

Isolation and Characterization of Microbial Flora Associated With Traditional Wheat Fermentation in Submerged Condition

Pruthviraj J. Khade

P.G.T.D. (Microbiology), S.G.B. Amravati University
Amravati, India
pruthvikhade@gmail.com

Niteen V. Phirke

P.G.T.D. (Microbiology), S.G.B. Amravati University
Amravati, India
phirkenv@gmail.com

Abstract: In India, Maharashtra state is popular for the fermented wheat product especially *kurdai* and *popdum*. The aim of this study to optimize the biochemical activity of wheat fermentation to achieve improved the flavor, texture and shelf- life and to determine how the bacteria are responsible for fermentation of wheat and to check the prevalence of different microflora from the procedure of steeping and fermentation, attempts were made to isolate fermenting microorganisms. The wheat grains were soaked in water in 1:2 proportions and its slurry allowed fermenting. From this, the bacteria especially lactic acid bacteria were tried for its isolation morphological and biochemical characterization. The total 37 lactic acid bacterial species from fermentation batter were isolated. Out of 37 isolates, 28 were identified. Among these isolates 24.57% of isolates found to be *P. pentosaceus*, 24.57% *L. plantarum*, 21.42% *L. brevis*, 14.28% *L. lactis*, 7.14% *L. fermentum*. At this preliminary study, it can be atleast seen clearly that *P. pentosaceus* prevails dominantly during fermentation of steeped wheat grains.

Keywords: Wheat, *Kurdai*, *Popdum*, Lactic acid bacteria.

1. INTRODUCTION

India is one of the major wheat production and consumption countries in the world. After the Green Revolution in the 1970s and 1980s, wheat production has shown a huge increase. The principal states involved in the cultivation of wheat, those who are in the plains like Uttar Pradesh, Punjab and Haryana. They account for almost 70 percent of total wheat production in the country. Punjab and Haryana yield the greatest amount of wheat due to the presence of more irrigation facilities. Wheat production in other states such as West Bengal, Kerala, Assam, Odisha and Andhra Pradesh has grown from providing more irrigation facilities in the area. Wheat is a Rabi crop, which is grown in the winter season. Sowing of wheat takes place from October to December and harvesting is done during the months of February and May. The wheat crop should cool winters and hot summers, so the fertile plains region of the Indo-Gangetic are the most favorable for growing it. While well-drained loam and clay loam soil is considered ideal for wheat, good crops of wheat have also been raised in a sandy loam and black earth region of the peninsula [1]. In India, there is only the Rabi (winter) wheat, which is made of wheat crop in September, October, November, December and harvesting of wheat per month January, February, March and April. Wheat is the most important cereal crop grown in the world. Wheat is one of the major plant nutrients play a role in their healthy growth. This is used as the main products the rural fermented wheat product as *popdum*, *kurdai*, bread etc. Fermentation metabolic process serving for a microorganism to obtain energy through digestion simple fermentation sugars, primarily glucose and fructose etc. Fermenting sugar present in wheat like Fructose, glucose, galactose, sucrose, D-fructose, maltose. The fermented wheat thousands of bacteria, among those participating, we try to determine a few of the bacteria. Although species belonging to *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus* genera have been isolated from the fermentation of wheat. *Lactobacillus* strain is most frequently observed in this ecosystem. Bacteria occurs during propagation, which leads to the creation of typically one or two digits to the

genus *Lactobacillus*, that three or four orders of magnitude higher than those of adventitious microbial flora [2]. Fermentation process digestion of a certain substance that causes chemical conversion of organic matter into simpler compounds [3]. The aim of this study to optimize the biochemical activity of wheat fermentation to achieve improved the flavor, texture and shelf- life and to determine how the bacteria are responsible for fermentation of wheat and to check the prevalence of different microflora from the procedure of steeping and fermentation, attempts were made to isolate fermenting microorganisms.

2. METHODS

2.1. Materials

MRS agar (deMan, Rogosa and Sharpe), MRS broth and sugar (biochemical analysis). Composition of MRS media [4] is as follows (Table 1).

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 ± 0.2

2.2. Laboratory Preparation and Set up of Wheat Fermentation [5]

The wheat 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 wheat was mixed with sterile distilled water in ratio of 1:2 (dry w/v). Steeping of wheat grains for 18h. At 30°C temperature in a sterile covered flask. Decanting of that water was done. After decanting, the grains were transferred to sterile pestle and mortar and denting of these grains was done after denting of grains, sieving of the water was done and slurry was formed and allowed to stay of overnight. This fermented mixture was aseptically withdrawn at 18h intervals for its physico-chemical 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.

2.3. Isolation and Biochemical Characterization [6]

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 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

3. RESULTS AND DISCUSSION

In the present study, 30 g of wheat was subjected for fermentation in the 50 mL of sterile water. This type of fermentation was done for about 15 times so the numbers of sample were 15. Among these 15 samples 37 isolates are examined with the help standard morphological, cultural and biochemical tests. Among these bacteria 28 were identified using different cultural media and sample technique. The most important media for isolation of fermentative bacteria in wheat was MRS media. It was used for isolation purpose. Serial dilution was done to obtain the isolated colony of bacteria in the sterile distilled water. Identification of isolates was based on cultural, morphological and biochemical characteristic (Table 2).

The bacterial floras comprising mainly species of LAB (lactic acid bacteria) were involved in traditional fermentation. This result was shown by Nout, [7]. In present study, it was observed that,

Isolation and Characterization of Microbial Flora Associated With Traditional Wheat Fermentation in Submerged Condition

LAB were involved in traditional fermentation of wheat. *P. pentosaceus* was found to be at highest pick and then other species of LAB. The lactic acid bacteria such as *L. lactis*, *L. plantarum*, *L. brevis* and *L. fermentum* were isolated and identified similarly by Salovaara, [8] in cereal base product. By inducing a lactic acid fermentation of wheat grains from the epiphytic microbiota the lactic acid bacteria colonies have been selectively distinguish by cultivation on MRS with 1% agar in double layer. After preparing the microscopic slides to certify the existence of the gram positive lactic acid bacteria. These are non motile non endospore, catalase and oxidase negative. Five LAB were isolated from wheat fermentation like *L. brevis*, *L. plantarum*, *Pediococcus pentosaceus*, *L. Lactis* and *L. fermentum*. The lactic acid bacteria prevent the growth of others bacteria [9].

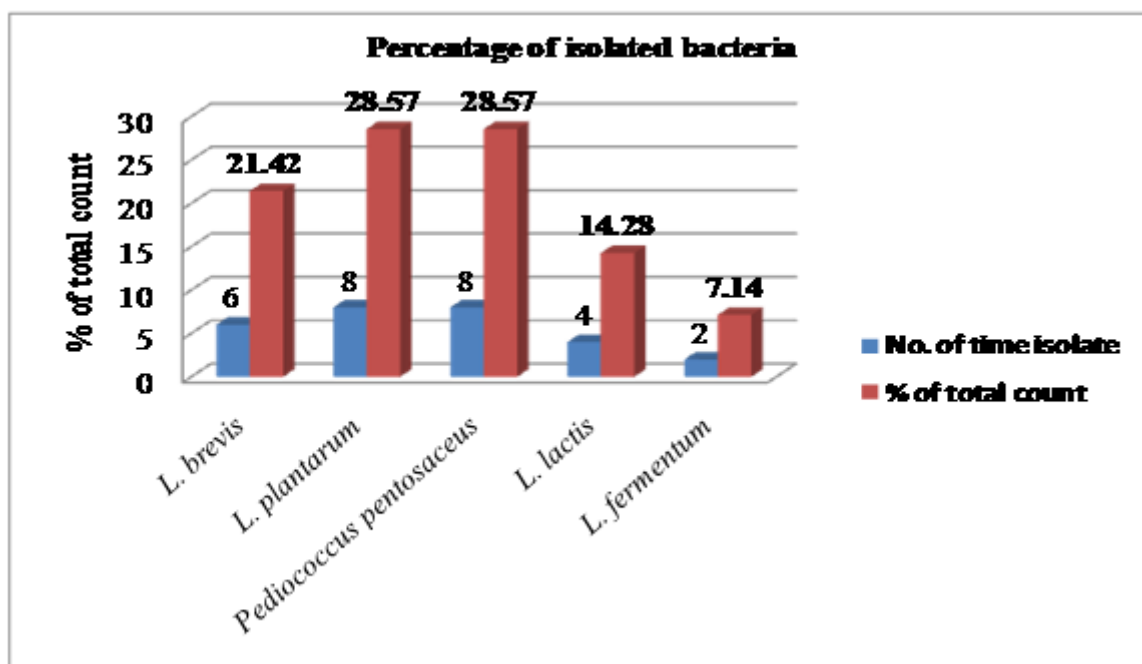
Table2. Morphological Cultural and Biochemical characteristics of isolated bacteria

Table2. Morphological Cultural and Biochemical characteristics of isolated bacteria																	
Morphological characteristic						Cultural characteristic					Biochemical characteristic						
Sr. No.	No. of time Isolates	Gm character	Shape	Motility	Endospore	Colour	Density	Margin	Elevation	Colony	Catalase	Oxidase	Maltose	Glucose	Mannitol	Raffinose	Organism
W 1	8	+	R o d	N. M.	N. S.	Pal e yel lo w	Opaq ue	Ent ire	Rai sed	Circ ular	-	-	-	-	+	-	<i>L. brevis</i>
W 2	9	+	R o d	Mo tile	N. S.	Pal e yel lo w	Opaq ue	Ent ire	Rai sed	Circ ular	-	-	+	-	+	+	<i>L. plan taru m</i>
W 3	9	+	C oc ci	N. M.	N. S.	Pal e yel lo w	Opaq ue	Ent ire	Co nve x	Circ ular	-	-	-	-	-	-	<i>Pedi ococ cus pent osac eus</i>
W 4	7	+	R o d	N. M.	N. S.	Pal e yel lo w	Trans lucent	Ent ire	Co nve x	Circ ular	-	-	+	-	+	+	<i>L. lacti s</i>
W 5	4	+	R o d	N. M.	N. S.	Pal e yel lo w	Trans lucent	Ser rat e	Rai sed	Irre gula r	-	-	+	-	+	+	<i>L. ferm entu m</i>

Abbreviation: NM-Non motile, NS-Non Sporing, Negative (-), Positive reaction (+)

Table3. Number of isolates from fermentation of wheat grain

Sr. No.	Name of Isolates	No. of time isolates	% of total count	No. of isolates	No. of sample
1	<i>L. brevis</i>	6	21.42%	28	15
2	<i>L. Plantarum</i>	8	28.57%		
3	<i>Pediococcus pentosaceus</i>	8	28.57%		
4	<i>L. lactis</i>	4	14.28%		
5	<i>L. fermentum</i>	2	7.14%		



L. plantarum and *Pediococcus pentosaceus* was found several time and having highest pick level. So the observation shows that *L. plantarum* and *Pediococcus pentosaceus* was present in more extend than other microorganism in wheat fermentation. After *L. fermentum*, were found in the wheat fermentation 5.88% and 11.76% were found to be *L. lactis* and *L. brevis* were found to be 17.64% (Table3) So with the help of microorganism breakdown of substances were done and fermentation of wheat was carried out.

4. CONCLUSION

Wheat fermentation was done because of bacteria. These were LAB. The most important and predominant species were *pediococcus pentosaceus*, *L. plantarum* and *L. brevis* which gives the typical sour flavor, texture, aroma and improve the nutritional quality of food. Lactic acid bacteria were considered as a beneficial microorganism for health and improve digestibility. These lactic acid bacteria were naturally produced in the fermentation of wheat to promote the health. These bacteria were considered to act against the pathogen microbes in food because of production of acid in the food and inhibit the growth of other harmful pathogens in food. LAB have an ability to ferment the soluble sugar in to various type of acid, so the medium become acidic. In this acidic medium very less pathogenic bacteria were survive and ultimately they were inhibited their growth. And due to acidic pH food get good texture, aroma and improve digestibility of food from the above study it was concluded that microbial fermentation helps to improve the quality of food and digestibility.

ACKNOWLEDGEMENTS

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.

REFERENCES

- [1] <http://www.archive.india.gov.in>
- [2] W. Hammes and M. Gannzle, Microbiology of fermented foods, ed. Wood, London: Blackie Academic and Professional 1998, Vol. 1, pp. 199-216.
- [3] Vandamme E. J. and Derycke D. G. Microbial inulinases: fermentation process, properties, and applications. Adv. Appl. Microbiol, 29(139176), 729-733 (1983).
- [4] Kunene N. F., Geornaras I., von Holy A. and Hastings J. W., 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(2000).

Isolation and Characterization of Microbial Flora Associated With Traditional Wheat Fermentation in Submerged Condition

- [5] Khade P. J. and Phirke N. V., Attempts to study naturally prevailing lactic acid bacteria in fermentation of steeped sorghum grains. *Indian journal of applied research*, 4(9): 253-255 (2014)
- [6] Khade P. J. and Phirke N. V., Comparison between Natural *Aambil* production without and with curd. *international journal of advances in pharmacy, biology and chemistry*, 3(4): 847-850 (2014)
- [7] Nout M. J. R. and Sarkar P. K., Lactic acid food fermentation in tropical climates. *Antonie van Leeuwenhoek* 76(1):395-401 (1999).
- [8] H. Salovaara. *Lactic acid bacteria in cereal-based products*. In: Salminen, S., von Wright, A. and Ouwehand, A. (eds.) *Lactic acid bacteria Microbiological and Functional Aspects*, 3rd edition, Marcel Dekker, New York, 2004, pp. 431-451.
- [9] Vandenberg, P. A., Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiology Reviews*, 12(1-3), 221-237 (1993).

AUTHORS' BIOGRAPHY



Mr. Pruthviraj Jagannath Khade is doing his DST- Major Research project work under the supervision of Dr. Niteen Vinay Phirke P. G. Department of Microbiology, S.G.B. Amravati University, Amravati-444602, India.



Mr. Niteen Vinay Phirke is working as an Associate Professor at P.G. Department of Microbiology, S.G.B. Amravati University, Amravati-444602, India.