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Isolation, screening and optimization of extracellular glucoamylase from *Paenibacillus amylolyticus* strain NEO03

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ABSTRACT

Extracellular glucoamylase producing microorganisms were screened from soil. The potent organism was identified as *Paenibacillus amylolyticus* strain NEO03 based on microscopic examinations and 16S rDNA molecular sequencing. The production of glucoamylase from the isolated bacterium was optimized by one factor at a time approach and the significant factors were evaluated by response surface methodology. Under submerged fermentation conditions, maximum activity was observed after 48 h of incubation, pH 7.0, temperature 37 °C and inoculum level of 6% (v/v). The optimum nutrient sources were rice bran and yeast extract. Furthermore, the significant medium factors were statistically evaluated. The fast growing *P. amylolyticus* strain possessed good fermenting ability to utilize agro-wastes for enzyme production. The extracellular glucoamylase can be used as a beneficial candidate for industrial applications, especially for saccharification processes.

1. Introduction

Glucoamylases (EC 3.2.1.3) are exo-acting enzymes which release $_D$-glucose from the non-reducing ends of starch. The exo-amylase hydrolyzes α-1, 4 glycosidic linkages in a consecutive pattern and produces $_D$-glucose as the single end product. The enzyme also hydrolyzes α-1, 6 and rarely α-1, 3 linkages in a slower manner. Glucoamylases are also referred as γ-amylase, amyloglucosidase, starch glucogenase, glucogenic enzyme and exo-1, 4-α-$_D$- glucanohydrolase (Mohamed et al., 2017). Glucoamylases have several applications in the starch processing industry, food processing industry, fermentation industry, ethanol industry, paper and textile industry. The enzyme is also applicable in baking, confectionaries and as well as pharmaceutical industries (Kumar and Satyanarayana, 2009). The enzyme is of economic interest due to its ability to hydrolyze polymers to simple monomers, to specifically form $_D$-glucose as the sole product.

The microorganisms such as fungi, yeasts and some bacteria are used for the production of glucoamylase. Glucoamylases from *Aspergillus* sp. and *Rhizopus* sp. are the widely used microbial strains for industrial activities. Microbial glucoamylases can be produced by both submerged fermentation (SmF) and solid-state fermentation (SSF) systems. The reported bacterial glucoamylases were mostly produced by SmF (Feng et al., 2002; Kim et al., 2004). Traditionally, the fungal glucoamylases were produced by SmF (Pavezzi et al., 2008; Pasin et al., 2017), whereas numerous studies have been carried out by using SSF conditions (Kiran et al., 2014; Banerjee and Gosh, 2017).

The glucoamylases from bacterial strains are the least studied microbial origin. Also, the use of agro-wastes in fermentation processes for the production of enzymes are of high importance. Since several studies are carried out on fungal glucoamylases, the aim of this study was to screen and identify a potent bacterial extracellular glucoamylase producer, and evaluate the enzyme biosynthesis under submerged fermentative conditions by utilization of agro-industrial residues.

2. Materials and methods

2.1. Isolation and screening of glucoamylase producing microorganisms from soil

The isolation of the microbial colonies was carried out from soil collected from different regions cultivating potato, rice and corn corps. The samples were serially diluted up to 10^{-5} dilutions on starch agar plates and the isolated colonies were primarily screened for starch hydrolysis. A secondary screening was performed wherein the positive amylase producers were inoculated in starch liquid medium. The medium composition was prepared as per Ghani et al. (2013) comprising of the following components (g L^{-1}): soluble starch 15.0, yeast extract 10.0, peptone 10.0, K_2HPO_4 1.0, $MgSO_4{\cdot}7H_2O$ 1.0 were adjusted

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to pH 5.0 prior to sterilization. The colonies were inoculated with 2% (v/v) of pre-inoculum and incubated at 30 °C for 24 h. The glucoamylase activity was estimated by assaying the reducing sugars (Bernfeld, 1955) and D-glucose by glucose oxidase-peroxidase (GOD-POD) kit (Glucose Eco-Pak, Mumbai, India). Furthermore, the enzyme was treated with 1% (w/v) soluble starch (1:1) and incubated overnight at 37 °C. After the incubation period, thin layer chromatography (TLC) was performed for all the positive glucoamylase producing bacterial strains. The standards (1% w/v, D-glucose and D-maltose) were prepared afresh and spotted with a capillary on the TLC plate. The mobile phase used was a mixture of butanol/ethanol/water (5:3:2, v/v), and the developer 0.2% (w/v) orcinol dissolved in methanol and sulphuric acid (9:1) was sprayed. The plate was heated up to 80 °C for development of bands (Gill and Kaur, 2004). The potent isolates were maintained in 40% glycerol and stored at −20 °C.

2.2. Identification of the bacterium

The glucoamylase producing bacterium was morphologically identified and biochemically characterized by referring to the Bergey's Manual of Systematic Bacteriology. Further, the culture was subjected to 16S rDNA molecular analysis and sequencing (Sanger et al. 1977). The similarity search was carried out using BLAST, while the percentage identity and similarity index of the reported sequence was performed using Clustal Omega. The phylogenetic tree inferred by the Neighbor-joining method was constructed using Molecular Evolutionary Genetics Analysis 7.0 (MEGA) (Tamura et al., 2011).

2.2.1. Determination of glucoamylase activity

Briefly, 0.5 mL of the glucoamylase fraction was added to 1.5 mL of sodium acetate buffer (pH 5.0) containing 0.5% (w/v) soluble starch and incubated at 55 °C for 15 min (Bernfeld, 1955). The reaction was stopped by addition of 1.0 mL of 3, 5-dinitrosalicylic acid solution, followed by heating in boiling a water bath for 3–5 min and the tubes were cooled to room temperature (Miller, 1959). The absorbance was measured at 540 nm by a Spectrophotometer (Systronics Double Beam 2202, India). The amount of D-glucose released was estimated by the GOD-POD assay and read at 505 nm. One unit of glucoamylase activity was defined as the amount of enzyme required to release one μmol of D-glucose from soluble starch per minute under the assay conditions.

2.2.2. Determination of protein and carbohydrate content

The protein concentration was estimated as per the method of Lowry et al. (1959) using bovine serum albumin (BSA) as the standard. The total carbohydrate content was determined by phenol sulphuric acid method, using D-glucose as a standard (Dubois et al., 1956).

2.3. Optimization of glucoamylase production by OFAT and a statistical design

Firstly, the one-factor-at-a-time (OFAT) approach was used to study the effects of incubation period (12–72 h), initial pH (5.0–9.0), initial temperature (30, 37, 45, 55 °C), inoculum size (2–12%, v/v), carbon sources (soluble starch, corn starch, wheat starch, potato starch, wheat bran and rice bran) and nitrogen sources (yeast extract + peptone, yeast extract, beef extract, peptone, ammonium chloride, sodium nitrate and urea).

A statistical approach was performed to test the optimum medium factors responsible for enhancement of extracellular glucoamylase production. The experimental design was set-up by Response Surface Methodology (RSM) to determine the factors between the average values of the measurement, made at high (+1) and low (−1) levels. The variable factors chosen in the present study were pH, temperature and carbon source. The experimental design is illustrated in Tables 3A and 3B. Furthermore, the experimental factors were evaluated by Central composite design (CCD) in order to obtain the quadratic model. The statistical software tool used was Design Expert (v.10) Stat-Ease, Inc. Minneapolis, USA.

2.3.1. Validation of the RSM model

The optimized factors resulting in maximum response were investigated in triplicate runs to verify the predicted and experimental results which confirms the validity of the model.

2.4. Raw starch adsorbability

The adsorption of the crude enzyme (50 μL) was tested on 1% and 5% of each raw starch (450 μL) prepared in buffer. The reaction mixture was incubated at 40 °C for 30 min with intermittent shaking. The enzyme activity from the supernatant was measured by assaying the reducing sugars as per Miller (1959). The percentage of adsorption rate was calculated as follows:

$$\text{Adsorption (\%)} = (A - B) \times 100/A$$

where, A = enzyme activity of the control without raw starch, B = residual enzyme activity in the supernatant after adsorption.

2.5. Raw starch hydrolysis

The crude enzyme was tested for its ability to hydrolyze raw starch (rice bran, rice flour starch) and soluble starch was observed as control. The reaction mixture containing the enzyme (0.1 mL) and 5% of each raw starch (0.9 mL) freshly prepared in the buffer, were incubated at 40 °C from 0 to 72 h. The enzyme activity was determined at different time intervals by estimation of the reducing sugars (Miller, 1959). The activity of the bacterial glucoamylase was compared with the commercial glucoamylase (AUM Enzymes, Gujarat, India) which was treated under identical conditions.

2.6. Statistical analyses

The experiments were performed in triplicates and the average values were represented as mean ± standard deviation (SD). One way analysis of variance (ANOVA) at $P_{0.05}$, was calculated using IBM SPSS Statistics V20.0.0.

3. Results and discussion

3.1. Screening of potent glucoamylase producing bacteria

Among the 75 isolates screened from soil samples, five bacterial strains with highest extracellular glucoamylase activity were chosen as potent glucoamylase producers. The primary screening results showed the maximum zone of hydrolysis for the positive isolate IS-44 (Fig. 1 A). The isolate IS-44 showed highest glucoamylase activity (Table 1). Further, the thin layer chromatogram confirmed the presence of only D-glucose units (Fig. 1 B, lane 5). Upon microscopic examinations of the isolate, IS-44 was characterized as aerobic, Gram-positive, spore forming rods bacterium. The glucoamylase producing bacterium was identified by the Bergey's Manual of Systematic Bacteriology and 16S rDNA molecular sequencing technique as *Paenibacillus amylolyticus*. The isolate was deposited in GenBank as the strain NEO03 (GenBank accession no. MG020758.1). The phylogenetic tree and evolutionary history was inferred using the Neighbor-Joining method and the tree was created using MEGA 7.0 (Fig. 2).

Some bacterial glucoamylases are reported in *Bacillus* sp. (Gill and Kaur, 2004), *Picrophilus* sp. (Dock et al., 2008) and *Streptosporangium* sp. (Stamford et al., 2002). There are also reports on glucoamylase production from *Clostridium* species, *Lactobacillus* sp., *Flavobacterium* sp., *Halobacterium* sp. (Kumar and Satyanarayana, 2009). Comparatively, the eukaryotic hosts have been extensively studied and are the

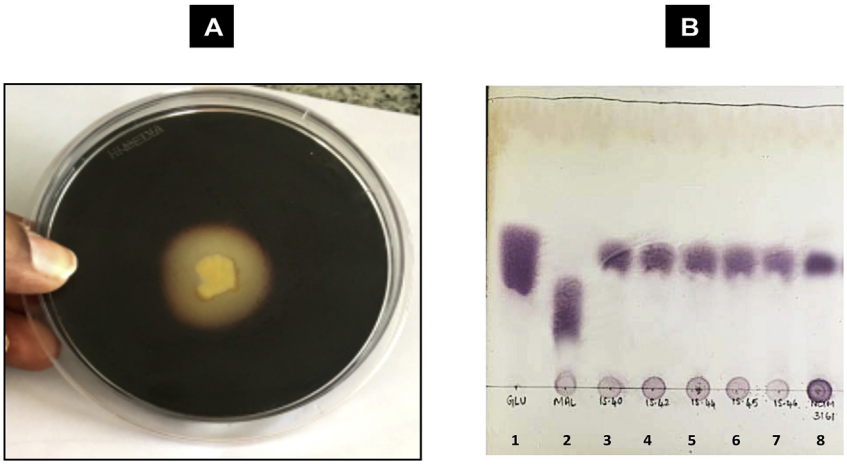

Fig. 1. Screening of glucoamylase-producing microbial isolates. **(A)** Starch hydrolysis test of IS-44 exhibiting maximum zone of clearance **(B)** Thin layer chromatogram of the positive glucoamylase producing bacterial strains. Lane 1: standard $_D$-glucose; Lane 2: standard $_D$-maltose; Lane 3: isolate 40; Lane 4: isolate 42; Lane 5: isolate 44; Lane 6: isolate 45; Lane 7: isolate 46; Lane 8: positive control (*Saccharomycopsis fibuligera*, NCIM 3161).

Table 1
Potential glucoamylase-producing isolates screened from soil.

Microbial isolates	Relative activity (%)
Control (NCIM 3161)[a]	100.00
IS-40	52.41
IS-42	87.47
IS-44	87.56
IS-45	70.57
IS-46	62.31

[a] NCIM 3161: Positive control (*Saccharomycopsis fibuligera*).

most common sources of glucoamylase due to their extracellular secretion. The commercially available glucoamylases for industrial applications are preferably produced from *Aspergillus* and *Rhizopus* species (Norouzian et al., 2006; Pavezzi et al., 2008). However, the enzymatic mechanisms and structure of bacterial glucoamylases has shown to have distinct advantage over eukaryotes (Ohnishi et al., 1992). Since studies on extracellular glucoamylases from bacteria are scarce, there arises a need to identify potent bacteria in order to determine its use for industrial applications.

3.2. Optimization of medium components by OFAT approach

The optimization of fermentation conditions involves various biochemical and physical factors. Under submerged conditions, the optimum incubation time was at 48 h of fermentation with 157.14 (U mL^{-1}) of glucoamylase production (Fig. 3 A). The enzyme activity was enhanced at pH 7.0 (184.9 U mL^{-1}), whereas the higher or lower pH conditions resulted in slight reduction in activity (Fig. 3 B). Glucoamylase activity was elevated at 37 °C (242.62 U mL^{-1}) than at 30 °C (184.03 U mL^{-1}), and increased temperatures lowered enzymic activity (Fig. 3 C). An inoculum level of 6% (v/v) was found to be ideal for glucoamylase production (295.66 U mL^{-1}), as higher concentrations declined the activity (Fig. 3 D).

A crucial strategy was adopted for enhancement in glucoamylase production by optimization studies. The further increase in incubation hours caused reduced activities, which might have occurred due to exhaustion of medium nutrients required for bacterial growth, or accumulation of toxic build-up of byproducts (Matthias, 2013). The initial pH 7.0 was also optimum for a thermophilic *Bacillus* sp. which produced extracellular glucoamylase by SmF (Gill and Kaur, 2004). In another study, *T. thermosaccharolyticum* strain ATCC 27384 and strain ATCC 7956 were anaerobically grown at optimum pH 6.7 and 7.2, respectively (Feng et al., 2002). However, most of the fungal microbes preferred an acidic environment (Mohamed et al., 2017). The optimum temperature for glucoamylase production from *P. amylolyticus* strain was 37 °C. Similarly, certain glucoamylases preferred a moderate temperature as observed in *L. amylovorus* ATCC 33621 (James and Lee, 1996) and for *Aspergillus* sp. was 30 °C (Ellaiah et al., 2002). Thus, the glucoamylase release was influenced by pH and temperature changes, which could have affected the metabolic activities of the

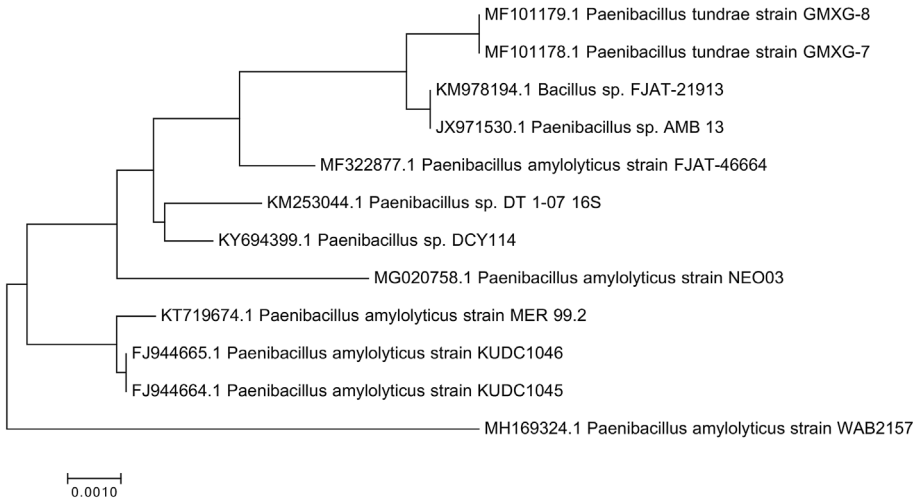

Fig. 2. The evolutionary history of *Paenibacillus subtilis* strain NEO03 inferred by the Neighbor-Joining method. Molecular Evolutionary Genetics Analysis (MEGA 7.0) was used to construct the phylogenetic tree.

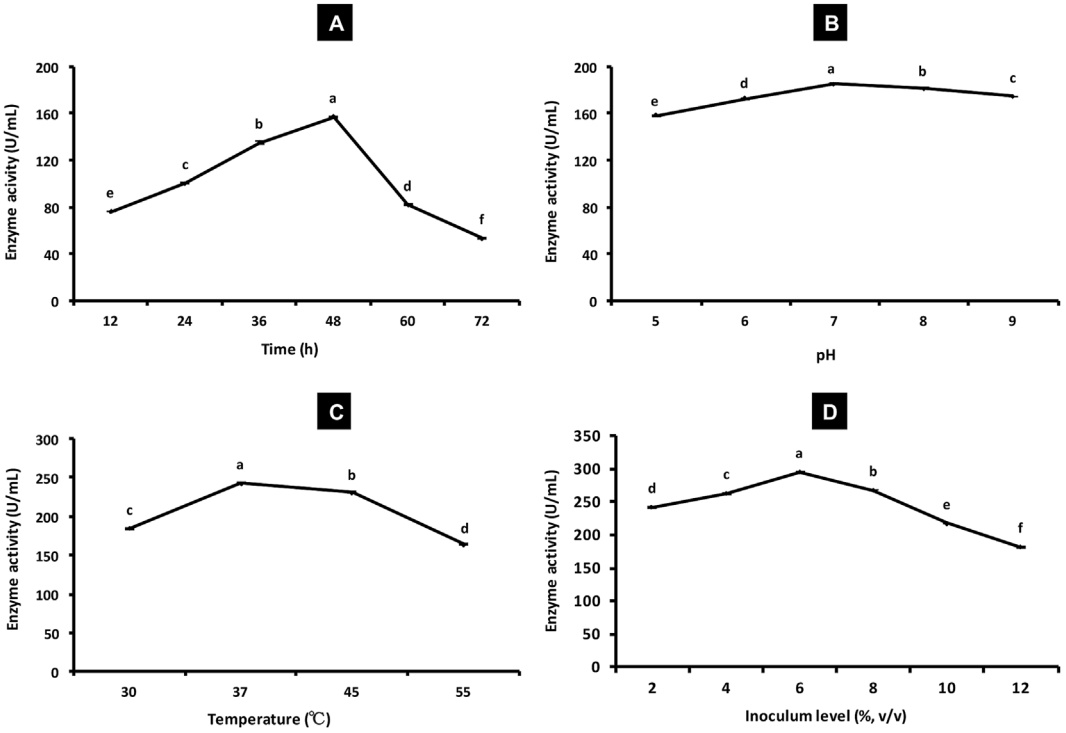

Fig. 3. Optimization studies by submerged fermentation for production of extracellular glucoamylase from *Paenibacillus amylolyticus* strain NEO03. **(A)** Effect of incubation time **(B)** Effect of initial pH **(C)** Effect of initial temperature (D) Effect of inoculum level.

Table 2
Effect of carbon and nitrogen sources on glucoamylase activity.

Carbon source (1.5%, w/v)	Enzyme activity (U mL-1)
Soluble starch (control)	295.92 ± 0.21[b]
Rice bran	343.41 ± 0.98[a]
Corn starch	230.54 ± 0.74[d]
Potato starch	301.22 ± 0.74[f]
Wheat starch	268.90 ± 0.57[c]
Wheat bran	181.32 ± 0.37[e]
Nitrogen source (1%, w/v)	**Enzyme activity (U mL-1)**
Yeast extract + Peptone (control)	344.15 ± 0.98[a]
Yeast extract	333.79 ± 0.98[b]
Peptone	323.79 ± 0.74[c]
Beef extract	278.52 ± 0.57[d]
NH_4Cl	181.94 ± 0.43[e]
$NaNO_3$	138.77 ± 0.98[f]
Urea	85.85 ± 0.37[g]

Table 3A
Ranges of variables used in RSM for bacterial glucoamylase production under submerged fermentation.

	- α	- 1	0	+1	+ α
A: pH	5.0	6.0	7.0	8.0	9.0
B: Temperature (°C)	23	30	37	44	51
C: C-source (w/v)	0.5	1.0	1.5	2.0	2.5

microorganisms. The inhibitory nature at increasing levels of inoculum was also observed in *Aspergillus* sp. (Ellaiah et al., 2002) and *A. niger* (Imran et al., 2017). The effect of inoculum levels influences enzyme release diversely among microorganisms, and shown to be dependent on the nature of the microbe.

Among all the tested substrates, rice bran was found to be an optimum carbon source for enhancement in enzymatic activity than the commercial soluble starch (Table 2). The use of nitrogen source (yeast extract) was found to be on par with the combination of yeast extract and peptone (Table 2). Therefore, a single nitrogen source was preferred rather than two sources.

The glucoamylase productivity was influenced with different carbon sources and it was crucial to identify the best substrate suitable for elevated enzyme production. Rice bran was found to be an ideal substrate for glucoamylase production, in several fungi especially *Aspergillus* spp. Various studies have evaluated the effect of rice bran in SSF systems in *Aspergillus* sp. and *Rhizopus* sp. (Nyamful et al., 2014) for glucoamylase production. Rice bran was tested for glucoamylase productivity in combination with wheat bran and also with corn flour in *A. niger* (Arasaratnam et al., 1997), and effects of rice bran: wheat bran; rice bran: paddy husk on *A. oryzae* (Puri et al., 2013) have been reported. On the contrary, Ellaiah et al. (2002) investigated different agro-waste substrates on glucoamylase production and showed rice bran produced least enzyme activity.

Starch is a renewable carbohydrate and the largest resource in plants. Starch is a major component in agricultural crops and an important substrate for enzyme production. It is the most basic source of chemical energy for sustenance of life. The potent sources of starch are present in rice, wheat, potato, corn, cassava, yam, grain and tuber crops. Rice is an important cereal in Asia and used as a staple food in most countries. Rice is grown in more than hundred countries across the world and accounts up to about 25% of the food grain production. Rice bran is the outer layer of rice, it is a brownish fragment removed during the process of milling and de-husking to form a fine grain. Rice bran comprises of carbohydrates (50%), proteins (15%), oils (20%) and many micronutrients (Hernandez et al., 2000). A recent study was carried out on isolation of starch from defatted rice bran by a wet-milling process showed 83% recovery of starch containing about 0.66% of protein. Rice bran is a rich source of starch, which was earlier not consumed as food due to high fiber and hull contamination. However, rice bran is now being incorporated in food stuffs as it is identified as a good nutrient and used in many food applications such as in the manufacture of rice bran oil, cereals, cookies, cakes and breads (Prasad et al., 2011).

Table 3B
Central composite design in coded levels with glucoamylase activity as response under submerged fermentation.

Experimental run	Factor 1 (A)	Factor 2 (B)	Factor 3 (C)	Experimental value (U mL-1)	Predicted value (U mL-1)
1	0	0	1.68179	220.92	218.92
2	0	0	0	343.16	344.17
3	0	1.68179	0	186.01	182.6
4	0	0	0	344.39	344.17
5	0	0	0	342.54	344.17
6	0	0	0	349.57	344.17
7	0	0	0	343.04	344.17
8	1.68179	0	0	194.65	196.75
9	0	−1.68179	0	96.58	99.25
10	−1	−1	1	143.95	145.58
11	−1.68179	0	0	200.81	197.97
12	1	−1	1	151.35	149.05
13	1	1	1	167.26	169.37
14	−1	1	−1	231.90	234.72
15	−1	−1	−1	157.64	155.93
16	0	0	0	342.17	344.17
17	1	1	−1	230.91	229.8
18	−1	1	1	209.45	211.79
19	0	0	−1.68179	277.17	278.43
20	1	−1	−1	198.72	196.9

Table 3C
Analysis of variables (ANOVA) for the experimental parameters of response surface methodology affecting glucoamylase production.

Source	Sum of squares	DF	Mean square	F-Value	P-Value	
Model	1.256E+05	9	13951.44	1292.11	< 0.0001	significant
A-pH	1.79	1	1.79	0.1655	0.6927	
B-Temperature	8384.28	1	8384.28	776.51	< 0.0001	
C-C-source	4275.53	1	4275.53	395.98	< 0.0001	
AB	1052.95	1	1052.95	97.52	< 0.0001	
AC	703.13	1	703.13	65.12	< 0.0001	
BC	79.13	1	79.13	7.33	0.0220	
A^2	38824.23	1	38824.23	3595.69	< 0.0001	
B^2	74411.01	1	74411.01	6891.54	< 0.0001	
C^2	16426.36	1	16426.36	1521.32	< 0.0001	
Residual	107.97	10	10.80			
Lack of Fit	69.82	5	13.96	1.83	0.2617	not significant
Pure Error	38.16	5	7.63			
Cor Total	1.257E+05	19				

The nitrogenous source yeast extract is an effective source for bacterial cell growth stimulation, and many glucoamylase producing bacteria reported previously have shown yeast extract as an ideal nutrient at 1% (Punpeng et al., 1992). However, a lower concentration of 0.2% was favourable for *P. subpelliculosa* ABWF-64 and higher concentrations resulted in inhibitory effects (Kumar and Satyanarayana, 2001).

3.3. Evaluation of significant fermentation medium factors by statistical approach

The response surface analysis and CCD were used to evaluate the interaction of the optimized factors of SmF. The factors selected as the most important variables for evaluation were pH, temperature and carbon source substrate. Table 3A shows the coded values of the three independent variables and Table 3B explains the experimental and predicted responses of SmF. The activity from the experimental (342.54 U mL^{-1}) was found to be on par with the predicted values (344.17^{-1}), respectively. The ANOVA of the regression model for glucoamylase production by SmF is summarized in Table 3C. The quadratic model was suggested as the most appropriate process order for further analysis. The factors B, C, AB, AC, A^2, B^2, C^2 were found to be significant, whereas factors A and BC were insignificant as per the quadratic model. The SmF model implied to be statistically significant ($p < 0.0001$) and lack of fit F-value 1.83 was found to be non-significant, suggesting that the model was adequate to predict glucoamylase production. The experimental responses of the co-efficient adjusted response (Adj R^2) 0.9984 was in reasonable agreement with the predicted response (Pred R^2) 0.9953. The F-value of the model was 1292.11.

Thus, the final equation in terms of coded factors for SmF are as follows:

$$\text{Glucoamylase activity} = 344.166 + -0.361712 * A + 24.7775 * B + -17.6937 * C + -11.4725 * AB + -9.375 * AC + -3.145 * BC + -51.9039 * A^2 + -71.8567 * B^2 + -33.7613 * C^2$$

where, A = pH, B = Temperature, C = Carbon source substrate.

The interactive effects of the three variables were analyzed by the 3D and contour graphs in CCD. The graphs were generated by combination of two factors when the third factor was constant, at the midpoint level. The response plot in Fig. 4(A and B) shows the interaction of factors A (pH) and B (temperature), which were significant with a good curvature. The interaction of the factors A (pH) and C (C-source substrate) depicted a slightly flattened curvature Fig. 4(C and D). The response surface plot of factors B (temperature) and C (carbon source substrate) generated good curvatures (Fig. 4E and F). Furthermore, the glucoamylase production was performed with the optimized factors of RSM and thereafter the predicted and experimental results were substantiated.

Most of the findings for glucoamylase production by SmF conditions involves the use of commercial soluble starch as reported for *T. thermosaccharolyticum* ATCC 27384 (Feng et al., 2002) and *B. licheniformis* KIBGE-IB3M67 (Ghani et al., 2013). However, several studies have demonstrated the use of agro-wastes such as wheat bran (Negi and

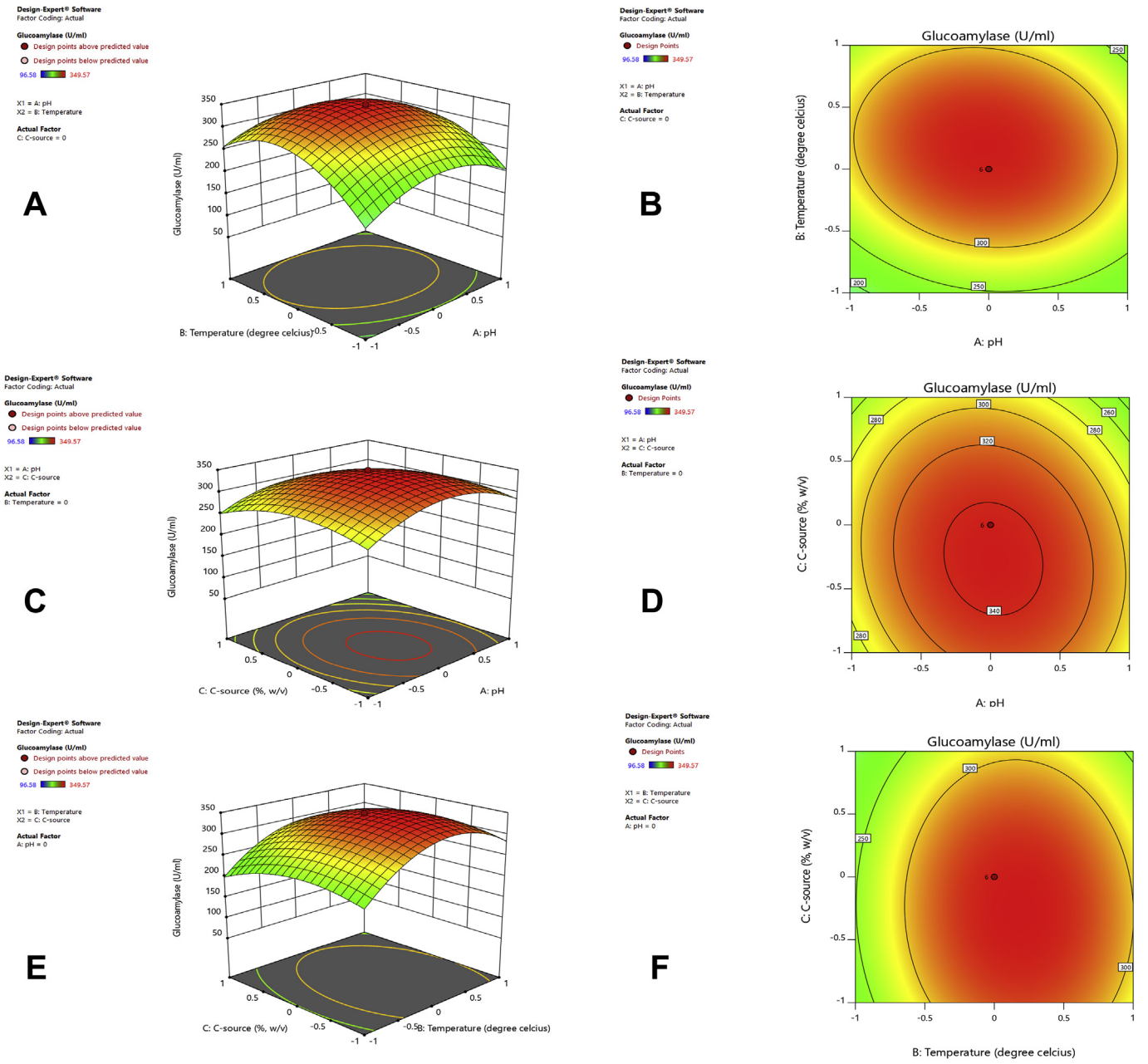

Fig. 4. Response surface plots: 3D and contour graphs of the variable factors (pH, temperature, rice bran) on extracellular glucoamylase production by SmF from *Paenibacillus subtilis* strain NEO03. **(A, B)** Interaction between pH and temperature; **(C, D)** Interaction between pH and carbon source; **(E, F)** Interaction between temperature and carbon source.

Table 4
Raw starch adsorbability of *P. amylolyticus* NEO03 glucoamylase on different starches.

Starch	Relative activity (%)	
	Concentration (1%)	Concentration (5%)
Soluble starch	59.69 ± 0.66	58.59 ± 0.58
Rice bran starch	40.53 ± 0.44	114.83 ± 0.77
Rice flour starch	21.59 ± 0.58	5.65 ± 0.34

Data is represented as are mean ± standard deviation from three replicates.

Banerjee, 2009), food wastes (Wang et al., 2008), food waste hydrolysates (Kiran et al., 2014) and other substrates such as cassava starch with sugarcane bagasse, groundnut shell, garden pea peel (Banerjee and Gosh, 2017) by SSF systems, especially using fungal microorganisms. Thus, the utilization of agro-wastes aids in reduction of production costs and value-added products are produced.

The use of agro-industrial residues in SmF systems are rare. However, some studies have shown the inclusion of solid-substrates in SmF conditions for production of glucoamylases. The amylolytic enzymes acts on various types of starches which differ in the composition of amylose and amylopectin, molecular weight, length and size of the chains. As per some recent reports, the type of nutrient source used in a fermentation medium can impact the enzymic properties of glucoamylase (Pavezzi et al., 2008).

3.4. Raw starch adsorbability and hydrolysis

The adsorbability of the crude enzyme was tested at two different concentrations on the raw starches. The adsorption rate was highest for

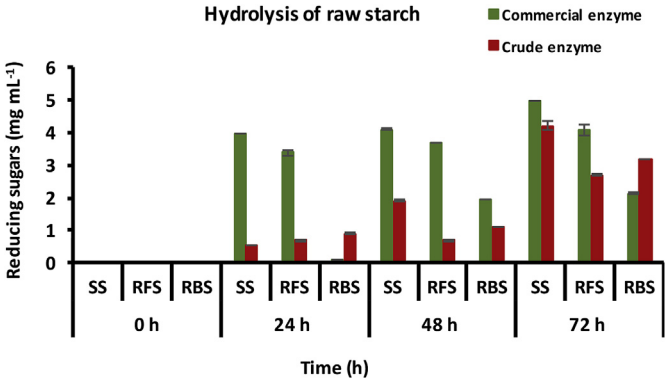

Fig. 5. Comparative analysis of hydrolysis of raw starch substrates (SS: Soluble starch; RFS: Rice flour starch; RBS: Rice bran starch) by commercial and crude glucoamylase enzymes monitored from 0 to 72 h.

rice bran at 5% concentration, however relatively low adsorption rate was observed with rice starch (Table 4). The rate of adsorption increased with increasing concentration of starch, especially for rice bran. The efficiency of crude glucoamylase from *P. amylolyticus* strain was comparatively evaluated with the commercial glucoamylase enzyme. High amounts of reducing sugars were released from all the tested starches at 72 h than 24–48 h of incubation (Fig. 5). The commercial enzyme rapidly hydrolyzed soluble starch followed by rice starch, whereas acted slowly up on rice bran. On the other hand, the crude glucoamylase acted moderately on all the tested starches at 24 and 48 h, whereas at 72 h the enzyme hydrolysis increased. Interestingly, the crude enzyme hydrolyzed rice bran significantly than the commercial glucoamylase.

The results of the raw starch adsorbability and raw starch hydrolysis of the crude glucoamylase were found to be in correlation. The results obtained were in accordance with α-amylase of *B. amyloliquefaciens* (Gangadharan et al., 2009), have showed raw-starch digesting ability. Increase in the adsorption rate by the crude glucoamylase was identical to a purified glucoamylase which exhibited adsorption on to various raw starch granules (cassava, rice, corn and potato) (Xu et al., 2016). Thus, the adsorbability and hydrolysis potential of *P. amylolyticus* glucoamylase indicates the presence of the starch-binding domain which is crucial for enzymatic action.

4. Conclusion

The study demonstrated isolation and screening of a potent bacterial glucoamylase producer from soil. The extracellular glucoamylase from *P. amylolyticus* strain NEO03 was optimized by SmF process using a crucial strategy for enhancement of enzyme production. The use of agro-industrial residues is a common practice for glucoamylase production mostly observed in fungal microorganisms. However, this study has showed the utilization of rice bran as the sole carbon source substrate in the fermentation medium, thereby making the process economically attractive. To the best of our knowledge, the use of industrial wastes in SmF for bacterial glucoamylase production are none. Glucoamylase from *P. amylolyticus* strain NEO03 hydrolyzed the starches considerably, and hydrolyzed rice bran extensively than the commercial enzyme. Thus, the crude glucoamylase exhibits good raw-starch hydrolyzing potential and can be applicable in saccharification industries. Furthermore, the glucoamylase efficiency can be increased by immobilization studies. Also, the purification and biochemical characterization studies can be performed to unravel the enzymic behaviour.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101054.

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