



EFFECT OF PARAMETERS ON THE DIVERSITY OF LACTIC ACID BACTERIA IN THE SORGHUM FERMENTATION PROCESS

Microbiology

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ABSTRACT

In India specially in Vidarbha Khandesh region during summer season sorghum fermented food items are made. Is the summer season advantageous or not? In this study, we tried to find out the effect of different parameters like temperature time on the sorghum fermentation on the basis of lactic acid bacterial count on selected media. The sorghum grain was soaked at (1:2 w/v) ratio in water and placed at different temperature and time and allowed to ferment, due to increasing temperature and time, the pH also changed. The temperature and time for fermentation are applied at different ranges: 10, 20, 25, 32, 37, 40, 45 and 50 °C. Time: Zero hours, 24 h, 48 h, 72 h, 96 h, 120 h and 144 h. The temperature ranges 32 °C and 37 °C and time 120 hours (5 days) and 144 hours (6 days) and pH are 4 and 3.5 where optimum bacterial colony count is observed. This observation is helpful for traditional homemade Sorghum fermentation.

KEYWORDS

fermentation, sorghum grains, parameters, lactic acid bacteria.

INTRODUCTION

Sorghum is a crop that grows in drought condition. It is hard to digest, to make digestion easier sorghum fermented food items are made in India, so that it becomes easier to digest. The problem is that people made their Sorghum fermented food items on the basis of their local traditional knowledge that's why each region the procedure may vary. It may be good or not, is any effect of parameter like temperature and time for making traditional fermented food items? Different researchers have different opinions about the parameters. The pH influences the microorganisms at the time of Sorghum fermentation (Hadiyanti *et al.*, 2021). Temperature also affects fermentation rate as well as product formation and their ecological association of microorganisms (Hofvendahl *et al.*, 2000 & Taleghani *et al.*, 2016). Time is also a very important factor in Sorghum fermentation. In Sorghum fermentation time is lower it does not become a proper product and if there is too much product quality decreases and kept optimum product quality improves, growth of microorganism is maintained and their association with them helps in increasing the fermentation rate (Adebo *et al.*, 2018). In this study our aim is to find out the effect of different parameters temperature, time and pH on fermentation of Sorghum grains. From this we can probably show which temperature and time is better for the fermentation process.

MATERIALS AND METHODS

Materials

Cereals (Sorghum), MRS agar (deMan, Rogosa and Sharpe), Glass Utensil, Plastic Utensil, Steel Utensil, Brass Utensil, Clay Utensil, Aluminum Utensil, Sterile water, Cotton Cloth, Crystal violet, iodine, Safranin (0.25%), Alcohol (0.95%), Hydrogen peroxide (0.3%) and Oxidase reagent (1% tetramethylene paraphenylene diamine dihydrochloride).

MRS Media

MRS agar (deMan, Rogosa and Sharpe 1960), MRS broth. Composition of MRS media (Kunene *et al.*, 2000, Khade, and Phirke, 2014) is shown in table no.1. Developed by de Man, Rogosa and Sharpe in 1960, MRS has been used enormously in the enumeration and isolation of Lactobacilli.

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 250C)	6.5 to 6.7

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 is washed by sterile distilled water 2 to 3 times. Then this sorghum was mixed with sterile distilled water in a ratio of 1:2 (dry w/v). Then this mixture was incubated at 37°C temperature for seven days in sterile covered utensils. 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 (Khade *et al.*, 2023).

Isolation and Identification of Microbial Flora

Source of Isolates: A total 9 fermentation sets were subjected for study and analysis.

Enumeration and Isolation of Microbial Flora From Fermentation

De Man, Rogosa and Sharpe (MRS) medium are used anaerobically by using gas pack or microaerophilic condition. From the fermented medium flask, 0.1mL of sample was taken on the solid MRS medium with 1.5% (w/v) agar. It was spread with sterile glass rod until the suspension was adsorbed. Then, it was poured with 4-5mL liquefied agar 2% (w/v) the plates were incubated at 37°C for 48h. After incubation major the colony count. colonies were picked up from each plate and transfer to MRS broth and incubated at 37°C for 48h. The isolates were identified using standard morphological and cultural characteristics (Karthiekeyan and Santosh, 2009).

All the isolates were initially tested for Gram reaction, Catalase enzyme and production of acid from glucose (Hamed *et al.*, 1992).

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 determinative bacteriology (Holt *et al.*, 1994).

RESULTS & DISCUSSION

Study of steeped sorghum grains fermentation using different temperatures and time (pH during fermentation):

Fermentation of sorghum is highly appreciated as a condiment in Vidarbha. In this study, we find the effect of different parameters (temperature, time and pH) on sorghum grain fermentation. 50g of sorghum was used for fermentation in clean water. flask at different temperatures for about 144 h and among this sample examined. The fermentation process was checked by pH. Production of gas in the form of bubbles on the surface of the media and turbidity of the flask.

Total 9 isolates were isolated from sets of Sorghum.

Staphylococcus spp and *Leuconostoc spp.*

The isolates were separated according to morphological and biochemical test into lactic acid bacteria such as *Lactobacillus spp.*

Isolation and characterization from steeped sorghum grains fermentation using different parameters:

Table 2: Morphological, Culture and Biochemical Characterization

No. of Isolates	Test	S1	S2	S3	S4	
Morphological Characteristics	Cell shape	Short rods	Cocci in bunches	Rod shape	Short rods	
	Gram Reaction	+ve	-ve	+ve	+ve	
	Motility	NM	NM	NM	NM	
Cultural Characteristics	Colour of Colony	Pale yellow	White	Pale Yellow	White	
	Margin	Entire	Entire	Entire	Entire	
	Elevation	Convex	Convex	Convex	Convex	
	Colony Shape	Circular	Circular	Circular	Circular	
	Density	Opaque	Opaque	Opaque	Opaque	
Biochemical Characteristics	Catalase	-ve	-ve	-ve	-ve	
	Oxidase	-ve	-ve	-ve	-ve	
	Gelatin	+ve	+ve	+ve	+ve	
Sugar Fermentation	Dextrose	A	+	+	-	+
		G	+	-	+	+
	Lactose	A	+	+	+	+
		G	+	-	+	+
	Mannitol	A	+	+	-	+
		G	+	-	-	-
	Sucrose	A	+	+	+	+
		G	-	-	+	+
	Trehalose	A	+	+	+	+
		G	-	-	-	-
	Probable bacteria		<i>Lactobacillus spp.</i>	<i>Streptococci spp.</i>	<i>Leuconostoc spp.</i>	<i>Lactobacillus spp.</i>

Mohammed *et al.*, (1991) found some lactic acid bacterial species in sorghum fermentation, *Pediococcus pentosaceus*, *Lactobacillus confusus*, *Lactobacillus brevis*, *Lactobacillus sp.*, *Erwinia ananas*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*. In sorghum we also found some lactic acid bacterial species *Lactobacillus spp.*, *Pediococcus spp.* and *Streptococcus spp.* They got dominant species *P. pentosaceus* and we observed *Streptococcus species* as dominant.

Taleghani *et al.* (2016) concluded that the four *Lactobacillus* species and one *Lactococcus* species were Screened for Lactic acid production The optimal growth of the selected organism for variable size of inoculant was examined. The effect of temperature was also studied 32.37, 42 and 47. Result showed that the concentration of cell dry weight increased of temperature from 32 to 42°C.

Mahapatra *et al.*, (2017) investigated that fermentation has proven, to reduce during preparation of most traditional dishes by natural or forced lactic acid bacteria fermentation, pH drops to below 4.0.

The morphological and biochemical characteristic isolates were studied and shown in table no.2 The isolates gave purple / violet color with staining showing Gram positive nature Gram positive cell walls

contain a thick layer of peptidoglycan with numerous teichoic acid cross linking which resist the decolorization. Thus, remaining purple in colour. The isolates coming from MRS plates were cocci, rods with long rounded ends. They appeared mostly as a chain of 3-4 cells or single or in pairs or in bunches. The hanging drop method showed that the bacteria were non-motile which is one of the unique characteristics of *Lactobacillus*. This might be due to the absence of unique propeller-like flagella in *Lactobacillus* responsible for motility. Culture Characteristic result shown in Table :2 Sorghum Fermentation is carried out for the preparation of fermented batter in different temperature sets observed at different times. No bubbles were observed indicating that the isolated bacteria that are catalase negative. and could not mediate the decomposition of H₂O to produce. Hence it is catalase negative. The citrate utilization test, the isolates were not able to use citrate as the sole source of carbon and couldn't lead to the production of sodium bicarbonate as well as ammonia. Thus, the isolates were gram positive, non-motile, catalase negative. and exhibited a negative pattern of citrate utilization H₂S formation, indole production, oxidase test, urease activity, NH₃ production from arginine and VP reaction.

These are the common characteristics of *Lactobacillus* Species.

Table 3: Colony Count Observed on MRS Agar

Fermentation set at Temperature	10°C			20°C			25°C			32°C		
	Incubate time in hours											
Fermentation setup time in hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
0	0	0	0	0	0	0	0	0	2 x 10 ⁷	0	11 x 10 ⁷	0
24	0	0	0	0	1 x 10 ⁷	1 x 10 ⁷	21 x 10 ⁷	23 x 10 ⁷	33 x 10 ⁷	17 x 10 ⁷	17 x 10 ⁷	14 x 10 ⁷
48	0	1 x 10 ⁷	1 x 10 ⁷	0	4 x 10 ⁷	10 x 10 ⁷	20 x 10 ⁷	36 x 10 ⁷	38 x 10 ⁷	51 x 10 ⁷	86 x 10 ⁷	69 x 10 ⁷
72	0	0	2 x 10 ⁷	10 x 10 ⁷	25 x 10 ⁷	26 x 10 ⁷	29 x 10 ⁷	31 x 10 ⁷	42 x 10 ⁷	96 x 10 ⁷	123 x 10 ⁷	131 x 10 ⁷
96	0	1 x 10 ⁷	1 x 10 ⁷	16 x 10 ⁷	32 x 10 ⁷	24 x 10 ⁷	26 x 10 ⁷	26 x 10 ⁷	31 x 10 ⁷	40 x 10 ⁷	44 x 10 ⁷	22 x 10 ⁷
120	0	0	0	36 x 10 ⁷	44 x 10 ⁷	47 x 10 ⁷	47 x 10 ⁷	25 x 10 ⁷	25 x 10 ⁷	133 x 10 ⁷	140 x 10 ⁷	141 x 10 ⁷

144	0	0	0	30 × 10 ⁷	43 × 10 ⁷	48 × 10 ⁷	48 × 10 ⁷	22 × 10 ⁷	23 × 10 ⁷	165 × 10 ⁷	168 × 10 ⁷	107 × 10 ⁷
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The number of colonies observed on MRS agar media are showed in above data table 3. The colonies were observed after 24 hrs,48 hrs and 72 hrs on the plates incubated. The colonies on the plate incubated at 32°C. The maximum growth of colonies of bacteria were observed at

temperature 32°C at 120 hrs & 144 hrs.

These findings suggest that this temperature is favorable for the growth of bacteria during steeped sorghum fermentation.

Table 4: Colony Count Observed on MRS Agar

Four sets of incubation hours at four different fermentation temperatures at 37, 40, 45 and 50°C Fermentation setup time in hrs	Incubation period in hrs											
	37°			40°			45°			50°		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
0	0	0	3 × 10 ⁷	3 × 10 ⁷	7 × 10 ⁷	13 × 10 ⁷	0	0	1 × 10 ⁷	12 × 10 ⁷	24 × 10 ⁷	26 × 10 ⁷
24	19 × 10 ⁷	20 × 10 ⁷	21 × 10 ⁷	11 × 10 ⁷	16 × 10 ⁷	30 × 10 ⁷	0	2 × 10 ⁷	9 × 10 ⁷	16 × 10 ⁷	18 × 10 ⁷	28 × 10 ⁷
48	19 × 10 ⁷	21 × 10 ⁷	21 × 10 ⁷	12 × 10 ⁷	12 × 10 ⁷	23 × 10 ⁷	14 × 10 ⁷	36 × 10 ⁷	47 × 10 ⁷	12 × 10 ⁷	11 × 10 ⁷	11 × 10 ⁷
72	14 × 10 ⁷	18 × 10 ⁷	19 × 10 ⁷	14 × 10 ⁷	26 × 10 ⁷	42 × 10 ⁷	30 × 10 ⁷	49 × 10 ⁷	50 × 10 ⁷	5 × 10 ⁷	6 × 10 ⁷	6 × 10 ⁷
96	18 × 10 ⁷	22 × 10 ⁷	22 × 10 ⁷	14 × 10 ⁷	18 × 10 ⁷	23 × 10 ⁷	45 × 10 ⁷	49 × 10 ⁷	40 × 10 ⁷	3 × 10 ⁷	3 × 10 ⁷	3× 10 ⁷
120	72 × 10 ⁷	84 × 10 ⁷	89 × 10 ⁷	19 × 10 ⁷	24 × 10 ⁷	29 × 10 ⁷	42 × 10 ⁷	40 × 10 ⁷	47 × 10 ⁷	0	0	0
144	113 × 10 ⁷	106 × 10 ⁷	110 × 10 ⁷	18 × 10 ⁷	25 × 10 ⁷	38 × 10 ⁷	39 × 10 ⁷	44 × 10 ⁷	42 × 10 ⁷	0	0	0

The number of colonies observed on MRS agar media are shown in above data table 4. The colonies were observed after 24 h, 48h and 72 h on the plates incubated. The maximum growth of colonies of bacteria were observed at temperature 37°C at 144 hrs.

These findings suggest that the 144 hrs (7day) at 37°C Temperature is the favorable for the growth of bacteria in the fermented batter.

Table 5: The pH Values of Fermented Batter.

Fermentation Time	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
Fermentation Setup temp.	pH						
10°C	6	6	6	6	5.5	5.5	5.5
20°C	6	6	5.5	5.5	5.5	5.5	5
25°C	6	5.5	5.5	5.5	5	5	4.5
32°C	6	5.5	5.5	5	4.5	4	4
37°C	6	5.5	5	4.5	4	3.5	3.5
40°C	6	5	5	5	4.5	4	4
45°C	6	5	5	4.5	3.5	3.5	3.5
50°C	6	5	4.5	4.5	3.5	3.5	3.5

The pH value of fermented batter obtains as shown in table 5. In all temperature such as 10°C , 20°C , 25°C , 32°C , 37°C, 40°C, 45°C & 50°C respectively observed at zero hrs to 144 hrs the pH slightly decreases as per increasing the temperature.

CONCLUSIONS

In this study, variable growth after fermentation is observed using different parameters (time, temperature and pH). This suggests the potential use of the parameter as a potential source for improving the fermentation rate and nutritional quality of members of the cereal genera lactic acid bacteria studied from sorghum seed germination and fermentation.

LAB were considered beneficial microorganisms for health and improved digestibility. These LAB are naturally occurring products of sorghum fermentation. These bacteria are believed to act against pathogenic microbes in fermentation by producing acid and inhibiting the growth of other harmful pathogens in fermentation.

Acidic pH results in better texture, aroma and improved digestibility of fermented food. The above study concluded that using different parameters which parameters are optimal, it was found that where the temperature ranged from 32°C to 37°C and 120h (5 days) and 144h (6 days) time and pH 4 -3.5, where optimum bacterial colony count is observed. lactic acid bacteria were the dominating microorganism at the end fermentation. We can recommend that using the above statistical analysis is useful for traditional household sorghum fermentation.

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