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Optimization and scale-up of fermentation of glucansucrase and branched glucan by *Pediococcus pentosaceus* CRAG3 using Taguchi methodology in bioreactor

Rishikesh Shukla
Arun Goyal

Authors' address:
Department of Biotechnology,
Indian Institute of Technology Guwahati, Guwahati 781039,
Assam, India.

Correspondence:
Arun Goyal
Department of Biotechnology,
Indian Institute of Technology Guwahati, Guwahati 781039,
Assam, India
Tel.: +361-258 2208
e-mail: arungoyl@iitg.ernet.in

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ABSTRACT

The present investigation focuses on screening and optimization of media components to enhance glucansucrase and glucan production by *Pediococcus pentosaceus* CRAG3 at shake-flask and bioreactor level using Taguchi orthogonal array design. A three-level Taguchi orthogonal array layout of $L_{27}(3^3)$ was employed, in which six variables were studied for their influence on glucansucrase and glucan production. The results showed that sucrose, K_2HPO_4 and Tween-80 were the most significant factors to improve glucansucrase production while the glucan production was mostly affected by sucrose, peptone and K_2HPO_4. The optimized medium composition for maximum glucansucrase and glucan production were: sucrose 3.5% and 5%; yeast extract 0.2% and 2.0%; beef extract 0.5% and 0.5%; peptone 3.0% and 1.0%; K_2HPO_4 0.2% and 0.2%, and Tween-80 1.0 and 0.1%, respectively. The optimized medium gave 10.1 U/ml and 10.2 U/ml glucansucrase activity while glucan concentrations were 56 mg/ml and 80 mg/ml in shake flask and bioreactor level, respectively which were in good agreement with predicted values (10.1 U/ml and 54.5 mg/ml). The optimized medium gave 2 fold enhancement in enzyme activity and 4 fold increase in glucan concentration as compared to non-optimized medium (4.5 U/ml and 15 mg/ml, respectively) at shake flask level.

Key words: Glucansucrase, glucan, *Pediococcus pentosaceus*, Taguchi orthogonal design, bioreactor

Introduction

Glucansucrases are large size extracellular enzymes which catalyze the formation of various glucans using sucrose (Purama & Goyal, 2005). *Streptococcus*, *Leuconostoc*, *Weissella* and *Lactobacillus* are four genera of lactic acid bacteria (LAB), majorly of which produce glucansucrases and in turn glucans (the exopolysaccharides excreted by LAB into their surroundings) (van Geel-Schutten et al., 1998; Monchois et al., 1999; Kralj et al., 2002; Tieking et al., 2003; Kralj et al., 2004; Kralj et al., 2005; Purama & Goyal, 2005). Exopolysaccharides (EPSs) have various applications as adhesives (Costerton et al., 1987), participants in certain cellular recognition processes as slime forming agents for protection against dehydration, phagocytosis, or toxins (Ceri et al., 1986) and also suitable for the food and dairy industry as thickeners, stabilizers, and gelling agents (Sandford & Baird, 1983). They have also numerous applications in pharmaceutical, food, agriculture and fine chemical industries (Sutherland, 1998). Korakli & Vogel (2006) studied their role as potential therapeutic agents and also as viscosifying, stabilizing, emulsifying, sweetening, gelling, or water-binding agents, in the food as well as in the non-food industries. The nature of glucans vary from species to species like the type of glucosidic linkages, degree and type of branching, length of glucan chains, molecular mass, and conformation of polymers (Monchois et al., 1999). Before the work of Smitinont and his coworkers, who had emphasized on dextran synthesizing ability of *Pediococcus* genus, there was not any distinct data revealing the dextran

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production by this genus (Smitinont et al., 1999). Later, Patel et al. (2010) reported the glucan producing ability of this genus. In our preliminary investigations we found the presence of α(1→6) linear chain and α(1→3) and α(1→4) linked branches in glucans from this strain and it had been reported that polysaccharides with such linkages have anti-cancerous properties (Cao et al., 2006; Liu et al., 2007). The glucan with α(1→6) and α(1→4) linkages has also been reported from *L. dextranicum* isolated from palm wine (Uzochukwu et al., 2002) and from *Lactobacillus reuteri* (Kralj et al., 2005).

It has been reported that the production of glucansucrases is affected by the number of factors like temperature, aeration, medium components (Tsuchiya et al., 1952; Lazic et al., 1993; Goyal et al., 1995; Rodrigues et al., 2003; Cortezi et al., 2005). Hence, there is a need of developing proper fermentation medium to improve its efficient utilization in fermentation technology. The first step in the process of medium optimization is to screen the important factors affecting the production. However alteration of one factor at a time, for conventional optimization of product formation, is a time and labor consuming process and also it does not effectively provide the combinatorial effect of studied factors (Beg et al., 2003). Hence to overcome such situation, statistical approaches have been developed using a combination of mathematical and analytical tools (Box & Hunter, 1975). In this respect, recently developed orthogonal array (OA) optimization methodology provides offline quality control of system, parameter, and tolerance designs that help in the identification of key factors and their levels for best performance (Rao et al., 2004). For this, simultaneous study of various control factors and their optimization by statistical experimental designs had been done (Stone & Veevers, 1994; Abdel-Fattah et al., 2005).

Taguchi experimental design is reported to be better over other statistical methods as it allows simultaneous examination of many factors and also allows the extraction of more quantitative information by employed only a few experiments (Cobb & Clarkson, 1994; Houng et al., 2006). Taguchi method examines the effects of many variables and identifies only those which have major effect on the process and it involves large number of experimental situation known as orthogonal arrays (OA) and reduces experimental errors and enhances the efficiency and reproducibility of the experiments (Prasad et al., 2005). Taguchi's method has been applied successfully on production of monoesters from lipase (Han et al., 1998), optimization of ELISA procedures (Jeney et al., 1999) and production of griseofulvin from *P. griseofulvin* (Venkata Dasu et al., 2003).

In the present study we report an optimization strategy for scale-up of glucansucrase and glucan production from *Pediococcus pentosaceus* CRAG3 using statistical method. For this Taguchi orthogonal array design was used to analyze the effect of various medium components on their production. The validation of data was done both at flask and bioreactor level. The optimized medium gave a significant enhancement in both glucansucrase activity and glucan concentration by this strain.

Materials and Methods

Microorganism and production of glucansucrase and glucan

The microbe was isolated from fermented cucumber and identified as *Pediococcus pentosaceus* CRAG3 (GenBank ID: JN944736.1) (hereinafter CRAG3). The culture was maintained in modified MRS medium (with sucrose as carbon source) (Goyal & Katiyar, 1996), as a stab at 4°C and subcultured every 15 days. One loopful culture from stab was inoculated in 5 ml enzyme production medium as described by Tsuchiya et al. (1952) and incubated at 25°C and 180 rpm for 12h. One percent from this broth was transferred to 100 ml sterile Tsuchiya medium (Tsuchiya et al., 1952) in 250 ml Erlenmeyer flask and incubated at 25°C and 180 rpm for 6h. The cells were removed by centrifuging at 8000g and 4°C for 10 min and culture supernatant was analyzed for glucansucrase activity and glucan concentration.

Enzyme assay

The enzyme assay was carried out in 1 ml reaction mixture containing 5% (w/v) sucrose in 20 mM sodium acetate buffer (pH 5.4) and 20 µl cell-free supernatant. The enzymatic reaction was performed at 30°C for 15 min. 100 µl aliquot was taken from the reaction mixture to estimate reducing sugar. The enzyme activity was determined by the estimation of released reducing sugar. The reducing sugar was estimated by method of Nelson (1944) and Somogyi (1945).

Analysis of glucan content of the isolated strain

To 200 µl of the culture supernatant, three volumes of the prechilled ethanol was added and centrifuged at 12000g and 25°C for 30 min. The supernatant was discarded, and the precipitate was resuspended in 200 µl distilled water. The process was repeated twice. The final precipitate was air

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dried and dissolved in 200 µl distilled water. The polysaccharide content of cell-free supernatant from CRAG3 was determined by phenol-sulphuric acid method as described previously by DuBois et al. (1956) and Fox & Robyt (1991).

Taguchi orthogonal array design

Taguchi methodology for optimization was performed by the method described by Prakasham et al. (2005). It consisted of four interconnected sequential phases: planning (selection of factors), conducting (designing of experiment and defining of data analysis procedure), analysis (analysis of data by fitting the results in Taguchi software) and validation (use of optimized parameters in experiment). In the present Taguchi design of experiment (DOE), six fermentation factors sucrose (%, w/v), yeast extract (%, w/v), beef extract (%, w/v), peptone (%, w/v), dipotassium hydrogen orthophosphate (K_2HPO_4) (%, w/v) and Tween-80 (%, v/v) were selected to analyze their effect on glucansucrase and glucan production. Three levels of factor variations were considered. An L27 orthogonal array (OA) was designed consisting of 27 different experimental trials. The total degree of freedom available in an OA was equal to the number of trials minus one. Fermentations were carried out by using 12-16h old culture of CRAG3 grown in enzyme production medium described by Tsuchiya et al. (1952). 1% culture was inoculated in each of 27 different compositions of media and incubated at 25°C and 180 rpm for 6h. After incubation all the media were analyzed for their glucansucrase activity and glucan concentration as described above. All experiments were performed in triplicates. The design for the L27 orthogonal array was developed and analyzed using "MINITAB 15" software for predicted values, individual and interactive influences, ANOVA and to determine the contribution of each selected factor in glucansucrase and glucan production by CRGAG3. The validation of data was done by using optimized parameters of fermentation media components and levels by software in shake flask.

Validation of experiments at bioreactor level

The validation of data was also done by using optimized medium at bioreactor level. Two 3L bioreactors (Applikon, model Bio Console ADI 1025) with 1L working volume were used for fermentation. One bioreactor with optimized medium for glucansucrase production and another for glucan were used. The bioreactors were autoclaved at 121°C, 15 lb/in^2 pressure for 20 min and allowed to cool at room temperature. The dissolved oxygen (DO) was adjusted to 100% before inoculation. 1% inoculum from 12h grown culture was inoculated in each bioreactor. pH 7.0 and temperature of 25°C were kept constant throughout the fermentation process. The agitation was set to 180 rpm at the beginning of the run but changed accordingly to keep the DO above 40%. During fermentation, the parameters like cell optical density, enzyme activity, sucrose concentration and glucan concentration were analyzed at every 2h interval. The cell optical density (OD) was observed by analyzing the absorbance (A_{600}) at 600 nm. The enzyme activity of cell free supernatant was analyzed by the method discussed previously. The sucrose profile of media was observed by estimating the reducing sugars by the method of Sumner & Sisler (1944). The glucan concentration of culture supernatant was measured as described in earlier section.

Results

Estimation of glucansucrase activity and glucan concentration of CRAG3

The maximum glucansucrase activity and glucan concentration showed by CRAG3 after 6h were 4.5 U/ml and 15 mg/ml, respectively when the culture was grown in enzyme production medium (Tsuchiya et al., 1952) at 25°C and 180 rpm.

Taguchi orthogonal array design for glucansucrase production

Screening of media components

Selected fermentation factors and their levels for optimization of glucansucrase production by CRAG3 are presented in Table 1.

Table 1. *Selected fermentation factors and their assigned levels on glucansucrase and glucan production by Pediococcus pentosaceus CRAG3.*

No.	Factor	Level 1	Level 2	Level 3
1	**Sucrose**	2.0	3.5	5.0
2	**Yeast Extract**	0.2	1.0	2.0
3	**Beef Extract**	0.1	0.5	1.0
4	**Peptone**	1.0	2.0	3.0
5	**K_2HPO_4**	0.2	2.0	4.0
6	**Tween-80**	0.1	0.5	1.0

Wide variation in glucansucrase production was observed in L27 orthogonal array experiments (Table 2), which showed the importance of optimization for attaining maximum production.

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Table 2. *Fractional factorial design of L-27 (6^3) orthogonal array used for glucansucrase activity and glucan production optimization by Pediococcus pentosaceus CRAG3.*

Run	Suc*	YE*	BE*	Pep*	K_2HPO_4	T80*	Glucansucrase (U/ml)		Glucan (mg/ml)	
	Concentration (%)						Experimental	Predicted	Experimental	Predicted
1	2	0.2	0.1	1	0.2	0.1	2.90	2.92	29.40	26.19
2	2	0.2	0.1	1	2	0.5	1.23	1.05	13.65	15.34
3	2	0.2	0.1	1	4	1	3.08	3.25	1.05	2.55
4	2	1	0.5	2	0.2	0.1	2.90	3.59	24.15	24.44
5	2	1	0.5	2	2	0.5	2.71	1.73	10.50	13.60
6	2	1	0.5	2	4	1	3.64	3.93	4.20	0.81
7	2	2	1	3	0.2	0.1	3.27	3.41	22.05	21.72
8	2	2	1	3	2	0.5	1.79	1.54	6.30	10.87
9	2	2	1	3	4	1	3.64	3.74	2.31	-1.92
10	3.5	0.2	0.5	3	0.2	0.5	7.71	7.34	29.40	30.40
11	3.5	0.2	0.5	3	2	1	7.52	7.50	30.45	22.25
12	3.5	0.2	0.5	3	4	0.1	1.79	2.18	4.20	11.40
13	3.5	1	1	1	0.2	0.5	5.67	6.04	43.05	36.35
14	3.5	1	1	1	2	1	6.41	6.20	34.65	28.20
15	3.5	1	1	1	4	0.1	1.05	0.89	4.20	17.35
16	3.5	2	0.1	2	0.2	0.5	5.12	5.67	43.05	42.64
17	3.5	2	0.1	2	2	1	4.75	5.83	23.10	34.50
18	3.5	2	0.1	2	4	0.1	2.16	0.52	34.65	23.65
19	5	0.2	1	2	0.2	1	9.90	8.95	38.85	41.15
20	5	0.2	1	2	2	0.1	0.81	1.59	36.75	34.95
21	5	0.2	1	2	4	0.5	1.60	1.77	19.95	19.45
22	5	1	0.1	3	0.2	1	10.10	9.59	34.65	31.35
23	5	1	0.1	3	2	0.1	2.72	2.23	24.15	25.15
24	5	1	0.1	3	4	0.5	1.42	2.40	7.35	9.65
25	5	2	0.5	1	0.2	1	9.56	9.59	42.00	52.35
26	5	2	0.5	1	2	0.1	1.97	2.23	51.45	46.15
27	5	2	0.5	1	4	0.5	2.71	2.41	35.70	30.65

*Suc - Sucrose, YE - Yeast Extract, BE - Beef Extract, Pep - Peptone, T80 - Tween-80

There was hardly to observe any variation between software predicted and experimental values for glucansucrase production. Response table of the selected factors on glucansucrase production is shown in Table 3. The effect of factors on response has been determined by rank and delta (Table 3, last two rows). The size of effect is measured by delta taking the difference between the highest and lowest characteristic average for a factor. Higher delta means higher effect of that component. Based on the delta values, rank arranges the factors from the highest effect to the least effect on response characteristics. In present study, the order of medium components for their effect on glucansucrase production was Tween-80 > K_2HPO_4 > Sucrose > Beef extract > Peptone > Yeast extract, which suggested that Tween-80 had an ample effect and the yeast extract had least effect on glucansucrase production by CRAG3.

ANOVA data indicated the significance of selected parameters on enzyme production, which varied with factor to factor. Sucrose, Tween-80 and K_2HPO_4 with least P-value were found to be most significant factors for glucansucrase production (Table 4). The experimental data revealed that selected level 1 values of yeast extract and K_2HPO_4, level 2 values of beef extract and sucrose, and level 3 values of peptone and Tween-80 in the medium are optimal for glucansucrase production (Figure 1A-F).

Table 3. *Response table of means for glucansucrase production by Pediococcus pentosaceus CRAG3.*

Level	Sucrose	YE*	BE*	Peptone	K_2HPO_4	Tween 80
1	2.797	4.062	3.721	3.846	6.348	2.174
2	4.688	4.070	4.503	3.733	3.326	3.332
3	4.533	3.887	3.795	4.440	2.345	6.513
Delta	1.891	0.183	0.783	0.707	4.003	4.338
Rank	**3**	**6**	**4**	**5**	**2**	**1**

*YE - Yeast Extract, BE - Beef Extract

The optimum conditions for maximum glucansucrase production are shown in Table 5. The sucrose with a final concentration of 3.5% (w/v) showed maximum glucansucrase activity. Increasing further the sucrose concentration leads to

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decreased activity of the enzyme (Figure 1A). This is in agreement with previous reports where the strains of *Leuconostoc mesenteroides* showed maximum activity at low concentration of sucrose and no enhancement in activity was observed above 4% (Goyal & Katiyar, 1997; Lopretti et al., 1999).

Table 4. *Analysis of variance (ANOVA) of experimental data on glucansucrase production by Pediococcus pentosaceus CRAG3.*

Source	DF*	Seq SS*	Adj SS*	Adj MS*	F	P*
Sucrose	2	19.843	19.8427	9.9213	14.55	0.000
YE*	2	0.193	0.1934	0.0967	0.14	0.869
BE*	2	3.360	3.3603	1.6801	2.46	0.121
Peptone	2	2.596	2.5963	1.2981	1.90	0.186
K_2HPO_4	2	78.377	78.3767	39.1884	57.49	0.000
Tween-80	2	90.841	90.8411	45.4206	66.63	0.000
Residual error	14	9.543	9.543	0.6817		
Total	26	204.754				

*YE - Yeast Extract, BE - Beef Extract, DF - Degree of Freedom, Seq SS - Sequential Sum of Squares, Adj SS - Adjusted Sum of Squares, Adj MS - Adjusted Mean of Squares, P - Probability

K_2HPO_4 acts as a buffering agent to the lactic acid produced during the fermentation (Tsuchiya et al., 1952; Rodrigues et al., 2003).

The increase in concentration of K_2HPO_4 above 0.2% (w/v) resulted in decreased glucansucrase activity (Figure 1E). 1% (v/v) Tween-80 gave maximum glucansucrase activity (Figure 1F). Tween-80 is used as a surfactant in industries and its presence in medium enhances secretion of glucansucrase from cells by altering the fatty acid composition of cell membrane (Sato et al., 1989; Goyal & Katiyar, 1997).

The validation of data was done at flask level with 100 ml medium in which the activity observed was 10.1 U/ml that was in good agreement with the predicted results (10.5 U/ml) of software. This showed a significant 2 folds increase in glucansucrase activity in optimized medium than in non-optimised medium (4.5 U/ml) (Table 6). This was further confirmed by performing the experiment at bioreactor level.

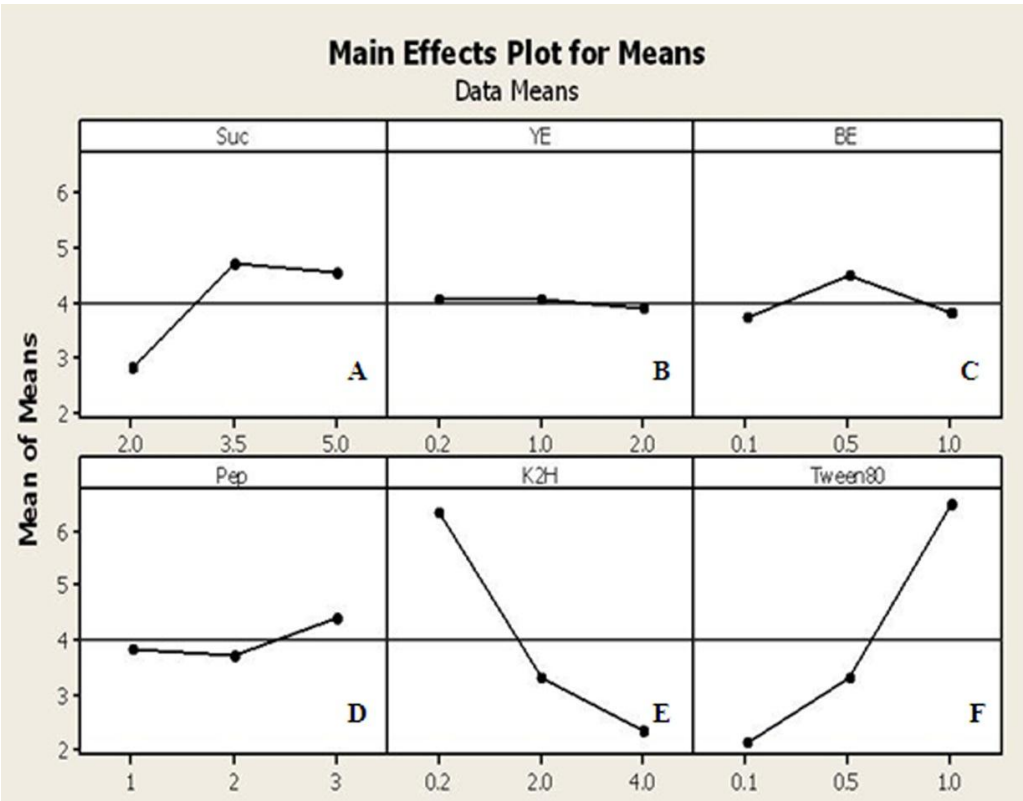

Figure 1. *Impact of selected fermentation factors and their assigned level on glucansucrase production by Pediococcus pentosaceus CRAG3. X-axis represents assigned levels of selected factor (%, w/w or w/v) and Y-axis represents glucansucrase production (U/ml). (a) Sucrose, (b) Yeast extract, (c) Beef extract, (d) Peptone (e) K_2HPO_4 and (f) Tween-80.*

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Table 5. *Optimized fermentation conditions for glucansucrase and glucan production by Pediococcus pentosaceus CRAG3.*

Components (%)	Suc*	YE*	BE*	Pep*	K_2HPO_4	T80*
Glucansucrase	3.5	0.2	0.5	3.0	0.2	1.0
Glucan	5.0	2.0	0.5	1.0	0.2	0.1

*Suc - Sucrose, YE - Yeast Extract, BE - Beef Extract, Pep - Peptone, T80 - Tween-80

Table 6. *Comparison of glucansucrase activity and glucan production by Pediococcus pentosaceus CRAG3 in non-optimized and optimized media at flask and bioreactor level.*

Medium	Glucansucrase activity (U/ml)	Glucan concentration (mg/ml)
Non-optimized	4.5	15.0
Taguchi predicted value	10.5	54.5
Optimized (in flask)	10.1	56.0
Optimized (in bioreactor)	10.2	80.0

Experimental validation of the model in bioreactor

The optimized medium for glucansucrase production contained (%, w/v): sucrose, 3.5%; yeast extract, 0.2%; beef extract, 0.5%; peptone, 3%; $MgSO_4$.7H2O, 0.02%; $MnSO_4.4H_2O$, 0.001%; $FeSO_4.7H_2O$, 0.001%; $CaCl_2.2H_2O$, 0.001%; NaCl 0.001%; K_2HPO_4, 0.2% and Tween-80, 1%. The glucansucrase production by CRAG3 in optimized medium was compared with that of non-optimized medium. The fermentation profile is shown in Figure 2. The maximum glucansucrase activity (10.2 U/ml) was observed at 8th hour of fermentation. It was also in good agreement with predicted values of glucansucrase activity. The sucrose profile showed that it started being consumed from the 1st hour of fermentation and a little sucrose remained in medium after 12h. The pH and temperature remained constant throughout the fermentation while DO was controlled by varying the agitation speed.

Taguchi orthogonal array design for glucan production

Screening of medium components

The selected fermentation factors and their levels for optimization of glucan production by CRAG3 are shown in Table 1. In this case, wide variation in glucan production had been also observed in L27 orthogonal array experiments (Table 2). The software predicted and experimental values for glucan production were matched. Table 7 showed response table of selected factors for glucan production. The order of medium components for their effect for glucan production was K_2HPO_4 > Sucrose > Peptone > Yeast extract > Beef extract >Tween-80, which suggested that K_2HPO_4 showed most significant effect and Tween-80 had least effect for glucan production by CRAG3.

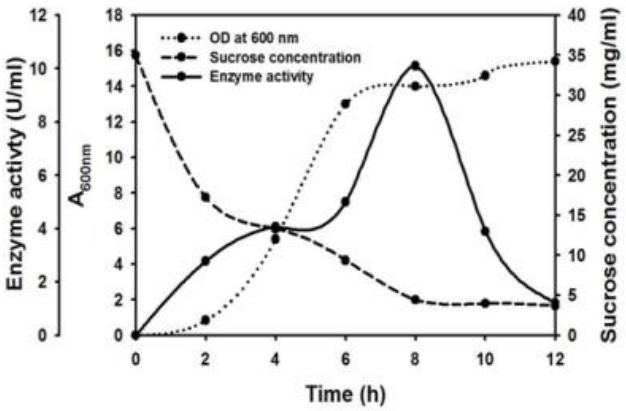

Figure 2. *Validation of data for glucansucrase production in bioreactor. Variation of glucansucrase activity (U/ml), sucrose concentration (mg/ml) and cell optical density (A_{600}) during batch fermentation of Pediococcus pentosaceus CRAG3 in bioreactor.*

Table 7. *Response table of means for glucan production by Pediococcus pentosaceus CRAG3.*

Level	Sucrose	YE*	BE*	Peptone	K_2HPO_4	Tween 80
1	12.62	22.63	23.45	28.35	34.07	25.67
2	27.42	20.77	25.78	26.13	25.67	23.22
3	32.32	28.96	23.12	17.87	12.62	23.47
Delta	19.69	8.19	2.66	10.48	21.44	2.45
Rank	**2**	**4**	**5**	**3**	**1**	**6**

*YE- Yeast Extract, BE- Beef Extract

ANOVA data revealed that sucrose, peptone and K_2HPO_4 had least P-value hence were found to be most significant factors for glucan production (Table 8). The experimental data showed that selected level 1 values of peptone, Tween-80 and K_2HPO_4; level 2 value of beef extract and level 3 values of sucrose and yeast extract in the medium, were optimal for maximum glucan production by CRAG3 (Figure 3A-F). Table 5 showed the optimum medium for maximum glucan production. The peptone in its final concentration range from 1 to 3% (w/v) showed its effect on glucan production. It has been shown that 1% (w/v) peptone gave maximum glucan concentration, which further decreased after increasing the concentration (Figure 3D). The effects of sucrose and K_2HPO_4 on glucan concentration showed that

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5% sucrose and 0.2% K_2HPO_4 gave maximum glucan concentration (Figure 3A and 3E). The validation of data was done at flask level in which the maximum glucan concentration observed was 56 mg/ml. This was in good agreement with the predicted results (54.5 mg/ml) of software. The glucan concentration in optimized medium was 4 fold higher (56 mg/ml) than in non-optimized medium (15 mg/ml) (Table 6). This was further validated at bioreactor level.

Table 8. *Analysis of variance (ANOVA) of experimental data on glucan production by Pediococcus pentosaceus CRAG3.*

Source	DF*	Seq SS*	Adj SS*	Adj MS*	F	P*
Sucrose	2	1892.04	1892.04	946.02	14.83	0.000
YE*	2	331.64	331.64	165.82	2.60	0.110
BE*	2	37.88	37.88	18.94	0.30	0.748
Peptone	2	548.741	548.741	274.35	4.30	0.035
K_2HPO_4	2	2101.52	2101.52	1050.76	16.48	0.000
Tween-80	2	32.64	32.64	16.32	0.26	0.778
Residual error	14	892.90	892.90	63.78		
Total	26	5837.31				

*YE - Yeast Extract, BE - Beef Extract, DF - Degree of Freedom, Seq SS - Sequential Sum of Squares, Adj SS - Adjusted Sum of Squares, Adj MS - Adjusted Mean of Squares, P - Probability

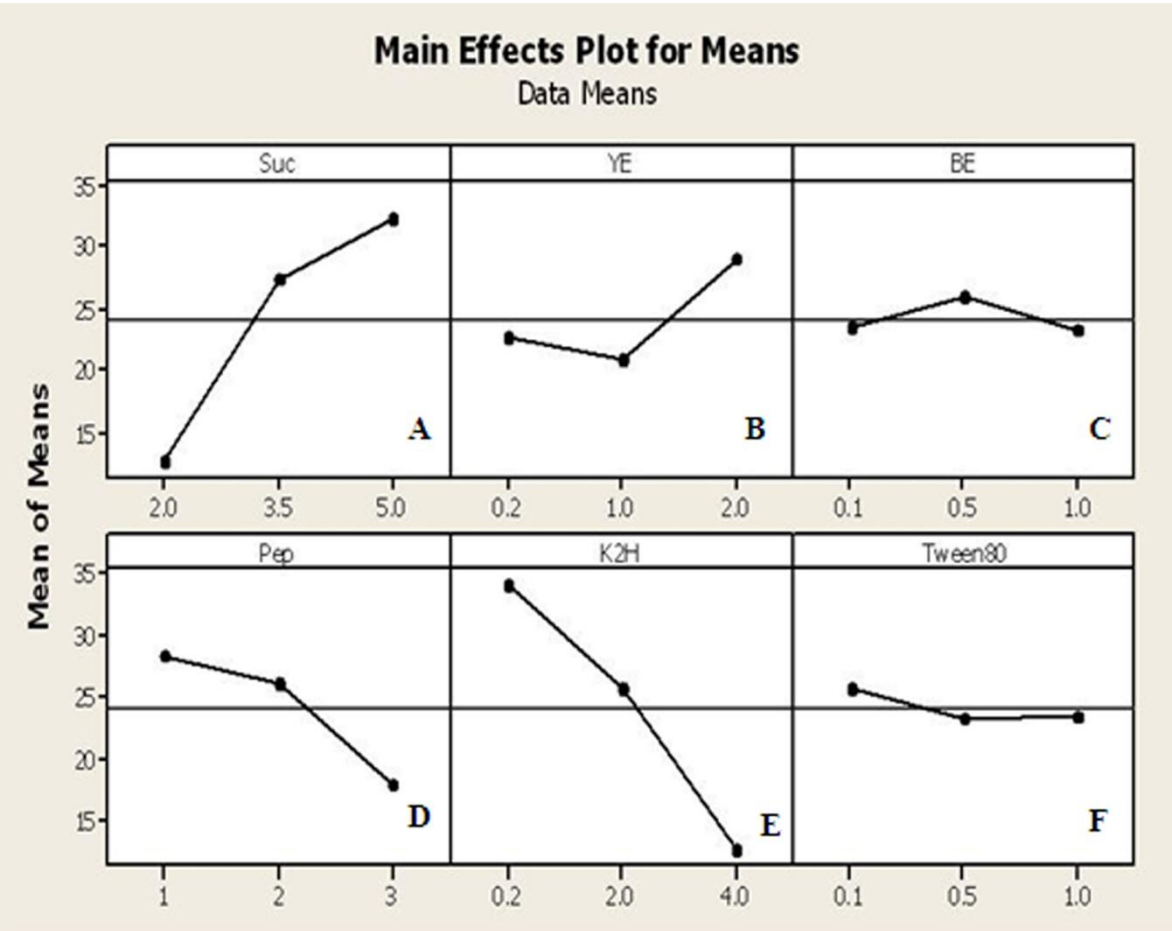

Figure 3. *Impact of selected fermentation factors and their assigned level on glucan production by Pediococcus pentosaceus CRAG3. X-axis represents assigned levels of selected factor (%, w/w or w/v) and Y-axis represents glucansucrase production (U/ml). (a) Sucrose, (b) Yeast extract, (c) Beef extract, (d) Peptone (e) K_2HPO_4 and (f) Tween-80.*

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Experimental validation of the model in bioreactor

The optimized medium for glucan production consisted (%, w/v): sucrose, 5%; yeast extract, 2%; beef extract, 0.5%; peptone, 1%; K_2HPO_4, 0.2%; $MgSO_4.7H_2O$, 0.02%; $MnSO_4.4H_2O$, 0.001%; $FeSO_4.7H_2O$, 0.001%; $CaCl_2.2H_2O$, 0.001%; NaCl 0.001% and Tween-80, 0.1%. Comparing the glucan production by CRAG3 in optimized medium (80 mg/ml) with non-optimized medium (15 mg/ml), an increase of 5 fold was observed. The fermentation profile showed that the glucan concentration reached to its maximum level (80 mg/ml) during 6^{th}-8^{th} hour of fermentation (Figure 4). The glucan concentration in bioreactor was 1.4 fold higher than that observed at flask level (56 mg/ml). The increase in concentration might be due to more growth of cells in medium (Figure 4). The sucrose profile showed that it started getting consumed from 1^{st} hour of fermentation and after 4^{th} hour almost 50% of sucrose was consumed. 2-3% sucrose remained in medium after 12 h. The pH and temperature remained constant throughout the fermentation while DO was controlled by varying the agitation speed.

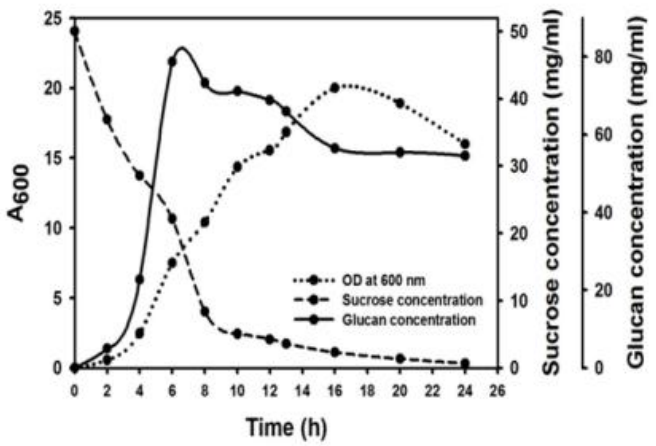

Figure 4. *Validation of data for glucan production in bioreactor. Variation of glucan concentration (mg/ml), sucrose concentration (mg/ml) and cell optical density (A_{600}) during batch fermentation of Pediococcus pentosaceus CRAG3 in bioreactor.*

Discussion

The branched glucan producer strain of *Pediococcus pentosaceus* CRAG3 was selected for optimization of media components for enhanced glucansucrase and glucan production using Taguchi orthogonal array deign. The effect of factors on response showed that Tween-80 had an ample effect and yeast extract had least effect on glucansucrase production by CRAG3 while K_2HPO_4 showed most significant effect and Tween-80 had least effect on glucan production by CRAG3. ANOVA data showed significant effect of sucrose, Tween-80 and K_2HPO_4 on glucansucrase production. On the other hand, sucrose, peptone and K_2HPO_4 were found to be most significant for glucan production. Significant increase in glucansucrase activity and glucan concentration was observed in optimized medium (10 U/ml and 54 mg/ml, respectively) than in non-optimized medium (4.5 U/ml and 15 mg/ml, respectively). Validation of data was done at flask and bioreactor levels. The glucansucrase activity was validated at both flask and bioreactor level. Interestingly, the enhancement in glucan concentration was observed in both flask (56 mg/ml) and bioreactor (80 mg/ml). The glucan concentration of CRAG3 in non-optimized medium was comparatively higher (15 mg/ml) than previously reported strains of lactic acid bacteria like *L. mesenteroides* NRRL B-512F, 6.9 mg/mL (Santos et al., 2000); *L. mesenteroides* NRRL B-640, 7.0 mg/mL (Majumder et al., 2009) and *Pediococcus pentosaceus*, 10.2 mg/mL (Patel et al., 2010). However, after optimization this became an economically and industrially important strain. The glucan produced by this strain was highly branched and hence can be used for the production of branched glucooligosaccharides by acceptor reaction, which could serve as potential prebiotics.

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