Remarkable improvement of levansucrase production from a newly isolated *Aspergillus niger* MK788296 strain using agro-industrial wastes through statistical optimization techniques

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Background and objective
Microbial levansucrase (LS) is a good source for the production of biologically active fructo-oligosaccharides and levan, which have diverse applications in pharmaceutical industries. Therefore, recent studies have focused on the enhancement of LS production through searching for potent microbial producers and optimization of the fermentation conditions. The present study aimed to use agro-industrial waste as a cost-effective carbon source for LS production and maximize the enzyme yield by optimization of the cultural conditions.
Materials and methods
A potent fungal producer of LS was isolated from an Egyptian soil sample that was collected from Giza Governorate at a depth of 5 cm and identified based on internal transcribed spacer identification and then submitted to the gene bank database. The production of LS by the isolated strain was optimized by evaluating the best fermentation state and agro-industrial waste to be used in the fermentation process. After that, further optimization of culture medium composition was established by two statistical designs: the Plackett–Burman design followed by central composite design.
Results and conclusion
The isolated strain was identified as *Aspergillus niger* MK788296. The first optimization approach declared that using the submerged fermentation technique and utilizing potato peels as the main carbon source led to a 2.4-fold increase in LS production. The statistical optimization resulted in a massive LS production (18870.3 U/ml) with a 59.4-fold increase in enzyme yield than the nonoptimized culture conditions.

Keywords:
agro-industrial wastes, central composite design, levansucrase, Plackett–Burman design, statistical optimization

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Introduction

Levansucrase (LS; E.C.2.4.1.10) is a fructosyltransferase that belongs to the glycoside hydrolase family 68 [1]. LS can be produced by plants, bacteria, and fungi. It is widely distributed in gram-positive and negative bacteria. The most common bacterial sources of this enzyme are Pseudomonas, *Zymomonas*, *Bacillus*, and *Corynebacterium*. Fungal fructosyltransferase can be found in *Aspergillus*, *Penicillium*, and *Fusarium* [2–4]. LS catalyzes three reactions, including hydrolysis, transfructosylation, and polymerization, and according to the acceptor molecule, fructo-oligosaccharides (FOS) or levan polymers were synthesized. FOS and levan have promising applications in the pharmaceutical and food industries due to their several biological activities, including prebiotic, anticancer, and antioxidant activities [5,6].

LS production using *Bacillus* and *Aspergillus* spp. in several studies was carried out by submerged fermentation (SMF), and fewer studies could produce LS by solid-state fermentation (SSF) [1,7–11]. Use of agro-industrial waste as the main carbon source and using statistical factorial designs for optimization of the fermentation medium constituents exert a significant positive effect on microbial enzyme production [12]. Agricultural by-products such as corn cobs, wheat bran, sugarcane bagasse, rice straw, aguamiel, banana peels, coffee by-products, lemon waste, and orange peels were utilized as the main substrates for LS production by many authors [8,13–16]. These wastes are eco-friendly

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and reduce the production cost at the industrial scale. The growing need for FOS and levan in many industrial fields urged many researchers to optimize LS production. The traditional one-factor-at-a-time methods are time consuming; therefore, statistical optimization is preferable for significant enhancement of microbial enzyme production as many variables could be studied with a low number of experiments [17,18]. Several studies focused on the optimization of medium composition for microbial levan production. Meanwhile, fewer studies are concerned with the optimization of the LS [4,19–22]. Response surface methodology was used by Erdal *et al*. [23] to evaluate the best level of initial pH, sucrose concentration, and fermentation temperature for maximum LS production by *Zymomonas mobilis* NRRL B-14023. Box-Behnken design was used by Gonçalves *et al*. [24] to optimize LS production by *Bacillus subtilis* Natto CCT 7712.

Materials and methods

Isolation of microbial strains

Several sources were used for the isolation. The microbial strains used in this study were isolated from environmental samples, including soil (local sample from Giza Governorate), Red Sea water (collected at a depth of 10 m in winter season from Hurghada Governorate, Egypt), sugarcane bagasse, soil-sugarcane bagasse mixture, sugarcane juice, and sugarcane juice-sucrose mixture. Samples (0.1 ml) were collected directly from liquid sources. On the contrary, the solid samples (10 g) were mixed with 100-ml sterile distilled water in a shaker incubator at 200 rpm for 30 min before being spread on both sucrose agar and PDA. The microbial isolates colonies were picked and purified.

Screening for microbial levansucrase producer

Microbial suspensions of the isolated strains were prepared by scratching one slant with 10-ml sterile distilled water. A volume of 1 ml of each microbial suspension was transferred to a sterile 250-ml Erlenmeyer flask containing 2-g crushed lemon peels and 50 ml of the basal nonoptimized medium. The basal medium used was composed of (g/l) yeast extract, 5; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; sucrose, 10; and the initial pH was 7.0 [25]. The flasks were incubated in a shaker incubator at 150 rpm for 7 days for fungi and 48 h for bacteria at 30°C. Cooling centrifugation was performed to get the culture filtrates, which were assayed for LS activity.

Levansucrase assay

The assay of LS activity was established according to the method by Yanase *et al*. [26], where 0.5-ml enzyme solution was mixed with 0.5 ml of 0.2 M acetate buffer (pH 5.2), 0.5 ml of distilled water, and 1 ml of 20% sucrose solution. The mixture was incubated for 15 min at 30°C. The produced sugars were measured via a glucose oxidase kit. One unit of LS activity was defined as the amount of LS that produces 1 μmole fructose per minute.

Identification of the fungal isolate

The fungus with the potent LS production was identified based on morphological characterization and internal transcribed spacer (ITS) identification by Sigma Scientific Services Company, 6th of October city, Egypt. The fungal DNA extraction was established by Quick-DNA Fungal/Bacterial Microprep Kit. The PCR was made using Maxima Hot Start PCR Master Mix, ITS 1 forward primer, and ITS 4 reverse primer [27,28]. The PCR product was cleaned up using GeneJET PCR Purification Kit. The sequencing was made by the use of an ABI 3730xl DNA sequencer. The sequence was aligned with NCBI (National Centre for Biotechnology Information) database using the BLAST tool (https://www.ncbi.nlm.nih.gov), and a phylogenetic tree was constructed using the Phylogeny.fr website.

Preliminary physiological optimization of levansucrase culture conditions

SSF and SMF techniques were used to investigate the LS production utilizing several agro-industrial wastes (lemon peels, potato peels, Molokhia stalks, orange peels, sugarcane bagasse, wheat straw, banana peels, and corn cobs) as the main carbon source. In SSF, a weight of 2 g of each waste was moistened with 10 ml of the basal medium in static conditions, whereas in SMF, the waste was moistened with 50 ml of the basal medium and shaken at 150 rpm.

Statistical optimization of levansucrase culture conditions

Two-step experimental designs were carried out to maximize LS production. The first design [Plackett–Burman design (PBD)] was used to detect the significant medium constituents and production conditions for LS. The second step was central composite design (CCD), which was used to detect the best concentrations for the significant factors determined by the PBD. The design of the two statistical experiments and the analysis and calculations of the results were performed using Design Expert software (version 13) from Stat-Ease Inc.

Plackett–Burman design

The PBD was used to study the qualitative effect of factors that influence LS production [21]. Eleven

factors were selected for the study, and each variable was presented at two levels, high (+1) and low (−1). The total number of experiments was 12 runs based on the rule n+1, where n represents the number of factors under investigation. The 11 factors studied were yeast extract (A), ammonium sulfate (B), sucrose (C), fructose (D), $MgSO_4.7H_2O$ (E), KCl (F), $MnCl_2.4H_2O$ (G), $FeSO_4.7H_2O$ (H), tween 80 (J), plant waste weight (K), and incubation period (L).

The following equation developed by PBD showed the dependence of production of LS by *Aspergillus niger* isolate on the medium constituents:

$$Y\ (\text{LS activity U/ml}) = \beta_0 + \beta_1 * A - \beta_2 * B - \beta_3 * C - \beta_4 * D + \beta_5 * E + \beta_6 * F - \beta_7 * H + \beta_8 * K + \beta_9 * L$$

Where β_0 is the intercept, β1–β9 are the coefficient estimates, and A, B, C, D, E, F, H, K, and L are the independent variables.

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs.

Central composite design

The CCD was used to investigate the quantitative effect of the most suitable significant factors detected by PBD. Five factors were detected in the study. Each variable was presented at five levels. The total number of experiments was 50 runs including eight central points in a quadratic design model. The five factors studied were yeast extract, plant waste (potato peels), KCl, $MgSO_4.7H_2O$, and incubation period.

Results and discussion

Screening and identification of the fungal isolate

A. niger was the most LS promising producer isolate among the isolated strains in the presence of lemon peels as the main carbon source in SMF. It was noticed that the ITS sequence of the isolated strain had 99.05%

Figure 1

Description	Max score	Total score	Query cover	E value	Ident	Accession
Aspergillus niger strain IR3_11 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	756	756	100%	0.0	99.05%	MK461093.1
Aspergillus niger strain IR3_8 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr	756	756	100%	0.0	99.05%	MK461090.1
Aspergillus niger strain IR2_9 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr	756	756	100%	0.0	99.05%	MK461080.1
Aspergillus niger strain IR1_6 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr	756	756	100%	0.0	99.05%	MK461067.1
Aspergillus niger strain IS3_19 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	756	756	100%	0.0	99.05%	MK461062.1
Aspergillus niger strain IS2_18 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	756	756	100%	0.0	99.05%	MK461043.1
Aspergillus niger strain IS1_9 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra	756	756	100%	0.0	99.05%	MK461017.1
Aspergillus niger strain ISR2_14 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and interna	756	756	100%	0.0	99.05%	MK460996.1
Aspergillus niger strain ISK2_14 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal	756	756	100%	0.0	99.05%	MK460961.1
Aspergillus niger strain CR3_6 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	756	756	100%	0.0	99.05%	MK460929.1
Aspergillus niger strain CR2_11 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal	756	756	100%	0.0	99.05%	MK460923.1
Aspergillus niger strain CR1_15 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal	756	756	100%	0.0	99.05%	MK460913.1
Aspergillus niger strain CS3_10 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal	756	756	100%	0.0	99.05%	MK460897.1
Aspergillus niger strain CS2_7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	756	756	100%	0.0	99.05%	MK460883.1
Aspergillus niger strain CS1_25 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal	756	756	100%	0.0	99.05%	MK460878.1
Aspergillus niger strain BIONCLD27 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete s	756	756	100%	0.0	99.05%	MK460263.1
Aspergillus niger isolate LBM 134 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and intern	756	756	100%	0.0	99.05%	MK457457.1
Aspergillus niger isolate RSBL4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complet	756	756	100%	0.0	99.05%	MH220543.1
Aspergillus niger strain yicha19 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequ	756	756	100%	0.0	99.05%	MK421544.1
Aspergillus niger voucher TIP 2018 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	756	756	100%	0.0	99.05%	MK418237.1
Aspergillus niger strain yicha1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete seque	756	756	100%	0.0	99.05%	MK418221.1
Aspergillus niger isolate CBR16-4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete se	756	756	100%	0.0	99.05%	MK397044.1

Similarity between the *Aspergillus niger* isolate and other *A. niger* strains registered in NCBI database.

similarity to more than 20 different *A. niger* strains registered in the NCBI database (Fig. 1).

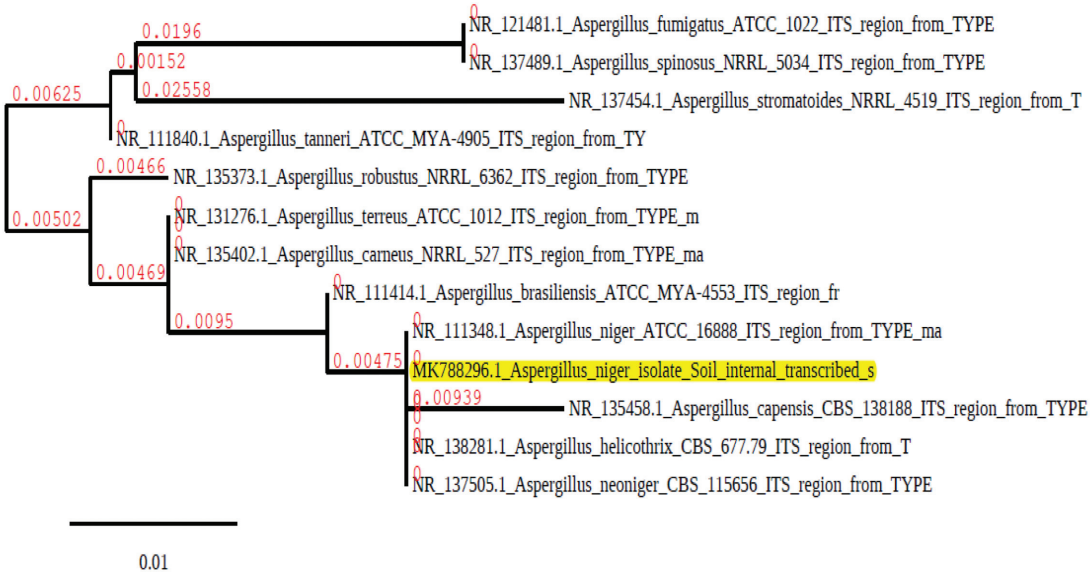
This similarity affirms the isolated strain identification. The partial ITS sequence was submitted to the gene bank database (NCBI) as *A. niger* MK788296. The phylogenetic tree illustrates the relation between *A. niger* isolate and other related strains (Fig. 2). The present work detected a potent fungal source (*A. niger* MK788296) for the microbial production of LS, which is mainly produced by bacteria as reported by Mostafa *et al.* [15] and Charoenwongpaiboon *et al.* [29]. Some studies investigated fructosyltransferase production from fungal strains, especially *A. niger* isolates [9,30]. The presented isolated fungus can be used as a promising source of LS enzyme as it can produce 317.49 U/ml LS in the nonoptimized medium, whereas the maximum LS production from recombinant *Bacillus licheniformis* RN-01 was 65.7 U/ml as reported by Sangmanee et al. [31]. Hamdy *et al.* [32] screened 13 bacterial isolates for LS production, and the best enzyme production was 94.1 U/ml from *B. subtilis* HMNig-2. LS produced by *Pediococcus acidilactici* bacteria showed 12.64 U/ml enzyme activity as illustrated by Youssef *et al.* [33].

Effect of the fermentation state condition and the type of agro-industrial wastes

In the present study, there was a variation in LS production yield depending on the production technique and the agro-industrial wastes used. The preliminary screening of *A. niger* isolate MK788296 in SMF using the basal medium and lemon peels resulted in the production of LS by 317.49 U/ml. On the contrary, SSF resulted in a massive decrease in LS yield by 5.3 folds (60.214 U/ml). This result is similar to observations reported by Prapulla *et al.* [7] and Ganaie *et al.* [34] and in contrast to Sangeetha *et al.* [8], Lateef *et al.* [9] and Dahech *et al.* [1]. The preference of SMF may be due to the need of the microorganism to high moisture environment for enzyme production [35].

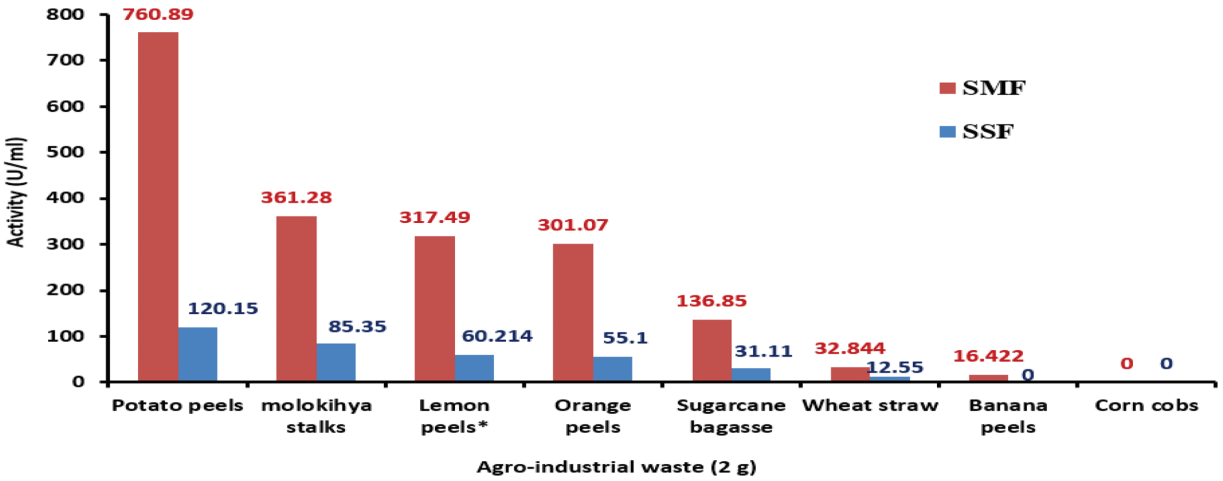
The major studies that used SMF for LS production mainly utilized soluble carbon source (sucrose) as the main substrate. Ganaie *et al.* [36] examined the production of fructosyltransferase by 20 different microorganisms in SMF using sucrose as the sole carbon source, and the highest enzyme activity was 35.64, 33.73, and 31.85 U/ml produced by *A. niger* (SI 19), *Aspergillus flavus* NFCCI 2364, and *Aspergillus terreus* (NFCCI 2347), respectively. Gonçalves *et al.* [24] produced LS from *B. subtilis* natto CCT 7712 in SMF using soluble carbon substrate in the culture medium (sucrose 300 g/l), and the highest activity detected was 8.53 U/ml. Bersaneti *et al.* [10] found that the best LS production (23.9 U/ml) by *B. subtilis* natto was detected in SMF in a medium containing sucrose (420.7 g/l) as the main carbon source. The present study is one of the rare studies that used agro-industrial wastes instead of the soluble carbon source (sucrose) as the main substrate for LS production in SMF as utilizing wastes is more common in SSF as reported by Mostafa *et al.* [15]. Among all the tested wastes in both SSF and SMF, potato peel in SMF was the carbon source with the

Figure 2



Phylogenetic tree of *Aspergillus niger* isolate.

Figure 3



Effect of agro-industrial waste and fermentation state condition on LS production. LS, levansucrase

highest LS productivity (760.89 U/ml) with a 2.4-fold increase than lemon peels under the same conditions (Fig. 3). Utilizing agro-waste instead of sucrose is a better substrate for LS production as it is an eco-friendly way to recycle agro-wastes and at the same time decrease the cost of enzyme production. In the present study, the pronounced increase observed in LS production upon utilizing agro-wastes can be attributed to the favorable environment for the microorganism that supplied by the agro-wastes as they are rich in carbon content, nutrients (protein, pectin, lipid, and polyphenols) and moisture [37,38]. The waste with the highest LS production was potato peel (760.89 U/ml). Meanwhile, Dahech *et al.* [1] used starch as the main carbon source for LS production by *B. licheniformis* in SSF, and the enzyme activity detected was 0.5 U/g. The type of the waste used in the fermentation medium can strongly affect the microbial enzyme yield. The use of potato peels by *A. niger* MK788296 in the present study led to a much better LS production than detected by *A. niger* strain used by Lateef *et al.* [9], which utilized plantain peel and kola nut pod waste. The reason for this difference can be attributed to the high moisture and carbohydrate contents in the potato peels than plantain peel and kola nut pod [39–41]. The fruit wastes can be considered suitable substrates for LS production [14]. Lemon peels and orange peels in the present work exhibited good LS productivities in SMF (317.49 and 301.07 U/ml) and SSF (60.21 and 55.1 U/ml). Meanwhile, banana peel as a substrate helped in the production of 16.4 U/ml LS in SMF and was not suitable for LS production in SSF. Kanakdande *et al.* [14] used banana peels, orange peels, and lemon peels for the production of LS by *Bacillus megaterium* isolate in SSF, and the activity detected was 0.05, 0.1, and 0.65 Umg/ml, respectively. Ganaie *et al.* [42] observed that fructosyltransferase activity produced by *A. flavus* NFCCI 2364 in SSF using banana peels and orange peels as the main carbon source was 102.6 and 31.15 U/gds, respectively. Sugarcane bagasse in the present study produced moderate LS in SMF (136.85 U/ml) relative to the other wastes examined. This result is in agreement with Ganaie *et al.* [42], who used sugarcane bagasse, and the enzyme activity reached up to 176.86 U/gds. On the contrary, Sangeetha *et al.* [8] stated that fructosyltransferase could not be produced by utilizing sugarcane bagasse, whereas corn cobs helped in the production of 7 U/ml enzyme by *Aspergillus oryzae* CFR 202. In the present study, corn cobs were not helpful for LS production in both SMF and SSF.

Statistical optimization by Plackett–Burman design

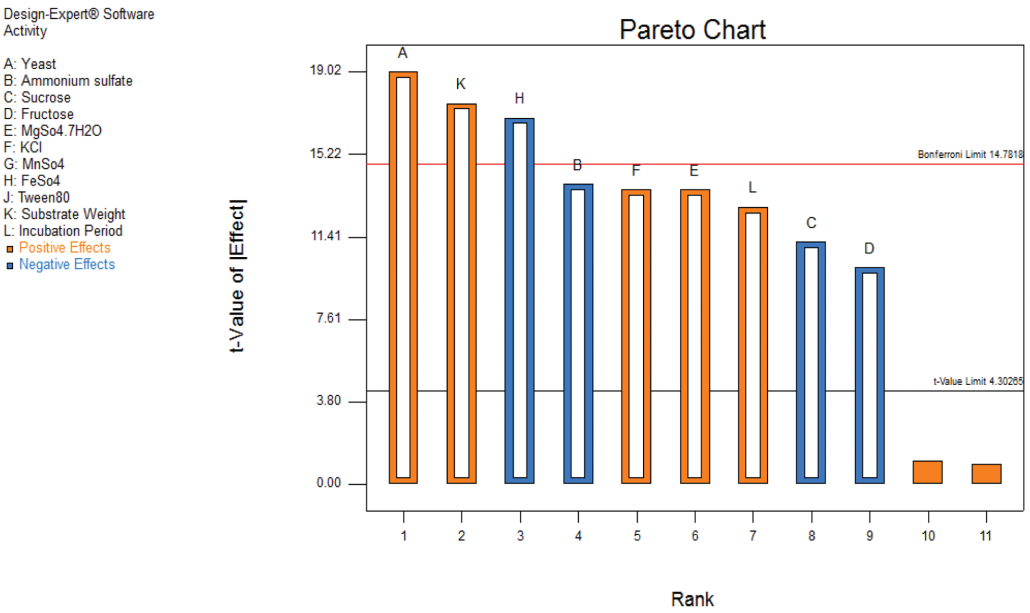
The statistical optimization of LS production was done by PBD followed by CCD. The maximum LS activity obtained after PBD was 1248 U/ml in run 12 with a 3.9-fold increase from the nonoptimized medium. The PBD examined the significance of 11 different variables (including medium constituents and culture conditions) on LS production (Table 1). The significant factors that positively affected the enzyme production were yeast extract, substrate weight, KCl, $MgSO_4.7H_2O$, and incubation period, as their 'Prob>*F*' values were lower than 0.05. Moreover, $FeSO_4.7H_2O$, ammonium sulfate, sucrose, and fructose had a negative effect. However, tween 80 and $MnCl_2.4H_2O$ had no significant effect on enzyme production, as illustrated in the Pareto chart (Fig. 4). The preference of yeast extract over

Table 1 Plackett–Burman design for optimization of levansucrase production

												LS activity (U/ml cf)	
Run numbers	A	B	C	D	E	F	G	H	J	K	L	Experimental	Predicted
1	5	0	5	10	0.25	0.5	0.001	0	1	2	7	1094.8	1081.11
2	10	2	5	10	0.5	0.5	0	0	0	2	4	1149.54	1163.23
3	5	0	10	5	0.5	0.5	0	0.001	1	2	4	843	843.91
4	5	2	10	5	0.5	0.5	0.001	0	0	1	7	821.1	820.19
5	10	0	10	10	0.25	0.5	0.001	0.001	0	1	4	541.93	541.02
6	10	2	5	5	0.25	0.5	0	0.001	1	1	7	815.63	816.54
7	5	2	5	10	0.5	0.25	0.001	0.001	1	1	4	257.28	243.59
8	5	2	10	10	0.25	0.25	0	0.001	0	2	7	306.54	320.23
9	5	0	5	5	0.25	0.25	0	0	0	1	4	602.14	615.83
10	10	0	10	10	0.5	0.25	0	0	1	1	7	947	947.91
11	10	2	10	5	0.25	0.25	0.001	0	1	2	4	788.26	774.57
12	10	0	5	5	0.5	0.25	0.001	0.001	0	2	7	1248.07	1247.16

A, yeast extract; B, ammonium sulfate; C, sucrose; D, fructose; E, $MgSO_4.7H_2O$; F, KCl; G, $MnCl_2.4H_2O$; H, $FeSO_4.7H_2O$; J, tween 80; K, plant waste weight; L, incubation period; LS, levansucrase.

Figure 4



Pareto chart for detection of significant variables.

ammonium sulfate as nitrogen source for LS production can be due to the slow assimilation of nitrogen from organic source than inorganic nitrogen source. Moreover, yeast extract contains certain growth factors, amino acids, and vitamins that enhance microbial enzyme production [43–45]. Similar effect of yeast extract was reported by Ademakinwa *et al.* [46] during statistical optimization for improving fructosyltransferase production from the fungal strain *Aureobasidium pullulans* NAC8. Similar effects of yeast extract, $MgSO_4.7H_2O$, ammonium sulfate, and $FeSO_4.7H_2O$ on LS production were observed by Esawy *et al.* [47] during the statistical optimization of LS production from *B. subtilis* M. However, Esawy *et al.* [48] reported the opposite results for the effects of both $FeSO_4$ and $MgSO_4$ on LS production by *B. subtilis* NRC1aza. In the present study, sucrose exerted a negative effect, which is contrary to the reports stated by both Esawy *et al.* [47] and Esawy *et al.* [48]. The presence of Mn^{2+} ion was insignificant in the present PBD. On the contrary, Esawy *et al.* [47] stated that Mn^{2+} had a positive effect on enzyme production, whereas Esawy *et al.* [48] stated the opposite.

The accuracy and validation of the PBD were affirmed by the closeness between the experimental LS activity values and the activity values predicted by the model (Table 1). The F-value was high (211.4), which exhibited the significance of the design model and indicated that there was only 0.47% probability that

the model is caused by noise as illustrated in analysis of variance results (Table 2). The strength of the model was affirmed by the closeness of the determination coefficient of the model (R^2=0.999) to 1. The proximity of both predicted R^2 (0.962) and adjusted R^2 (0.994) to each other advocated a good correlation between the observed and predicted enzyme activities. The signal to the noise (46.272) ratio was higher than 4, which indicated the adequate signal of the model. The equation in coded factors describing the relation between the 11 factors and LS production was as follows:

Activity=784.608
+130.464×A–94.883×B–76.636×C–68.426×D+93.058×E+93.059×F–115.866×H+120.428×K+87.583×L.

Statistical optimization by central composite design

The CCD detected the optimum concentrations for the five significant factors selected by PBD, as illustrated in Table 3. The optimum concentrations of the significant variables were g/l yeast extract 15, $MgSO_4.7H_2O$ 1.5, KCl 1.5, potato peels weight 5.7 g per flask, and incubation for 12 days. Application of the CCD resulted in a massive increase in LS activity, reaching 18870.3 U/ml (run 50). However, LS activities after statistical optimization of LS production by different *B. subtilis* isolates reached 8.53 U/ml, 1189 AU, and 1250 U/ml as reported by Neeta and Manjusha [11], Gonçalves *et al.* [24], and Mantovan *et al.* [49] respectively. Erdal *et al.* [23] used a response surface methodology statistical design to

Table 2 Analysis of variance results for Plackett–Burman design

Sources	Sum of squares	DF	Mean square	*F* value	*P* value Prob>*F*
Model	1 073 962	9	119 329.1	211.401	0.0047 Significant
Yeast extract	204 250.8	1	204 250.8	361.8466	0.0028
Ammonium sulfate	108 032.3	1	108 032.3	191.3878	0.0052
Sucrose	70 476.61	1	70 476.61	124.8549	0.0079
Fructose	56 185.14	1	56 185.14	99.53645	0.0099
$MgSO_4.7H_2O$	103 916.4	1	103 916.4	184.0962	0.0054
KCl	103 920.1	1	103 920.1	184.1028	0.0054
$FeSO_4.7H_2O$	161 098.7	1	161 098.7	285.3992	0.0035
Substrate weight	174 033.4	1	174 033.4	308.314	0.0032
Incubation period	92 048.33	1	92 048.33	163.071	0.0061
Residual	1128.936	2	564.4679		
Cor total	1 075 091	11			

SD=23.7, mean=784.6, coefficient of variance (%)=3.03, predicted residual sum of squares=40641.

Table 3 Central composite design for optimization of levansucrase production

Run	Yeast (g/l) (A)	Substrate weight (g/flask) (B)	KCl (g/l) (C)	$MgSO_4$ (g/l) (D)	Incubation period (days) (E)	Activity (U/ml)
1	15	4.5	1.5	1.5	12	9100.5
2	10	4	2	1	14	9561.25
3	10	4	1	1	14	5100.15
4	10	5	1	2	10	11 568.39
5	10	5	2	1	10	12 334.75
6	10	5	1	1	10	10 770.28
7	15	4.5	1.5	2.68	12	8046.78
8	15	4.5	1.5	1.5	7	6240.36
9	15	4.5	1.5	1.5	12	9241.94
10	15	4.5	2.68	1.5	12	8028.53
11	15	4.5	1.5	1.5	12	9100.5
12	15	4.5	1.5	1.5	12	9100.5
13	20	4	1	2	14	11 568.39
14	20	5	1	2	10	12 935.25
15	20	4	1	2	10	8767.523
16	20	4	2	1	10	10 373.23
17	15	4.5	1.5	1.5	12	9100.5
18	10	5	2	2	10	10 017.42
19	20	5	1	1	10	14 800.18
20	10	4	2	2	10	4452.187
21	20	4	2	2	10	6120.55
22	20	5	1	2	14	15 374.4
23	15	4.5	1.5	0.31	12	11 988.06

(*Continued*)

Table 3 (*Continued*)

Run	Yeast (g/l) (A)	Substrate weight (g/flask) (B)	KCl (g/l) (C)	$MgSO_4$ (g/l) (D)	Incubation period (days) (E)	Activity (U/ml)
24	20	5	2	2	14	14 706.81
25	3.1	4.5	1.5	1.5	12	5370.15
26	10	4	1	2	14	4141.99
27	20	4	1	1	14	11 477.15
28	10	4	2	2	14	4665.1
29	10	5	2	1	14	11 643.33
30	20	5	2	1	10	13 830.97
31	15	4.5	1.5	1.5	12	9100.5
32	20	4	1	1	10	8368.15
33	15	4.5	0.31	1.5	12	10 911.51
34	20	5	1	1	14	17 681.02
35	15	4.5	1.5	1.5	12	9100.5
36	26.89	4.5	1.5	1.5	12	12 554.8
37	10	4	1	2	10	3270.85
38	20	5	2	1	14	14 553.3
39	10	5	1	1	14	12 188.77
40	10	5	1	2	14	10 181.64
41	20	5	2	2	10	13 835.25
42	20	4	2	2	14	7955.55
43	10	4	1	1	10	5574.36
44	20	4	2	1	14	8000.25
45	15	4.5	1.5	1.5	17	9561.25
46	15	4.5	1.5	1.5	12	9100.5
47	10	5	2	2	14	11 458.91
48	10	4	2	1	10	3421.05
49	15	3.31	1.5	1.5	12	2919.47
50	15	5.69	1.5	1.5	12	18870.3

Table 4 Analysis of variance results for central composite design

Source	Sum of squares	DF	Mean square	*F* value	*P* value (Prob>*F*)
Model	585 793 802.08	5	117 158 760.42	74.39935	<0.0001 significant
Yeast extract	137 189 682.34	1	137 189 682.35	87.11959	<0.0001
Substrate weight	408 396 508.22	1	408 396 508.22	259.3441	<0.0001
KCl	4 330 418.54	1	4 330 418.54	2.749947	0.1044
$MgSO_4$	18 141 888.05	1	18 141 888.05	11.52065	0.0015
Incubation period	17 735 304.92	1	17 735 304.92	11.26245	0.0016
Residual	69 288 041.11	44	1 574 728.21		
Cor total	655 081 843.19	49			

SD=1254, mean=9762.7, coefficient of variance (%)=12.8, predicted residual sum of squares=92 388 599.

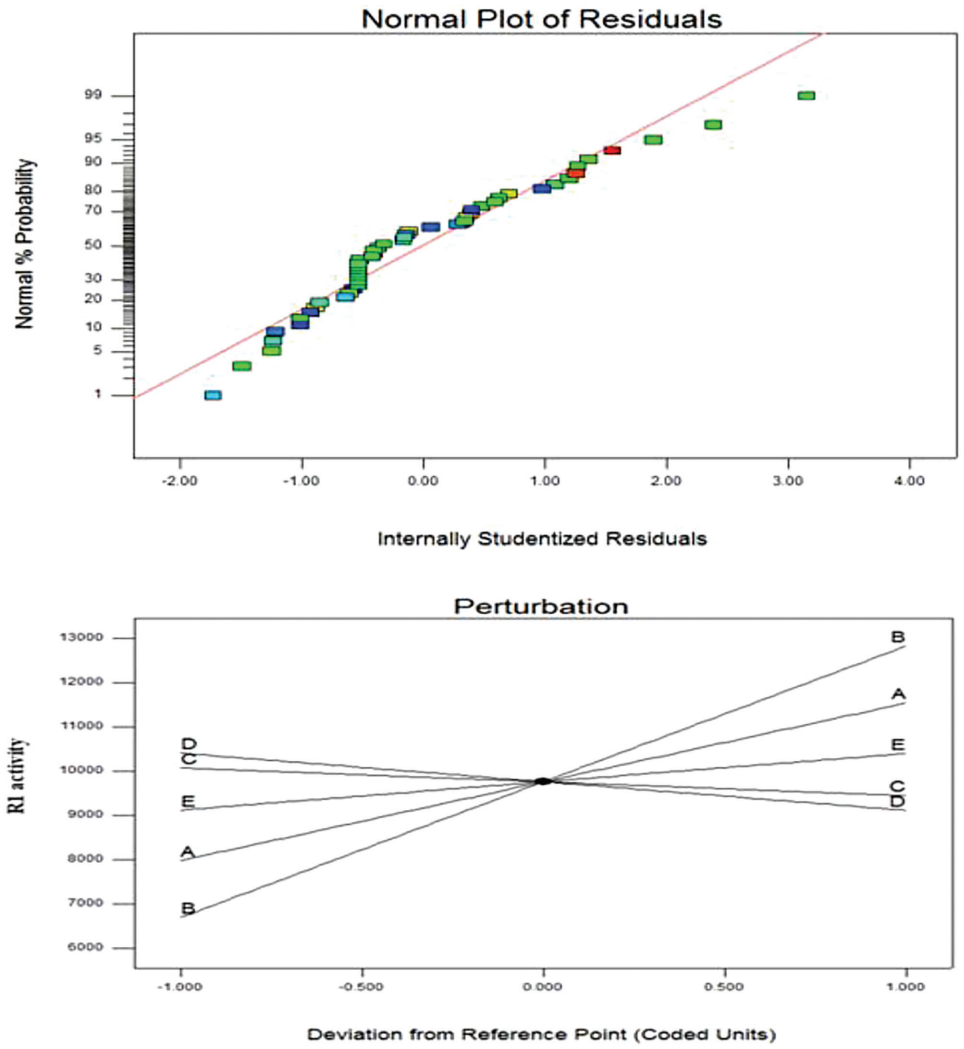
optimize culture conditions for LS production from *Z. mobilis* NRRL B-14023, and the maximum enzyme activity reached was 13.3 U. LS production in the present study was higher than the enzyme produced by the recombinant fructosyltransferase from *A. niger* SG610 (2294.7 U/ml) as investigated by Guo *et al.* [50].

The high *F*-value of the model (74.4) indicated the model significance (Table 4). The closeness of the determination coefficient (R^2=0.894) to 1 demonstrated the strength of the model. The proximity of both predicted R^2 (0.859) and adjusted R^2 (0.882) to each other advocated a good correlation between the experimental and predicted enzyme activities. The adequate precision (signal to the noise ratio=33.6) was higher than 4, which indicated the adequate signal of the model. The predicted LS activity can be estimated by the following coded factor equation:

$$\text{LS activity}\,(\text{U/ml}) = 9762.701 + 1779.705*\text{A} + 3070.636*\text{B} - 316.193*\text{C} - 647.185*\text{D} + 639.892*\text{E}$$

Both the normal plot of residuals and perturbation plot indicated the effectiveness and validity of the model (Fig. 5). The straight line of the normal distribution

Figure 5



Normal plot of residuals and perturbation plot.

plot confirms the normality of the data. The steep deviation from the center point of both yeast extract concentration (A) and potato peel weight (B) in the perturbation plot indicated their significance in the LS production process. The interaction effect of yeast extract and potato peels weight on LS activity at fixed values of other variables was graphically described at the three-dimensional surface and two-dimensional contour plots (Fig. 6). The validation of the CCD model was confirmed by the reproducibility of results and by the proximity among the experimental and predicted LS activities (Fig. 7).

Conclusion

The isolated *A. niger* MK788296 strain was a better LS producer than the commonly used bacteria. Optimization of the culture conditions positively affected the enzyme yield which was declared by using the SMF technique in the presence of potato peels as the main carbon source. Both PBD and CCD designs led to a massive increase in LS productivity from 317.49 U/ml under nonoptimized conditions to 18870.3 U/ml with an overall 59.4-fold increase in LS production. The optimum medium conditions were achieved by using 5.7 g potato peels in 50-ml statistically optimized medium (pH 7) for 12 days at 150 rpm at 30°C. The statistically optimized medium was composed of (g/l) yeast extract, 15; sucrose, 5; fructose, 5; KCl, 1.5; $MgSO_4.7H_2O$, 1.5; $MnCl_2.4H_2O$, 0.001; and $FeSO_4.7H_2O$, 0.001.

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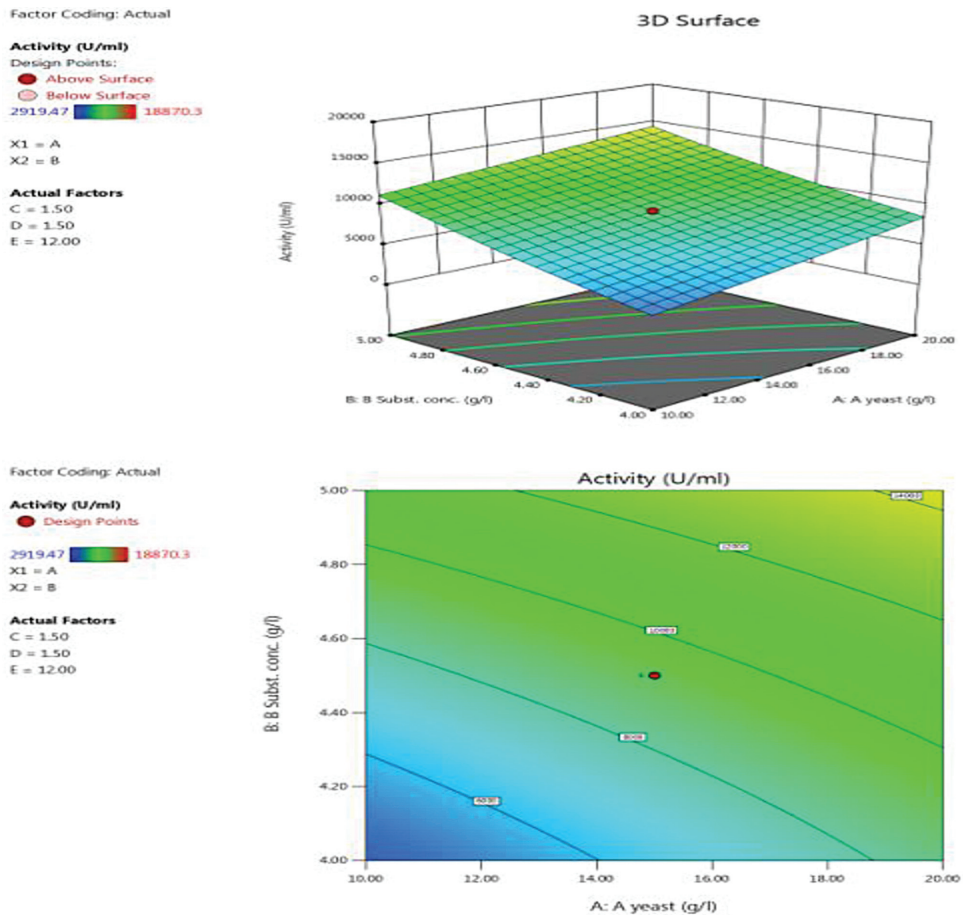
Conflicts of interest

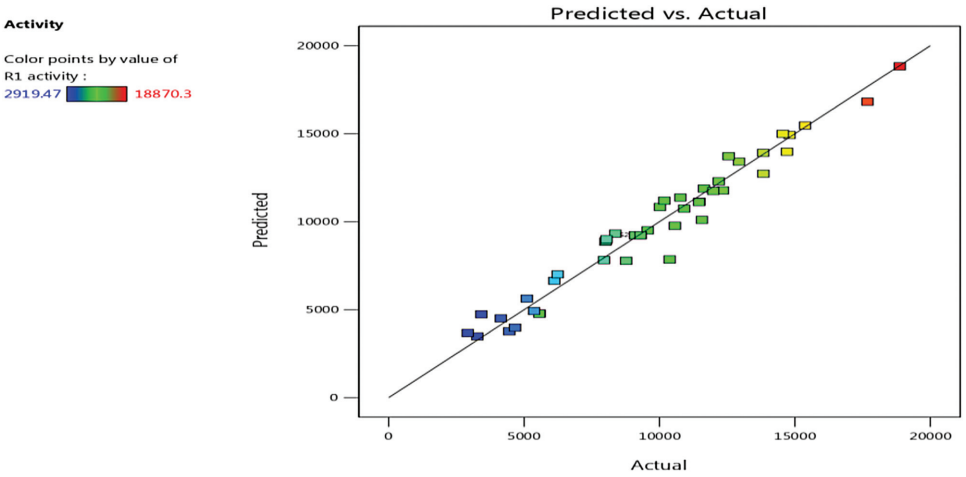
There are no conflicts of interest.

Figure 6



Three-dimensional surface and two-dimensional contour plots for CCD. CCD, central composite design.

Figure 7



Validation of the CCD model. CCD, central composite design.

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