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Statistical Optimization of Industrially Potent *Streptomyces plicatus* MK559563 Xylanase and α-Amylase Production Utilizing Agricultural Wastes

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SOIL isolate *Streptomyces plicatus* MK559563 succeeded in the biological conversion of agricultural wastes (rice straw RS and potato shells PS) into valuable by-products (reducing sugar and industrial enzymes) by sub merged fermentation technique (SmF). *Streptomyces plicatus* MK559563 xylanase and α-amylase production were statistically optimized through two designs Plackett-Burman (PB) and Central composite design (CCD). PB design showed that xylanase production by *S. plicatus* MK559563 was more affected by incubation time, PS weight, and $(NH_4)_2SO_4$ while α-amylase production was more affected by RS weight, $(NH_4)_2SO_4$, and $CaCl_2$. CCD improved *S. plicatus* MK559563 xylanase and α-amylase production by 39.69 and 61.60–fold increase compared to unoptimized media. The optimized media for *S. plicatus* MK559563 xylanase production composed of g/L: Baker's yeast (1), $(NH_4)_2SO_4$ (6.5), KCl (1), $ZnSO_4$ (0.001) with RS (2g/flask) and PS (3.5g/flask) for 7 days of incubation. The optimized media for *S. plicatus* MK559563 α-amylase production composed of g/L: glucose (10), baker's yeast (5), $(NH_4)_2SO_4$ (6.5), $CaCl_2$ (6.5) with RS (3.5g/flask) and PS (1g/flask) for 6 days of incubation. *Streptomyces plicatus* MK559563 can be used as an intermediate step for bioconversion of agricultural wastes into biofuel.

Keywords: α-amylase, Agricultural wastes, Statistical factorial design, *Streptomyces* sp., Xylanase.

Introduction

The continuous and rapid progress in the industrial field led to, first: The accumulation of agro-industrial wastes which accordingly caused serious environmental pollution and their disposal requires high costs, second: The requirement of huge quantities of cheap resources for fuel and industrial enzymes. Therefore, all the efforts are directed today to solve these obstacles by, utilizing the agro-industrial wastes for the production of valuable by-products. Since, Agro-industrial wastes are rich in polymers as lignin, cellulose, hemicelluloses (main xylan), pectin. Moreover, the utilization of microorganisms as tools for the production of industrial enzymes by consumption of agro-industrial wastes as fermentation substrates and this can be achieved by either solid state ferementation (SSF) or sub merged fermentation (SmF) techniques. Enzymes have many applications in industries, such as pharmaceutical, food, drink and textile, leather, and paper industry (Pandey et al., 2000; Sivaramakrishnan et al., 2006).

Hemicelluloses are the second most abundant polysaccharide next to cellulose. Xylan is one of the major components of hemicelluloses found in plant cell walls. Complete xylan degradation requires the combined action of different xylanolytic enzymes. Xylanase (E.C.3.2.1.8) is one of the enzymes used in the biodegradation of xylan. Xylanases have been widely used for clarifying fruit juices and wine (Hang &

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Woodams, 1997; Bhushan et al., 2008; Das et al., 2008; Diaz et al., 2011; Ahmed et al., 2016), with cellulases in food processing and textile industry (Biely, 1985; Abd El Aty et al., 2018), bleaching in the paper and pulp industry (Daneault et al., 1994; Viikari et al., 1994; Polizeli et al., 2005), and hydrolyzing agricultural waste to produce renewable energy products in the biofuel industry (Verma et al., 2010; Wang et al., 2011). Xylanases are also utilized for the production of rayon, cellophane and several chemicals including cellulose esters (acetates, nitrates, propionates and butyrates) and cellulose ethers (carboxymethyl cellulose and methyl and ethyl cellulose), which are all produced by dissolving pulp and purifying fibers from other carbohydrates (Subramaniyan & Prema, 2002). The xylose resulting from xylan hydrolysis can be converted into xylitol, a valuable sweetener that has applications in both the pharmaceutical and food industries (Parajó et al., 1998; Soleimani et al., 2006). Microbial xylanases are the preferred catalysts for xylan hydrolysis, due to their high specificity, mild reaction conditions, negligible substrate loss and side product generation.

α-amylases are extracellular enzymes that catalyze the hydrolysis of α-1,4-glycosidic linkages in starch liberating linear and branched oligosaccharides of varying chain lengths as well as glucose (Sharma & Satyanarayana, 2013). α-amylase fins extensive applications in industries like food, brewing, detergents, paper, textile, and dyeing (Abd El Aty et al., 2018). The enzyme is important from a clinical point of view to diagnose pancreatitis. Microbial amylase is more preferred for industrial purposes due to large scale production at a lower cost and in less time.

Various genera of actinomycetes have been reported to produce a wide array of potential industrial enzymes that can be used in biotechnological applications and biomedical fields (Nawani et al., 2013).

Actinobacteria are well known for their productive activities in nutrient recycling by the degradation of chitin, cellulose, starch, lipids, and complex carbohydrates and flouting them into simple sugars by the secretion of various kinds of hydrolytic enzymes in the rhizosphere (Vurukonda et al*.,* 2018). Genus *Streptomyces* possess a capacity to produce and secrete a variety of extracellular hydrolytic enzymes (Sathya & Ushadevi, 2014), so it plays a significant role in the recycling of nutrients and involve in the primary degradation of organic matter in compost and related materials (Sykes & Skinner, 1973), it is considered as one of the most important bacteria, due to their ability to develop the soil properties as well as producing several extracellular substances (enzymes) as secondary products such as α-amylases, cellulases, proteases, chitinases, xylanases and pectinase (Brzezinski et al.,1999; Syed et al., 2009; Mohamed et al., 2013; Mohamed et al., 2014).

Microbial enzyme production is usually done by two possible techniques, solid-state and submerged techniques. Nowadays, the submerged cultivation is more favorable due to the ease of sterilization. Optimization of the medium by changing one independent variable keeping the other factors constant has disadvantages as time-consuming and incapable of detecting the most effective factors (Liu & Tzeng, 1998). While Statistical experimental designs including Plackett-Burmanand response surface methodologies (RSM) can overcome the disadvantages of the classical method. Therefore, the Plackett-Burman design (Plackett & Burman, 1946) is a powerful statistical technique has been widely used for detecting the most effective medium components for fermentation optimization (Cui et al., 2010; Periyasamy et al., 2010; Chen et al., 2011; Salihu et al., 2011).

For all the mentioned above, this study focused on the utilization of isolated actinomycetes for the conversion of agro-industrial wastes into industrially valuable byproducts (α-amylase, xylanase, and reducing sugar). Besides, optimizing and investigating the most critical factors affecting the production of α-amylase and xylanase by two steps factorial design (Plackett-Burman and central composite design).

Materials and Methods

Collection of soil samples

Soil samples were collected from El- Boyette village- El-What and were air-dried on the benches for one week. Streptomycete isolates were collected from soil samples by serial dilution method (Hayakawa & Nonomura, 1987) using starch nitrate medium of the following composition (g/L): starch, 20; KNO_3, 2; K_2HPO_4, 1; $MgSO_4.7H_2O$, 0.5; NaCl, 0.5; $CaCO_3$, 3; $FeSO_4$

$7H_2O$, 0.01; agar, 20 .0.1ml inoculum of the appropriate dilution was plated on each plate. The plates were incubated at 28°C for 7 days. After the incubation period the plates were examined for typical streptomyces colonies. *Streptomyces* form typical round, small, opaque, compact, frequently pigmented (white, brown, gray pink or other colors) and appear dull looking (Bernard, 2007). Streptomycetes colonies were recognized on the basis of morphological characteristics by light microscopy (G X10). Representative colonies were selected and streaked on new plates of starch nitrate agar medium. Agar plates were inoculated with the strains, incubated at 28°C until good growth was observed. The isolates were identified as species belonging to the genus *Streptomyces* by analyzing their morphological characteristics. The isolates were identified morphologically to the genus level by comparing the morphology of spore bearing hyphae with entire sporechain as described in Bergey's Manual (Locci, 1989).

Waste samples

Waste samples potato shells (PS), banana peels (BP),and rice straw (RS) were collected, dried in an oven at 50°C for 24hrs, grinded in a laboratory grinder to ~0.5cm particle size.

Production of xylanase and α-amylase by submerged fermentation (SmF)

The purified streptomycetes slant surface were scratched using purified needle after adding 10ml pure distilled water in each streptomycete slants then transfer 2ml from each slant into sterile waste flasks prepared by weighting 2g from each dried waste and put them in sterile 250ml conical flasks containing 50ml sterile distilled water. All flasks containing wastes were autoclaved at 121°C for 20min. After inoculation, the flasks were incubated in a rotary incubator at 28°C for 7 days. At the end of the fermentation period each flask, was filtered and the resulted culture filtrate was centrifuged at 5,000rpm for 10min. The supernatant was used as enzymes source.

α-amylase assay

This was done according to Sajjad & Choudhry (2012) by mixing 0.5ml of clear culture filtrate (crude enzyme) and 0.5ml of 1% soluble starch (0.05M acetate buffer, pH 5.0). The mixture was incubated for 20min at 40°C and the released reducing sugars were determined by the Somogyi method (Somogyi, 1952). One unit of α-amylase (U) was defined as the amount of enzyme which librated 1µmol of glucose per min under the assay conditions.

Xylanase assay

This was done according to Warzywoda et al. (1983) by mixing 0.5ml of clear culture filtrateand 0.5ml of 1% xylan (0.05M acetate buffer, pH 5.00) and incubating for 30min at 50°C followed by measuring the released reducing sugars by Somogyi method (Somogyi, 1952). One unit of xylanase was defined as the amount of enzyme that liberates 1µmol of xylose equivalents per minute under assay conditions.

Molecular identification of streptomyces isolates

The genomic DNA was extracted from the cultures of streptomycete isolate by using a kit (MicroSeq 500 16S rDNA bacterial identification Kits) according to the manufacturer's instructions. Amplification of 16S rDNA by PCR was done using universal bacterial primer forward F (5`-CGGGCGGTGTGTAC -3´) and reverse R (5`-CAGCCGCGGTAATAC -3´) which amplify a ~800-bp. Amplification was carried out in a final volume of 50µl containing; PCR buffer (1x), *Taq* DNA polymerase (2.5 U), dNTPs (4mM), primers (0.4µM), and template DNA (4ng) with 100 bp ladder DNA marker. The thermal cycle (PCR) steps were applied as follows; 5min initial denaturation at 95° C, followed by 30 cycles of 1min denaturation at 95°C, 1min primer annealing at 55°C, 1min extension at 72°C, and a final 10min extension at 72°C. The amplified DNA fragment was separated on 1% (w/v) agarose gel electrophoresis, using TBE buffer containing ethidium bromide (1µg/ml). A single ~800 bp DNA fragment was cut and extracted from the gel, using a Core Bio-Gel Extraction Kit. The sequence was determined by the Cinna Gen Company.

Sequence data of partial 16S rDNA were aligned and analyzed for finding the closest homologous bacteria. The 16S rRNA nucleotide sequence was compared to nucleotide databases using the BLASTN program that is available from the National Center for Biotechnology Information (NCBI) and retrieved aligned using GeneDoc software version 2.6.002.

Statistical optimization of xylanase and α-amylase production

Plackett-Burman design (PB)

In this design we investigated the effect

of eleven factor on xylanase and α-amylase production including A: RS weight (g/flask), B: PS weight (g), C: Incubation time (hrs), D: Glucose, E: Lactose (g/L), F: Baker's yeast (g/L), G: $(NH_4)_2SO_4$ (g/L), H: $CaCl_2$ (g/L), J: KCl (g/L), K: $ZnSO_4$(g/L), L: $CuSO_4$ (g/L). Each of these factors was studied with low level (−1) and high level (+1) resulted in 12 trials. The success of the design was statistically analyzed by (ANOVA).

Central composite design (CCD)

In this design we studied the quantitative effect of the three most effective factors determined from the Plackett-Burman design including, (A) incubation time and (B) PS weight and (C) $(NH_4)_2SO_4$ for xylanase and, (A) RS weight, (B) $(NH_4)_2SO_4$ and (C) $CaCl_2$ for α-amylase. Variables were investigated with five levels, -1.682, -1, 0 and +1, +1.682 giving 20 trial. The success of the design was statistically analyzed by ANOVA.

Results and Discussion

Screening of actinomycetes isolates for xylanase and α-amylase production

As shown in Fig. 1, first, there was great variation in the ability of the tested isolates for the production of both enzymes according to the utilized agricultural waste. Second, not all the tested isolated were able to produce both enzymes (xylanase and α-amylase), i.e. all the isolates except isolate 11 were able to produce xylanase on all agricultural wastes while only isolate 5 was able to produce both enzymes on RS. Third, the highest xylanase and α-amylase production (1.02 and 0.55 U/ml, respectively) by isolate number 5. The variation in the ability of tested isolates to utilize the different agricultural wastes for enzyme production may be due to the variation in the availability and percent of xylan and starch in the used agricultural waste.

It can be observed that the highest xylanase and α-amylase production were obtained on utilizing rice straw. This may be due to that cereals such as rice and corn have a crystalline structure A, while tubers such as potatoes and cassava have a crystalline structure B which has higher water content and a relatively long chain of amylopectin which makes starch more resistant to enzyme attacks (Puspitasari et al., 2011).

Molecular identification of streptomycete isolates by 16S rRNA gene sequence analysis

The amplified DNA products separated on agarose gel electrophoresis revealed a single band of ~800 bp. PCR sequences of streptomycete isolate were compared with the other sequenced streptomycetes in the NCBI and the database showed similarity with some sequences of other streptomycetes upon the molecular characterization of 16S rDNA gene sequence. The sequence data indicated that isolate 5 showed 99% similarity with *Streptomyces plicatus*. The DNA sequences were published in the NCBI databases under the following specific accession numbers MK559563 (Fig. 2).

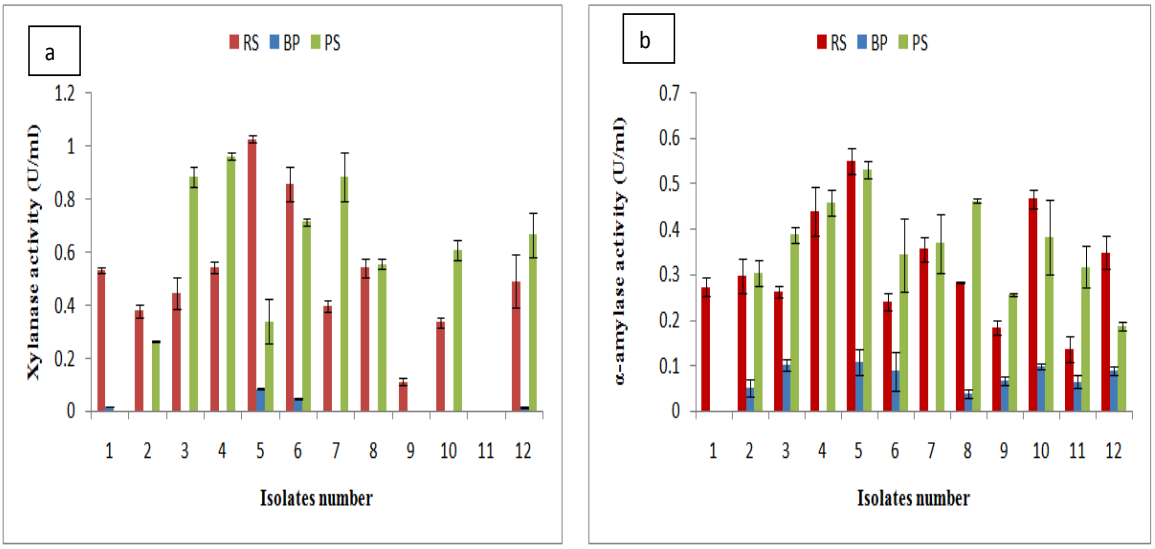

Fig. 1. Screening of different actinomycetes on different agricultural wastes for, a: Xylanase production; b: α-amylase production

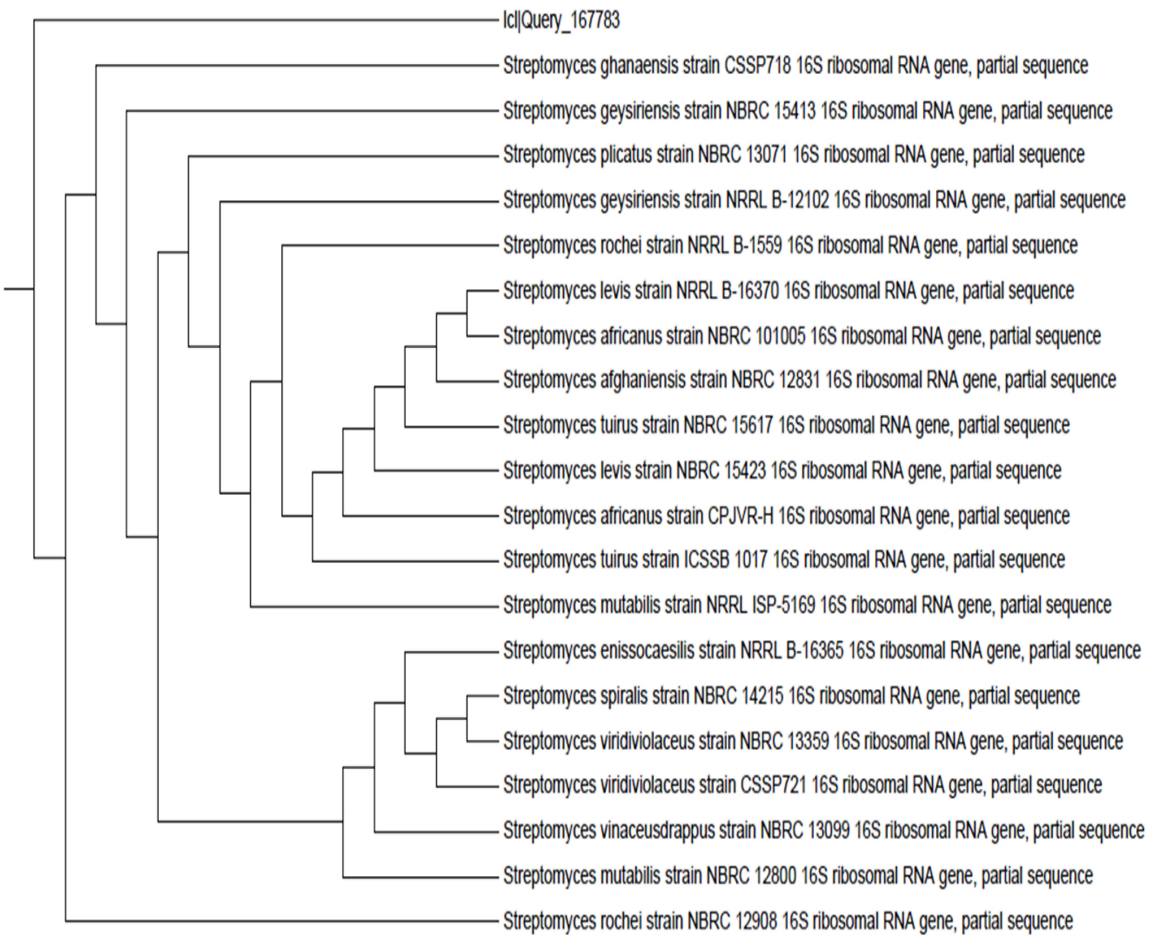

Fig. 2. Phylogenetic tree of *Streptomyces plicatus* MK559563.

Statistical optimization of S.plicatus MK559563 xylanase and α-amylase production

Plackett- Burman design (PB)

In this step as shown in Table 1, the qualitative testing of a different variable resulted in remarkable variation in both enzyme production. i.e. for xylanase and α-amylase production, the highest production was obtained in trial 11 (17.74U/ml) causing 17.32 -fold increase, and 6 (10.71U/ml) causing 19.49 -fold increase, respectively.

Xylanase and α-amylase activitiescan be calculated from the following equations

Xylanase activity (U/ml)=+7.21 +0.79 * PS weight +4.98 * incubation time -1.38 * glucose -0.58 * lactose +0.86 * $(NH_4)_2SO_4$ -0.81 * $CaCl_2$ +0.22 *KCl +0.35 * $ZnSO_4$ -0.64 * $CuSO_4$

α-amylase activity =+5.92 +1.38 * RS weight +0.34 * PS weight -0.81 * lactose +0.40 * baker's yeast +0.97 * $(NH_4)_2SO_4$ +0.83* $CaCl_2$ -0.61 * $CuSO_4$

From the Pareto chart (Fig. 3a, b) it can be observed that the different variables effect on production differed according to the enzyme. *i.e.*for xylanase production,it can be observed that among the tested 11 variables only 9 variables exerted significant effect distinguished into 5 positive variables that exerted enhancement effect (incubation time, $(NH_4)_2SO_4$, PS weight, $ZnSO_4$, and KCl) and 4 negative variables (glucose, lactose, $CaCl_2$, and $CuSO_4$). Also, the effect of the nutrients differed from microorganism to another. E.g. in our study $(NH_4)_2SO_4$ exerted positive and $CaCl_2$ negative effects on xylanase production similar to that reported by Ali et al. (2016) and in contrast to that reported by Cui & Zhao (2012) for *Penicillium*sp.WX-Z1 xylanase production.

TABLE 1. Plackett- Burman design for *Streptomyces plicatus* MK559563 xylanase and α-amylase production

Run	Factor 1 A:RS weight g/flask	Factor 2 B:PS weight g/flask	Factor 3 C: incubation time day	Factor 4 D: glucose g/L	Factor 5 E:lactose g/L	Factor 6 F:bakers yeast g/L	Factor 7 G:NH_4SO_4 g/L	Factor 8 H:$CaCl_2$ g/L	Factor 9J:KCl g/L	Factor 10 K:$ZnSO_4$ g/L	Factor 11 L:$CuSO_4$ g/L	xylanase activity U/ml	α-amylase activity U/ml
1	1	1	4	10	0	5	5	0	1	0.001	0.001	2.32±0.15	4.41±0.15
2	2	2	4	0	0	5	1	1	1	0	0.001	2.52±0.12	8.34±0.33
3	2	1	4	0	10	1	5	1	0	0.001	0.001	1.71±0.09	7.19±0.75
4	1	2	4	10	10	1	5	1	1	0	0	1.65±0.09	5.95±0.45
5	1	1	4	0	0	1	1	0	0	0	0	3.47±0.2	4.07±0.25
6	2	1	6	10	0	5	5	1	0	0	0	10.68±0.25	10.71±0.99
7	2	2	4	10	10	5	1	0	0	0.001	0	1.76±0.09	5.92±0.46
8	1	2	6	10	0	1	1	1	0	0.001	0.001	10.05±0.12	4.30±0.28
9	1	1	6	0	10	5	1	1	1	0.001	0	11.79±0.09	3.99±0.18
10	2	1	6	10	10	1	1	0	1	0	0.001	8.56±0.35	3.09±0.27
11	2	2	6	0	0	1	5	0	1	0.001	0	17.74±0.88	8.54±0.75
12	1	2	6	0	10	5	5	0	0	0	0.001	14.31±0.28	4.53±0.35

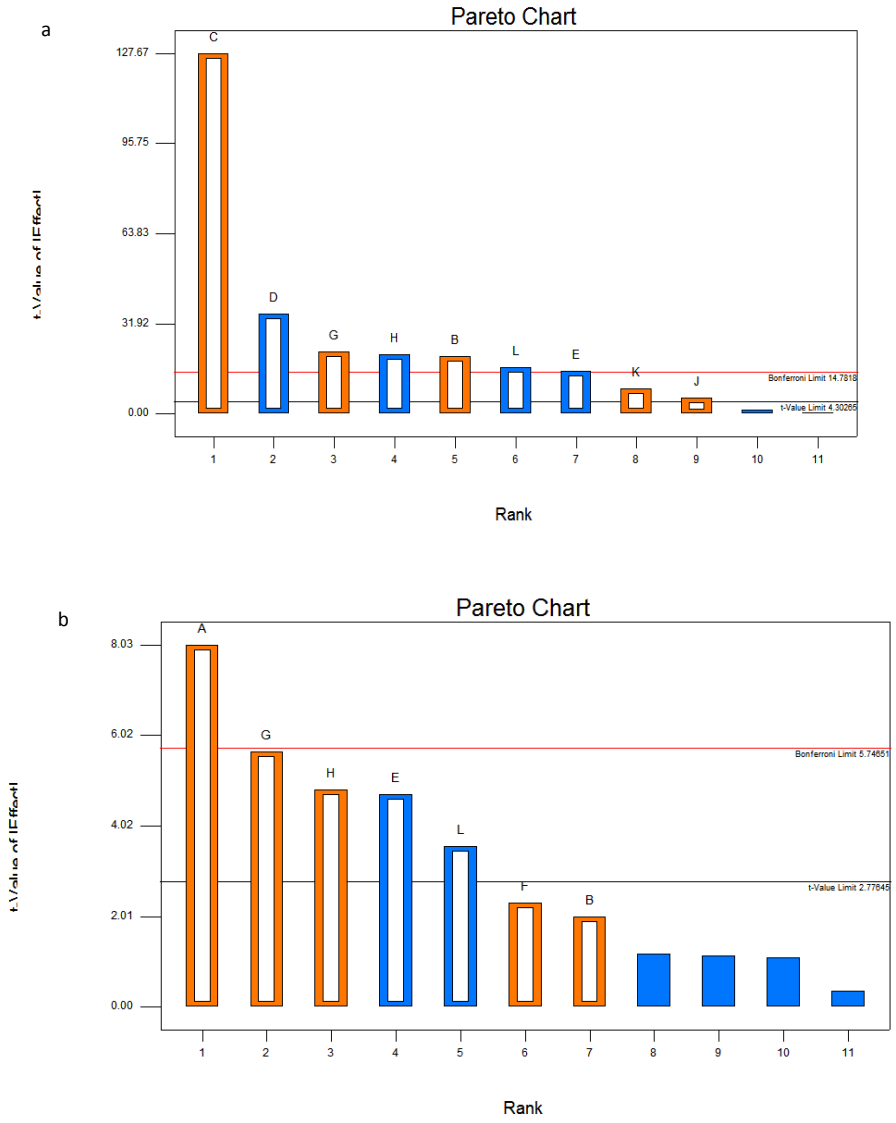

Fig. 3. Pareto chart showing significant variables affecting enzyme production (a, xylanase enzyme production; b, α-amylase production)

While for α-amylase production only 7 variables exerted a significant effect on enzyme production distinguished into 5 positive variables that exerted enhancement effect (RS weight, $(NH_4)_2SO_4$, $CaCl_2$, baker's yeast, and PS weight) and 2 negative variables (lactose, and $CuSO_4$). The enhancement effect of $CaCl_2$ on α-amylase production was also reported by Balkan et al. (2011) and Bekler et al. (2019). While, Retnaningrum & Purwestri (2016) and Hazaa et al. (2018) found α-amylase production was enhanced by the addition of, starch and yeast extract as carbon and nitrogen sources, maltose followed by lactose, respectively. In contrast to our study, Hassan & Abd Karim (2015) reported the enhancement effect of glucose on *Bacillus subtilis* α-amylase production.

The success of the design for *Streptomyces plicatus* MK559563 xylanase and α-amylase production was analyzed by ANOVA as shown in Table 2. Moreover, the success of the design was emphasized by some parameters included R^2, adj R^2, and pred R^2 which were 0.999, 0.9994, and 0.9963, respectively for xylanase and 0.9762, 0.9345, and 0.7855, respectively for α-amylase. As observed the predicated R^2 value for both enzymes wa sin reasonable agreement with the adjective R^2.

TABLE 2a. Analysis of variance (ANOVA) for PB designfor *Streptomyces plicatus* MK559563 xylanase production

Source	Sum of squares	df	Mean square	F value	P value Prob>F	
Model	355.0713223	9	39.45236915	2164.138	0.0005	Significant
B-PS weight	7.513094584	1	7.513094584	412.1267	0.0024	
C-incubation time	297.1331044	1	297.1331044	16299.08	< 0.0001	
D-glucose	22.73241851	1	22.73241851	1246.975	0.0008	
E-lactose	4.093018081	1	4.093018081	224.5203	0.0044	
G-$(NH_4)_2SO_4$	8.80306132	1	8.80306132	482.8872	0.0021	
H-$CaCl_2$	7.925433548	1	7.925433548	434.7454	0.0023	
J-KCl	0.562429795	1	0.562429795	30.85178	0.0309	
K-$ZnSO_4$	1.458585799	1	1.458585799	80.00993	0.0123	
L-$CuSO_4$	4.850176308	1	4.850176308	266.0538	0.0037	
Residual	0.036460117	2	0.018230059			
Cor Total	355.1077824	11				

R^2 0.999, adj R^2 0.9994 and pred R^2 0.9963

TABLE 2b. Analysis of variance (ANOVA) for PB design for *Streptomyces plicatus* MK559563 α-amylase production

Source	Sum of squares	df	Mean square	F value	P value Prob>F	
Model	57.90416539	7	8.272024	23.40377	0.0043	Significant
A-RS weight	22.79683289	1	22.79683	64.49834	0.0013	
B-PS weight	1.406504147	1	1.406504	3.979376	0.1168	
E-lactose	7.836676057	1	7.836676	22.17205	0.0092	
F-baker's yeast	1.880060558	1	1.880061	5.319194	0.0824	
G-$(NH_4)_2SO_4$	11.28021439	1	11.28021	31.91474	0.0048	
H-CaCl	8.219541748	1	8.219542	23.25528	0.0085	
L-$CuSO_4$	4.484335598	1	4.484336	12.68738	0.0235	
Residual	1.413793517	4	0.353448			
Cor Total	59.31795891	11				

R^2 0.9762, Pred $R^2$0.9345 and adj R^2 0.7855

Central composite design (CCD) for S. plicatus MK559563 xylanase and α-amylase production

CCD for S.plicatus MK559563 xylanase production

In this step, the quantitative effect of the most positive three significant factors incubation time, $(NH_4)_2SO_4$ and PS weight as determined from PB design was tested. As shown in Table 3a, the highest *Streptomyces plicatus* MK559563 xylanase production 40.65U/ml was obtained in the central trials (2, 11, 12, 13, 14, and 18) causing a 2.30- fold increase in xylanase production compared to the previous step in optimization. Xylanase activity can be calculated from the following equation:

Xylanase activity = +40.73 +1.84* incubation time +2.13 * $(NH_4)_2SO_4$-2.16 * PS weight +0.23* incubation time * $(NH_4)_2SO_4$ -2.97 * incubation time *PS weight -0.075* $(NH_4)_2SO_4$ *PS weight -5.07 * incubation time ^{2} -9.33 * $(NH_4)_2SO_4{}^2$ -5.32 * PS weight ^{2}

The interaction between the three variables was shown in Fig. 4a, b, and c. The success of the design was emphasized by statistical analysis (ANOVA) as shown in Table 3b. The model F-value of 91.49 implied the model was significant. Moreover, The values of R^2 (0.9880) means that 98.80 of the results can be explained by the design, and pred R^2 0.9087 was in reasonable agreement with the adj R^2 0.9772.

TABLE 3a.CCD for *Streptomyces plicatus* MK559563 xylanase production

Run	Factor 1 A: incubation time days	Factor 2 B:PS weight g/flask	Factor 3 C: $(NH_4)_2SO_4$ g/l	Xylanase activity U/ml	
				Actual	Predicated
1	10	2	10	14.87	15.44
2	7	3.5	6.5	40.65	40.73
3	7	3.5	0.61	30.66	29.32
4	4	5	10	20.85	21.81
5	7	6.02	6.5	19.15	17.92
6	10	5	10	19	20.01
7	7	0.98	6.5	12.44	10.74
8	12.04	3.5	6.5	30.25	29.49
9	4	2	3	15.33	16.38
10	10	2	3	24.44	25.54
11	7	3.5	6.5	40.65	40.73
12	7	3.5	6.5	40.65	40.73
13	7	3.5	6.5	40.65	40.73
14	7	3.5	6.5	40.65	40.73
15	1.95	3.5	6.5	25.45	23.29
16	7	3.5	12.39	23.66	22.07
17	10	5	3	30.15	30.42
18	7	3.5	6.5	40.65	40.73
19	4	2	10	16.35	18.15
20	4	5	3	18.85	20.34

TABLE 3b. Analysis of variance (ANOVA) for CCD for *S.plicatus* MK559563 xylanase production

Source	Sum of squares	df	Mean square	F value	P value Prob>F	
A- incubation time	46.32498	1	46.32498	19.21638	0.0014	Significant
B-PS weight	62.19742	1	62.19742	25.80053	0.0005	
C- $(NH_4)_2SO_4$	63.60405	1	63.60405	26.38402	0.0004	
AB	0.41405	1	0.41405	0.171755	0.6873	
AC	70.44845	1	70.44845	29.2232	0.0003	
BC	0.045	1	0.045	0.018667	0.8940	
A^2	370.6616	1	370.6616	153.7566	< 0.0001	
B^2	1255.454	1	1255.454	520.7835	< 0.0001	
C^2	407.1786	1	407.1786	168.9045	< 0.0001	
Residual	24.10703	10	2.410703			
Lack of Fit	24.10703	5	4.821406			
Pure Error	0	5	0			
Cor Total	2009.106	19				

R^2 0.9880, Adj R^2 0.9772, Pred R^2 0.9087

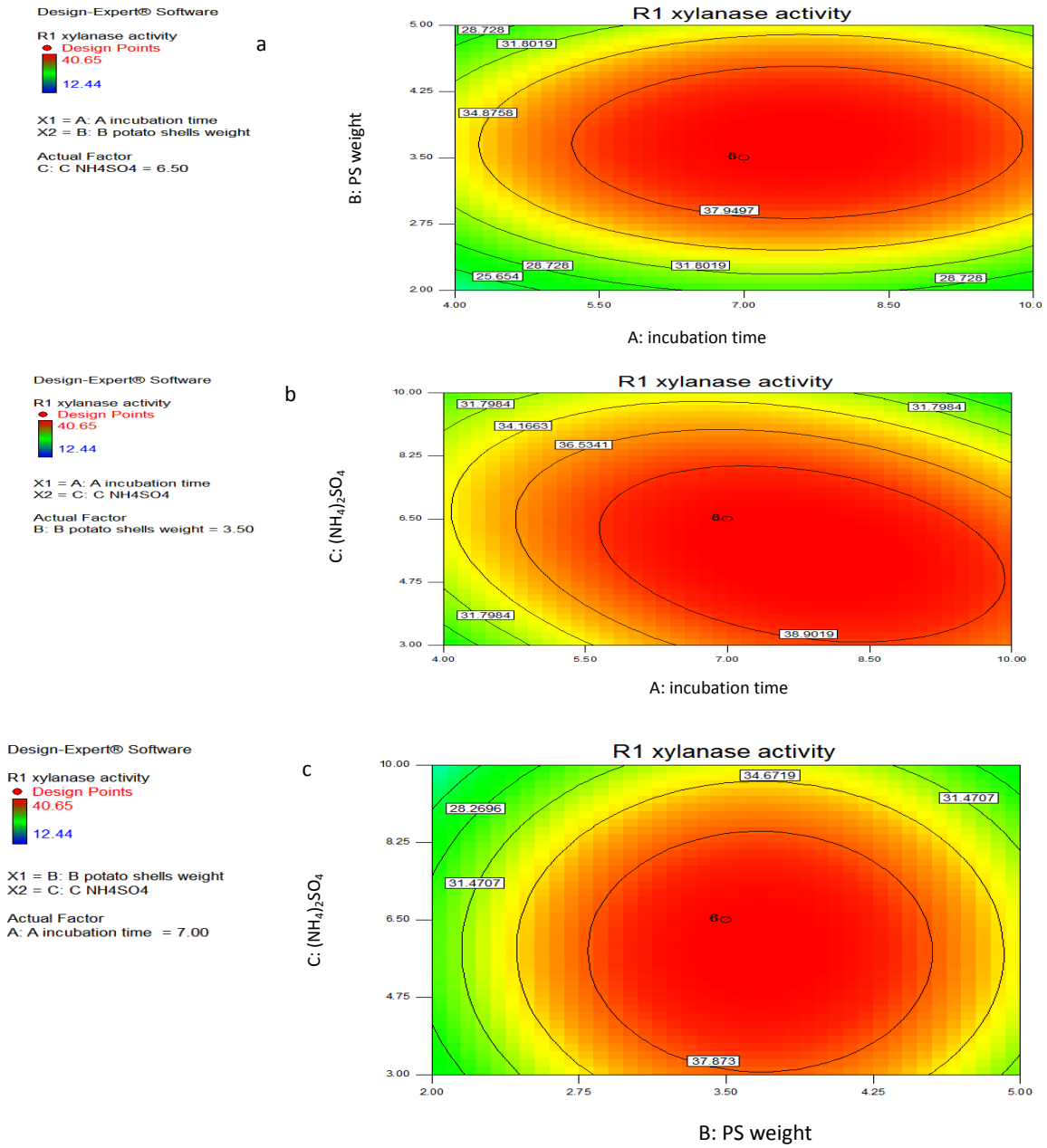

Fig. 4. Contour plot showing an interaction between, a: Incubation time and PS weight when $(NH_4)_2SO_4$ was kept at optimum concentration 6.50g/L; b: Incubation time and $(NH_4)_2SO_4$ when PS weight was kept at optimum concentration 3.5g/flask; c: PS weight and $(NH_4)_2SO_4$ when the incubation time was maintained at optimum value 7 days

CCD for S. plicatus MK559563 α-amylase production

For α-amylase production, the quantitative effect of RS weight, $(NH_4)_2SO_4$, and CaCl was examined. As shown in Table 4a. The highest *Streptomyces plicatus* MK559563 α-amylase production of 33.85 U/ml was obtained in central trials 1, 3, 6, 15, 16, and 20 causing 3.16- fold increase compared to the previous step of optimization. Our result was higher than that reported by Božić et al. (2011), Pathania et al. (2017) and Kwatia et al. (2017).

Theα-amylase activity can be calculated from the following equation :

α-amylase activity= +33.86 +3.52 * RS weight +3.05 * $(NH_4)_2SO_4$ +0.48 * $CaCl_2$ -3.57 * RS weight * $(NH_4)_2SO_4$ +4.61 * RS weight * $CaCl_2$+2.44 * $(NH_4)_2SO_4$ * $CaCl_2$ -3.20 * RS weight^2 -6.01 * $(NH_4)_2SO_4$ -1.77 * $CaCl_2^2$

TABLE 4a. CCD for *S. plicatus* MK559563 α-amylase production

Run	Factor 1 A: RS weight g/flask	Factor 2 B: $(NH_4)_2SO_4$ g/l	Factor 3 C: CaCl g/l	α-amylase activity U/ml	
				Actual	Predicated
1	3.5	6.5	6.5	33.85	33.85
2	5	10	10	33.75	33.4
3	3.5	6.5	6.5	33.85	33.85
4	3.5	0.61	6.5	12.35	11.72
5	2	10	3	28.55	11.72
6	3.5	6.5	6.5	33.85	33.85
7	3.5	12.39	6.5	21.66	21.98
8	5	3	3	24	24.26
9	5	10	3	17.44	18.34
10	6.02	6.5	6.5	31.76	30.72
11	3.5	6.5	0.61	28.45	29.55
12	2	3	3	18.75	19.31
13	2	10	10	24.33	24.28
14	0.98	6.5	6.5	18.15	18.88
15	3.5	6.5	6.5	33.85	33.85
16	3.5	6.5	6.5	33.85	33.85
17	5	3	10	28.45	29.55
18	3.5	6.5	12.39	29.55	29.65
19	2	3	10	6.85	6.16
20	3.5	6.5	6.5	33.85	33.85

TABLE 4b. Analysis of variance (ANOVA) forCCD for *Streptomyces plicatus* MK559563 α-amylase production

Source	Sum of squares	df	Mean square	F value	P value Prob>F	
Model	1251.566	9	139.0629	228.0257	< 0.0001	Significant
A-RS weight	169.0525	1	169.0525	277.2007	< 0.0001	
B- $(NH_4)_2SO_4$	127.1899	1	127.1899	208.5572	< 0.0001	
C- $CaCl_2$	3.084146	1	3.084146	5.057172	0.0483	
AB	101.8165	1	101.8165	166.9516	< 0.0001	
AC	170.0168	1	170.0168	278.7819	< 0.0001	
BC	47.72645	1	47.72645	78.25856	< 0.0001	
A^2	147.7369	1	147.7369	242.2489	< 0.0001	
B^2	520.9776	1	520.9776	854.2633	< 0.0001	
C^2	45.2343	1	45.2343	74.1721	< 0.0001	
Residual	6.09856	10	0.609856			
Lack of Fit	6.09856	5	1.219712			
Pure Error	0	5	0			
Cor Total	1257.664	19				

R^2 0.9952, Adj R^2 0.9908, Pred R^2 0.9604

The interaction between the three variables was shown in Fig. 5a, b, and c. The success of the design was emphasized by statistical analysis (ANOVA) as shown in Table 4b. The model F-value of 228.03 implied the model was significant. Moreover, The values of R^2 (0.9952) means that 99.52 of the results can be explained by the design, andpred R^2 0.9604 was in reasonable agreement with the adj R^2 0.9908.

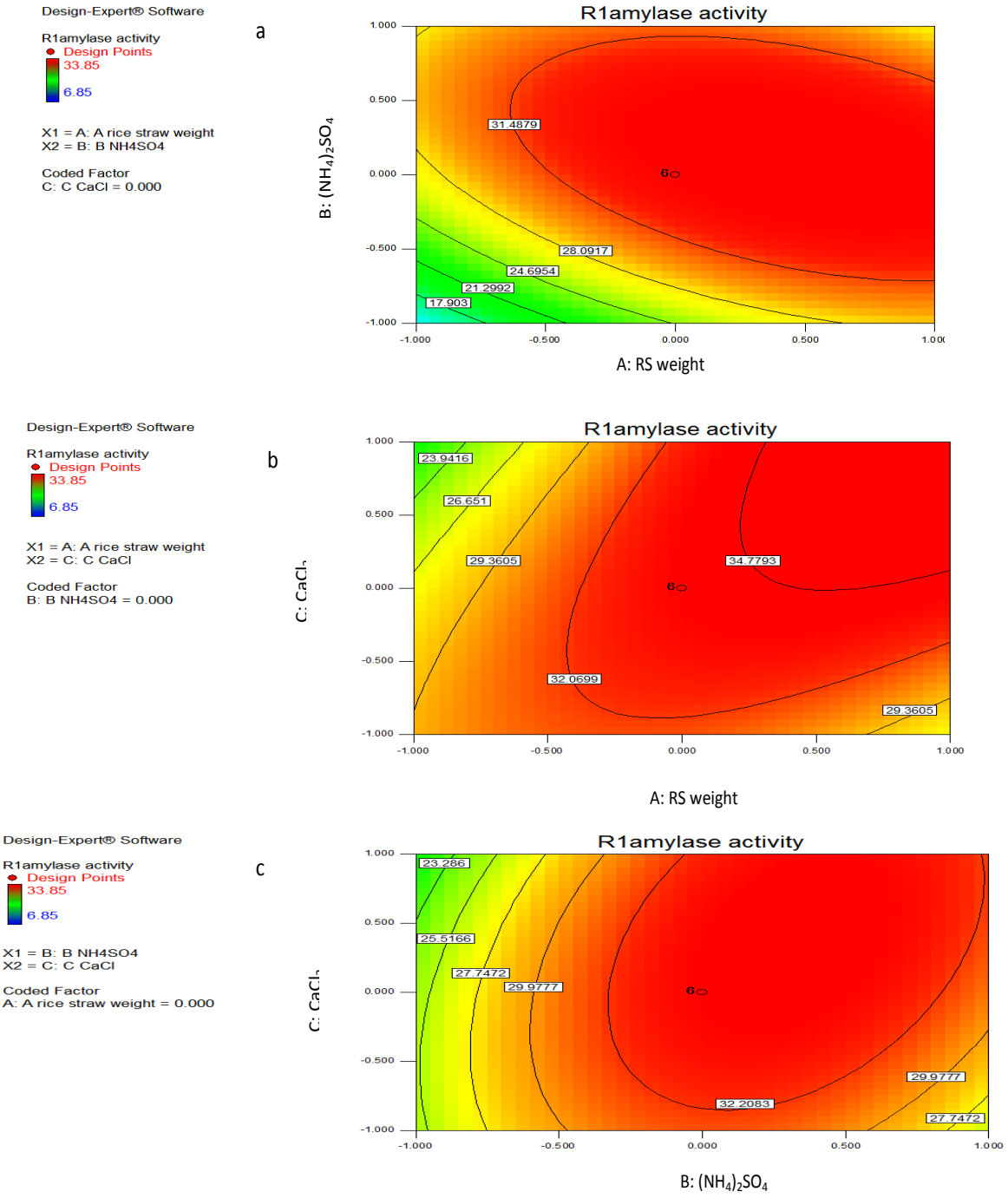

Fig. 5. Contour plot showing an interaction between, a: RS weight and $(NH_4)_2SO_4$ weight when $CaCl_2$ was kept at zero levels (6.5g/L); b: RS weight and $CaCl_2$ when $(NH_4)_2SO_4$ was kept at zero levels(6.5g/L); c: $CaCl_2$ and $(NH_4)_2SO_4$ when RS weight was maintained at zero levels (3.5 g/flask)

Conclusion

The industrially potent enzymes xylanase and α-amylase can be produced with low cost and the eco-friendly environmental method by the use of soil isolate *Streptomyces plicatus* MK559563. That was capable of producing xylanase and α-amylase by the SmFtechnique utilizing RS and PS as substrates. *Streptomyces plicatus* MK559563 xylanase and α-amylase production werestatistically optimized through two designs Plackett-Burman (PB) and Central composite design (CCD). The highest *S. plicatus* MK559563 xylanase production (40.65 U/ml) was obtained in media composed of g/L: Baker's yeast (1), $(NH_4)_2SO_4$ (6.5), KCl (1), $ZnSO_4$ (0.001) with RS (2 g/flask) and PS (3.5 g/flask) for 7 days of incubation. The highest α-amylase production was obtained in mediacomposed of g/L: Glucose (10), baker's yeast (5), $(NH_4)_2SO_4$ (6.5), $CaCl_2$ (6.5) with RS (3.5 g/flask) and PS (1g/flask) for 6 days of incubation.

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Conflict of interest: The authors declare that there is no conflict of interest.

References

Abd El Aty, A.A., Saleh, S.A.A., Eid, B.M., Ibrahim, N.A., Mostafa, F.A. (2018) Thermodynamics characterization and potential textile applications of *Trichoderma longibrachiatum* KT693225 xylanase. *Biocataly. Agric. Biotechnol.* **14**, 129–137.

Ahmed, S.A., Saleh, S.A.A., Mostafa, F.A., Abd ElAty, A.A., Ammar, H.A.M. (2016) Characterization and valuable applications of xylanase from endophytic fungus *Aspergillus terreus* KP900973 isolated from *Corchorus olitorius. Biocataly. Agric. Biotechnol.* **7**, 134–144.

Ali, M.B., Irshad, M., Anwar, Z., Zafar, M., Imran, M. (2016) Screening and statistical optimization of physicochemical parameters for the production of xylanases from agro-industrial wastes. *Advances in Enzyme Research*, **4**, 20-33.

Balkan, B., Balkan, S., Ertan, F. (2011) Optimization of parameters for α-amylase production under solid-state fermentation by *Trichothecium roseum. Romanian Biotechnological Letters,* **16**(5), 6591-6600.

Bekler, F.M., Yalaz, S., Güven, R.G. (2019) Optimization of the thermostable alkaline and Ca-dependent α−amylase production from *Bacillus paralicheniformis* by statistical modeling. J. *Serb. Chem. Soc.* **84**(0), 1–13.

Bernard, B. (2007) Access excellence @ the national health museum, Isolation of Antibiotic Strinas from Soils. (www. access excellence. org.) July.

Bhushan, S., Kalia, K., Sharma, M., Singh, B., Ahuja, P.S. (2008) Processing of apple pomace for bioactive molecules. *Crit. Rev. Biotechnol.* **28**, 285–296.

Biely, P. (1985) Microbial xylanolytic systems. *Trends Biotechnol.* **3**, 286–290.

Božić, N., Ruiz, J., López-Santín, J., Vujčić, Z. (2011) Optimization of the growth and α-amylase production of *Bacillus subtilis* IP 5832 in shake flask and laboratory fermenter batch cultures. *J. Serb. Chem. Soc*. **76**(7), 965–972.

Brzezinski, R., Dery, C.V., Beaulieu, C. (1999) Thermostable xylanase DNA, protein and methods in use," USA patent 5871730, 1999.

Chen, X.S., Tang, L., Li, S., Liao, L.J., Zhang, J.H., Mao, Z.G. (2011) Optimization of medium for enhancement of epsilon-Poly-L-Lysine production by *Streptomyces* sp. M-Z18 with glycerol as carbon source. *Bioresour. Technol.* **102**, 1727–1732.

Cui, F., Zhao, L. (2012) Optimization of xylanase production from *Penicillium*sp.WX-Z1 by a two-step statistical strategy: Plackett-Burman and Box-Behnken experimental design. *Int. J. Mol. Sci.* **13**, 10630-10646.

Cui, F.J., Liu, Z.Q., Li, Y., Ping, L.F., Ping, L.Y., Zhang, Z.C., Lin, L., Dong, Y., Huang, D.M. (2010) Production of mycelial biomass and exo-polymer by *Hericium erinaceus* CZ-2: optimization of nutrients levels using response surface methodology. *Biotechnol. Bioproc.* **15**, 299–307.

Daneault, C., Leduc, C., Valade, J.L. (1994) The use of xylanases in kraft pulp bleaching—A review. *Tappi J.* **77**, 125–131.

Das, M., Gupta, S., Kapoor, V., Banerjee, R., Bal, S. (2008) Enzymatic polishing of rice—A new processing technology. *Lwt-Food Sci. Technol.* **41**, 2079–2084.

Diaz, A.B., Bolivar, J., de Ory, I., Caro, I., Blandino, A. (2011) Applicability of enzymatic extracts obtained by solid state fermentation on grape pomace and orange peels mixtures in must clarification. *Lwt-Food Sci. Technol*. **44**, 840–846.

Hang, Y.D., Woodams, E.E. (1997) Xylanolytic activity of commercial juice-processing enzyme preparations. *Lett. Appl. Microbiol.* **24**, 389–392.

Hassan, H., Abd Karim, K. (2015) Optimization of alpha amylase production from rice straw using solid-state fermentation of *Bacillus subtilis.* Internat. *J. Sci. Environment Technol.* **4**(1), 1–16.

Hayakawa, M., Nonomura, H. (1987) Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J. Fermen. Technol*. **65**(5), 501-509.

Hazaa, M.M., Sabae, S.Z., Areej, I. F. T., Eldourghamy, A.S., Sayed, H. (2018) Production, purification and characterization of alfa-amylase produced by bacteria from pharaonic lake. *J. Basic Environmental Sciences*, **5**, 162-173.

Kwatia, S., Dzogbefia, V.P., Ofosu, I.W. (2017) Optimization of amylase production by *Aspergillus niger* cultivated on yam peels in solid state fermentation using response surface methodology. *Afr. J. Biochem. Res.* **11**(7), 34-42.

Liu, B.L., Tzeng, Y.M. (1998) Optimization of growth medium for production of spores from *Bacillus thuringiensis* using response surface methodology. *Bioprocess Eng.* **18**, 413–8.

Locci, R. (1989) Streptomycetes and related genera. In: "*Bergey's Manual of Systematic Bacteriology*", Williams, S.T., Sharpe, M.E., Holt, J.G. (Eds.), pp. 2451-93. Baltimore: Williams and Wilkins.

Mohamed, S.H., Seham, S.D.E.H., Mona, A.I., Sadik, A.S. (2013) Taxonomy of *Streptomyces* strains isolated from rhizospheres of various plant species grown in Taif region, KSA, having antagonistic activities against some microbial tissue culture contaminants. *Afr. J. Biotechnol.* **12**(14), 1657-1664.

Mohamed, Sonya H., Omran, W.M., Sadik, A.S. (2014) Use of 16S rRNA gene for molecular identification of some bacteria isolated from soil in Taif having biochemical and antibiosis activities. *Wulfenia J.* **21**(2), 173 185.

Nawani, N., Aigle, B., Mandal, A., Bodas, M., Ghorbel, S., Prakash, D. (2013) Actinomycetes: Role in biotechnology and medicine. *Bio. Med. Res. Int.* **Special Issue 2013**.

Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D., Mohan, R. (2000) Advances in microbial amylases. *Biotechnol. Appl. Biochem.* **31**, 135-152.

Parajó, J.C., Domínguez, H., Domínguez, J. (1998) Biotechnological production of xylitol. Part 1: Interest of xylitol and fundamentals of its biosynthesis. *Bioresourc. Technol.* **65**, 191-201.

Pathania, S., Sharma, N., Handa, S. (2017) Optimization of culture conditions for production of amylase by *bacillus amyloliquifaciens*sh8 using response surface methodology. *Proc. Indian Natn. Sci. Acad.* **83**(1), 203-210.

Periyasamy, A.K., Muthu, M., Kannan, V. (2010) Optimization of nutrients for the production of RNase by *Bacillus firmus* VKPACU1 using response surface methodology. *Biotechnol. Bioprocess,* **14**, 202–206.

Plackett, R.L., Burman, J.P. (1946) The design of optimum multifactorial experiments. *Biometrika,* **37**, 305–325.

Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A., Amorim, D.S. (2005) Xylanases from fungi: Properties and industrial applications. *Appl. Microbiol. Biotechnol.* **67**, 577–591.

Puspitasari, F., Nurachman, Z., Noer, A.S., Radjasa, O.K., Maarel, M., Natalia, D. (2011) Characteristics of raw starch degrading amylase from *Bacillus aquimaris*MKSC 6.2 associated with soft coral *Sinularia* sp. *Starch.* **63**, 461- 467.

Retnaningrum, E., Purwestri, Y.A. (2016) Molecular identification and optimization culture conditions

of amylase producing bacteria isolated from green algae in the coast side of southern sea, Yogyakarta, Indonesia. *AIP Conference Proceedings*, **1755**(1), 8p.

Sajjad, M., Choudhry, S. (2012) Effect of starch containing organic substrates on alpha amylase production in *Bacillus* strains. *Afr. J. Microbiol. Res.* **6**(45), 7285–7291.

Salihu, A., Alam, M.Z., AbdulKarim, M.I., Salleh, H.M. (2011) Optimization of lipase production by *Candida cylindracea* in palm oil mill effluent based medium using statistical experimental design. *J. Mol. Catal. Enzym.* **69**, 66–73.

Sathyar, R., Ushadevi, T. (2014) Industrially important enzymes producing *Streptomyces* species from mangrove sediments. Internat. *J. Pharmacy Pharmaceut. Sci.* **6**(10), 233-237.

Sharma, A., Satyanarayana, T. (2013) Microbial acid-stable α-amylases: characteristics, genetic engineering and applications. *Proc. Biochemi.* **48**, 201–211.

Sivaramakrishnan, S., Gangadharan, D., Kesavan, M.N., Ricardo, S.C., Pandey, A. (2006) α-amylases from microbial sources – An overview on recent developments. *Food Technol. Biotechnol.* **44**, 173-184.

Soleimani, M., Tabil, L., Panigrahi, S., Alberta, E. (2006) Bio-production of a Polyalcohol (Xylitol) from Lignocellulosic Resources: A Review written for presentation at the CSBE/SCGAB 2006 Annual Conference, Society; 2006.

Somogyi, M.J. (1952) Notes on sugars determinations. *J. Biol. Chem.* **195**, 19–23.

Subramaniyan, S., Prema, P. (2002) Biotechnology of microbial xylanases: Enzymology, molecular biology, and application. *Critical Rev. Biotechnol.* **22**, 33-64.

Syed, D.G., Agasar, D., Pandey, A. (2009) Production and partial purification of α-amylase from a novel isolate *Streptomyces gulbargensis. J. Ind. Microbiol. Biotech.* **36**(2), 189–194.

Sykes, G., Skinner, F.A. (1973) "*Actinomycetales: Characteristics and Practical Importance*". Academic Press. London. 339p.

Verma, D., Kanagaraj, A., Jin, S.X., Singh, N.D., Kolattukudy, P.E., Daniell, H. (2010) Chloroplast-derived enzyme cocktails hydrolyse lignocellulosic biomass and release fermentable sugars. *Plant Biotechnol. J.* **8**, 332–350.

Viikari, L., Kantelinen, A., Sundquist, J., Linko, M. (1994) Xylanases in bleaching: From an idea to the industry. *FEMS Microbiol. Rev.* **13**, 335–350.

Vurukonda, S.S.K.P., Giovanardi, D., Stefani, E. (2018) Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. *Internat. J. Molecular Sci*. **19**, 1-26.

Wang, H.Y., Fan, B.Q., Li, C.H., Liu, S., Li, M. (2011) Effects of rhamnolipid on the cellulase and xylanase in hydrolysis of wheat straw. *Bioresour. Technol.* **102**, 6515–6521.

Warzywoda, M., Ferre, V., Pourquie, J. (1983) Development of culture medium for large-scale production of cellulytic enzymes by *Trichoderma reesei. Biotechnol. Bioeng.* **25**, 3005-3011.

التحسين الأحصائى لأنتاج إنزيمات الزيلينيز والألفا أميليز الصناعية بواسطة *Streptomyces plicatus* MK559563 على مخلفات زراعية.

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نجح *Streptomyces plicatus* MK559563 المعزول من التربة في إنتاج مركبات مهمة مثل السكريات المختزلة وإنزيمات ذات أهمية صناعية بتقنية المزارع المغمورة على نفايات زراعية (قش الأرز RS وقشر البطاطس PS). تم تحسين إنتاج إنزيمات الزيلينيز والألفا أميليز *Streptomyces plicatus* MK559563 إحصائيًا من خلال تصميمين Plackett-Burman (PB) و (CCD). أظهر تصميم PB أن إنتاج الزيلينيز بواسطة *S. plicatus* MK559563 كان أكثر تأثرًا بفترة التحضين ووزن PSقشر البطاطس و كبريتات الأمونيوم $(NH_4)_2SO_4$ بينما تأثر إنتاج α-amylase بشكل أكبر بوزن قش الأرز RS و كبريتات الأمونيوم $(NH_4)_2SO_4$ و كلوريد الكالسيوم $CaCl_2$ تحسن إنتاج *S. plicatus* MK559563 xylanase و α-amylase بنسبة 39.69 و 61.60 ضعفًا بواسطة النظام الأحصائى CCDمقارنة بالوسائط غير المحسنة. الوسائط المحسنة لإنتاج S. plicatus MK559563 xylanase تتكون من جم / لتر: خميرة بيرة (1) $(NH_4)_2SO_4$ (6,5)،كلوريد البوتاسيوم (1) ، كبريتات الزنك (0,001) مع قش الأرز (2 جم / فلاسكة) وقشر بطاطس (3,5 جم / فلاسكة) لمدة 7 أيام من التحضين. الوسائط المُحسَّنة لإنتاج الألفا MK55956 *S.plicatus* تتكون من جم / لتر: الجلوكوز (10) ، خميرة بيرة (5) ،كبريتات الأمونيوم (6,5)، كلوريد الكالسيوم (6,5) مع قش الأرز (3,5 جم / فلاسكة) و قشر بطاطس (1 جم / فلاسكة) لمدة 6 أيام من التحضين. يمكن استخدام *Streptomyces plicatus* MK559563 كخطوة وسيطة للتحويل الحيوي للنفايات الزراعية إلى وقود حيوي.