Studies on Food Grade Dextran by *Weissella confusa* for Idli Batter Leavening

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Abstract - Dextran a polymer of glucose is produced using sucrose rich media. In the present study an efficient isolate was isolated from batter dumped soil. It was morphologically, culturally, biochemically and genetically identified as Weissella confusa. The dextran produced had a low molecular weight as indicated by HPLC analysis. A production medium containing cheaper sucrose sources like cane juice or molasses along with organic nitrogen source like yeast extract was used. The dextran produced was recovered from broth by alcohol precipitation and the compound was stable for more than six months. This sample dextran was used for leavening of idli batter and compared with standard dextran for leavening. Idli batter samples with both sample dextran and standard dextran were tested for dextransucrase activity, dextranase activity and volume increase with variable time. Results indicated that the sample dextran added to idli raw material powders (cereal and legume) increased volume up to 50% within four hours, with good dextransucrase activity and no dextranase activity. Standard dextran (Commercial dextran) added to the similar such idli raw material powders increased in volume up to 46% in six hours. Similar leavening in wet idli batter took twelve hours for 40% increase in volume and showed good dextranase activity. These studies indicate that low molecular weight dextran can be produced using cheaper production medium by the present isolate and then used for commercial production of idli in shorter time using dry cereal and legume powders.

Keywords: Dextran, Dextransucrase, Dextranase, Leavening, Idli, Sucrose, Weissella confusa.

I. INTRODUCTION

Dextran is structurally an exopolysaccharide [1], biochemically a branched glucan made up of glucose molecules joined into chains of varying length [2]. It is produced as low molecular weight and high low molecular weight dextrans (From 10 to 150 Kilo Daltons) [3]. It is produced by certain lactic acid bacteria like *Leuconostoc mesenteroides* [4], [5] *Lactobacillus brevis, Streptococcus mutants and Weissella sps* [6]. Dextran is of particular interest because of its use as blood-plasma volume expander [7]. It finds various other industrial applications in pharmaceutical food, and chemical industries as adjuvant, emulsifier, carrier and stabilizer [8]. Crossed linked Dextran known as Sephadex [9] is widely used for separation and purification of various products like proteins in research and industry. Food grade dextran is being used as thickener for jam and ice cream [10]. It prevents sugar crystallization, improves the retention of moisture and also maintains flavor and appearance of various food stuffs. As it has numerous industrial applications, it is being produced by commercially using the strain of *Weissella sps*. Dextran production depends on the composition of fermentation media [5]. Idli is one of the very widely used fermented foods of India, particularly in the South. It is prepared from rice and black gram [11]. Its (Idli) spongy texture, attractive appearance, appetizing taste and flavor, additional to its easy digestibility and good nutritive value contribute to its increasing popularity in India and also in some countries outside India [12]. In the present study commercial dextran and sample dextran were used to study leavening of wet idli batter and with dry powders of rice and black gram.

II. DESIGN OF THE STUDY

The design of the study was preparation of inoculum using the bacterial isolate identified as *Weissella confusa* by 16s rRNA gene sequencing analysis. A 5% inoculums containing 10^6 cells/ml was used for fermentative production of dextran using cortezi medium. Dextran produced by submerged fermentation was recovered by alcohol precipitation method. This sample dextran was used for idli batter (prepared using dry black gram and rice powders in 1:2ratio) leavening studies in comparison with standard dextran. Dextran production in wet idli batter with those in dry powders as batter was also compared.

III. PREVIOUS WORK

Studies on role microorganisms in fermentation batters was carried out in 1960s and different bacteria like *Lactobacillus delbrueckii, Lactobacillus lactis, Streptococcus lactis* and predominantly *Leuconostoc mesenteroides* were identified [13]. Work on role of *Leuconostoc mesenteroides* in leavening of Idli batter was done by of S.K. Mukherjee and

group. *Leuconostoc mesenteroides* was found to produce an exopolysaccharide called dextran which was essential for leavening of the batter, and also responsible for acid production mostly lactic acid in idli, dosa and such related products, together with *Streptococcus faecalis* [14]. Further studies revealed that a number of physico-chemical changes during fermentation of the idli batter including leavening (batter volume increase) and acid production are known to occur [15]. Authors Soni and Sadhu have reviewed numerous methods of idli prepration [16]. Balasubramanium, *et al*., focused on varied aspects such as effect of raw materials, fermentation or processing temperature, microorganisms involved in biochemical and nutritive changes [17]. Characteristics like texture, taste and flavor of idli depended largely on the acidity developed during fermentation of batter and also production of dextran that entrapped the CO_2 evolved [8]. Cost effective production of dextran commercially needs good production medium and so cheaper sucrose rich sources and also different nitrogen sources were studied for production of dextran [18], [19].

IV. PROPOSED METHODOLOGY

INOCULUM PREPERATION FOR DEXTRAN PRODUCTION*:* Bacterial culture was isolated from idli batter/black gram soaked water, using enrichment culture technique. The isolate was identified by microscopic, biochemical tests like resistance to vancomycin and confirmed by 16s rRNA gene sequencing analysis as *Weissella confusa*.

FERMENTATION: Broth studies for dextran production was done in 250ml Erlenmeyer flasks containing 50ml cortezi medium with sucrose as main carbon source. The inoculum size was 5% and it contained 10^6 cells/ml. The flasks were incubated at 30^0 C for 24 hours and later at 4^0C for next 24 hours. The broth sample was tested for dextran production by anthrone method [20] and fructose by resorcinol method [21]. Fructose in broth was tested only to prove that dextran is a polymer of glucose and fructose is left in broth when sucrose is taken in the medium.

RECOVERY: Dextran was recovered from broth by alcohol precipitation, dried under vacuum over $CaCl_2$ at 30^0C weighed [22]. Product was assayed and found to contain glucose polymer (Dextran) by using anthrone method. Dextran yield was determined in grams/100ml of fermented broth.

BATTER LEAVENING STUDIES: These were done in 100ml wet idli batter (prepared by 1:2 ratio of black gram to rice) and with dry powders of black gram and rice (1:2 ratio). Increase in volume, production of dextransucrase, dextranase and dextran yield were studied for a period of 12 hours of wet batter and 6 hours for dry powders. Studies were done with both standard dextran (commercial dextran) and sample dextran (10mg/100ml).

ENZYME ASSAYS: a) Dextransucrase activity was determined by measuring the reducing sugar released from sucrose [23]. Units of dextransucrase activity are represented in DSU/ml/hr. One unit of enzyme activity was defined as the enzyme quantity that converts 1.0 milligram of sucrose into fructose and dextran in 1 hour under standard conditions [24].

b) Dextranase activity was assayed by measuring the glucose released from dextran [25]. Units of dextranase activity are represented in DSU/ml/hr. One unit of enzyme activity was defined as the enzyme quantity that converts 1.0 milligram dextran into glucose.

V. RESULTS

Freshly prepared idli batter volume reaches its maximum in 12 hours. Dextransucrase and dextranase activity were also found from 1hr and 6hr respectively (Table-1). Batter leavening studies with rice and black gram powder using standard dextran revealed that the volume increased by 45% in 6 hours while for sample dextran it was also 45% increase in 3hrs. In both the cases there was no dextranase activity and dextransucrase activity was found from 3 hr (Table-2). Freshly prepared idli batter leavening studies revealed that the volume increased by 46% in 4hrs with standard dextran and 54% in 4hrs with sample dextran. Dextransucrase activity was observed from 1hr in both the samples but not dextranase activity (Table-3).

TABLE 1. LEAVENING OF FRESHLY PREPARED IDLI BATTER 100 ML (1:2 RATIO OF BLACK GRAM TO RICE).

Time (Hrs)	Dextransucrase activity (µm/ml/min)	Dextranase activity (µm/ml/min)	Volume of batter increased (in ml)	Dextran yield (mg/100 ml)
2	0.24	-	-	4
4	0.58	-	1.5	12
6	1.5	0.01	4.0	33
8	2.0	0.03	11.5	39
10	2.11	0.08	32	42
12	2.19	0.10	45	48

TABLE 2. LEAVENING OF RICE AND BLACK GRAM POWDER (2:1 RATIO) WITH STANDARD DEXTRAN AND SAMPLE DEXTRAN (10 mg/ml).

Time (Hrs)	Dextransucrase activity (µm/ml/min)		Volume of batter increased (in ml)		Dextran yield (in mg/100ml)	
	C.D	S.D	C.D	S.D	C.D	S.D
1	-	-	-	9	-	-
2	-	-	4	27	-	-
3	0.15	0.20	11	45	3.3	3.6
4	0.25	0.34	33	50	4	6
5	0.36	0.42	40	60	6	7.5
6	0.42	0.54	46	65	7.5	11

TABLE 3. LEAVENING OF FRESHLY PREPARED IDLI BATTER 100ML (2:1 RATIO OF RICE AND BLACK GRAM) WITH STANDARD DEXTRAN AND SAMPLE DEXTRAN (10 mg/ml).

Time (Hrs)	Dextransucrase activity (µm/ml/min)		Volume of batter increased (in ml)		Dextran yield (in mg/100ml)	
	C.D	S.D	C.D	S.D	C.D	S.D
1	0.012	0.014	1	3	3	3.1
11/2	0.15	0.16	5	9	3.3	3.4
2	0.24	0.26	12	18	4	4.2
21/2	0.29	0.31	19	24	4.9	4.9
3	0.33	0.34	27	32	6	6
31/2	0.42	0.54	39	45	7.5	9
4	0.54	0.60	46	54	9	11

VI. DISCUSSION

In Indian subcontinent, Idli is one of the very widely used fermented food mainly prepared from rice and black gram [26]. Many microorganisms like Leuconostoc *mesenteroides*. *Streptococcus thermophilus* produce lactic acid and carbon dioxide that make the batter anaerobic and leaven the product during the fermentation of idli batter [17]. The maximum leavening of wet idli batter takes 12 hrs as the microbes should grow and then produce dextran. Taking the fact that dextran is used in leavening of wet idli batter, use of commercially produced dextran for dry powders of rice and black gram minimizes the time for leavening. Dextran is an exopolysaccharide that acts as a mesh like structure, that prevents the release of gases namely CO_2 produced by microorganisms present naturally during fermentation of idli batter. Addition of dextran directly not only reduces leavening time but also may be helps in better entrapment of released gases thus giving more sponginess to the product idli.

VII. CONCLUSION

An efficient isolate was isolated that produced more amount of exo-polysaccharide by 48 hours in sucrose rich media. The isolate was identified as *Weissells sp* .by morphological, biochemical and genetic method like 16s-rRNA sequencing. Food grade dextran produced by present isolate can be used for leavening of idli batter and for the preparation of idlis using dry ingredients instead of wet batter. Use of dry ingredients instead of wet batter for preparation of traditional and nutritionally rich idlis with leavening by this food grade dextran has commercial significance and can also be used for automation of the whole process.

VIII. FUTURE SCOPES

The food grade dextran is commercially significant as it can also be used as jelling agent. Use of dry ingredients for idli preparation with leavening by this food grade dextran has commercial significance as this can also be used for automation of this traditional and high nutritional domestic process of idli making.

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