased only lactic acid bacteria observed because of low pH. Due to its patentability Pediococcus acidilactici might be used for food industry as a starter culture and for research field.

CONCLUSION

In this study, members of several genera of lactic acid bacteria were recovered from steeping and fermenting sorghum grains. At this preliminary study, it can be atleast seen clearly that Pediococcus acidilactici prevails dominantly during fermentation of steeped sorghum grains and might be used as a starter culture to reduce the fermentation time for next batch.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial assistance received from Science and Engineering Research Board (SERB), Department of Science and Technology (DST), New Delhi through its Fast Track Scheme No. SR/FT/LS-71/2010 dated 4th May, 2012 for conducting the current research, creating the infrastructural facilities and offering a fellowship to first author.

REFERENCE

1. Achi OK (1992). Microorganisms associated with natural fermentation of Prosopis africana seeds for the production of okpiye. Plant Foods Hum Nutr. 42: 297-304. | 2. Anglani C (1998). Sorghum for human food: a review. Plant foods human nutria.52: 85-89. | 3. Doggett H (1988). Sorghum. Longman Scientific and Tech London. | 4. Food and Agricultural Orientations, (FAO) (1995). Sorghum and Millet in Human. | 5. Kingamkono RE, Sjo¨gren U, Svanberg and Kaijser B (1995). Inhibition of different strains of enteropathogens in lactic-fermenting cereal gruel. World J Microbiol Biotechnol. 11:299–303 | 6. Kunene, N.F., Geornaras, I., von Holy, A., and Hastings, J.W. (2000) Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. Applied and Environmental Microbiology 66, 1084–1092. | 7. Nout M J R (1991). Ecology of accelerated natural lactic fermentation of sorghum-based infant food formulas. Int J Food Microbiol. 12:217–224 | 8. Olsen AM, Halm and Jakobsen M (1995). The antimicrobial activity of lactic acid bacteria from fermented maize (kenkey) and their interactions during fermentation. J Appl Bacteriol. 79:506–512. | 9. Oyewole OB (1995). Lactic fermented foods in Africa and their benefits Fermentation: assessment and research. Report of a joint FAO/WHO workshop on fermentation as a household technology to improve food safety. WHO/FNU/FOS/96.1. (Food Safety Unit, Division of Food and Nutrition, World Health Organization, Geneva, Switzerland), p. 44. | 10. Salama MST, Musafija-Jeknic W, Sandine and Giovannoni SJ (1995). An ecological study of lactic acid bacteria: isolation of new strains of Lactococcus including Lactococcus lactis subspecies cremoris. J Dairy Sci. 78: 1004–1017. | 11. Sanjeev KS and Sandhu KD (1990). Indian fermented foods, Microbiological and Biochemical Aspects. Ind J Microbiol. 30: 135-157. | 12. Vescovo MS, Torriani C, Orsi F, Macchiarolo and Scolari G (1996). Application of antimicrobial producing lactic acid bacteria to control pathogens in ready-to-use vegetables. J Appl Bacteriol. 81:113–119. |