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RESEARCH ARTICLE

Exo-polygalacturonase production enhancement by *Piriformospora indica* from sugar beet pulp under submerged fermentation using the response surface methodology

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Abstract

This study proposed a novel and cost-effective approach to enhance and optimize the exo-polygalacturonase from *P. indica*, a root endophytic fungus. In the current investigation, the impact of ammonium sulfate, sugar beet pulp (SBP), and glucose as variables on induction of exo-polygalacturonase from *P. indica* was optimized using the central composite design (CCD) of response surface methodology (RSM) under submerged fermentation (SmF). Additionally, determination of the exo-polygalacturonase molecular weight and in situ analysis was performed. The optimal reaction conditions, which resulted in the highest enzyme activity, were observed in the following conditions: ammonium sulfate (4 g/L), SBP (20 g/L), and glucose (60 g/L). Under the optimized condition, the maximum enzyme activity reached 19.4 U/ml (127 U/mg), which increased by 5.84 times compared to non-optimized conditions. The exo-polygalacturonase molecular weight was estimated at 60 KDa. In line with the bioinformatic analysis, the exo-polygalacturonase sequence of *P. indica* showed similarity with *Rhizoctonia solani's* and *Thanateporus cucumeris*. These results indicated that SBP acts as a cheap and suitable inducer of exo-polygalacturonase production by *P. indica* in submerged cultivation. The outcome of this study will be useful for industries to decrease environmental pollution with cost-effective approaches.

Keywords By-product · Eco-friendly · Environmental pollution · Optimization · Pectin · Pectinase

Introduction

Agricultural by-products as a rich source of carbohydrates, notwithstanding their attractive morphological, chemical, and mechanical properties commonly, remain unused. Due to the limitation of non-renewable resources, there is an urgent need to the management of the agricultural wastes. The agro-waste applications include construction material, bio-fertilizers, pulp and paper products, packaging products, tableware, heating applications, biocomposites, nano-cellulosic materials, soil stabilizers, bioplastics, fire-retardant additives, dye removal, and biofuels (Gupta et al. 2022).

Nowadays, considering the global sensitivity to the environment, enzyme production from wastes helped to overcome the problem of high-cost production in the industry and prevent environmental pollution (Tepe and Dursun 2014). With the increasing application of pectinase, decreasing cost production has become one of the most important targets. Previous studies have mentioned that pectin-containing agro-wastes, including sugar beet pulp, citrus pulp pellets, apple pomace, henequen pulp, lemon pulp, and other related materials as carbon source, could induce pectinase production by many microorganisms (Bai et al. 2004). Sugar beet pulp (SBP), the by-product of the beet sugar industry, is produced annually in large quantities. On the other hand, SBP could be an important renewable resource and its bio-conversion appears to be a great biotechnological advantage. The lignocellulosic portion of dried SBP consists 22–30% cellulose, 24–32% hemicellulose (essentially arabinan), 24–32% of pectin substances, and 3–4% of lignin (Hutnan et al. 2000). Due to the high pectin content of SBP, it could be used for pectinolytic

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enzyme production without adding any pectinaceous materials as enzyme inducers (Jacob 2009).

Pectinases or pectin depolymerases which are very important industrial enzymes have a broad range of applications in the food, pharmaceutical, textile, processing, and paper and pulp industries (John et al. 2020). The pectinolytic enzymes are produced by higher plants, bacteria, fungi, and yeasts under submerged and solid-state fermentation conditions. They have classified according to their mechanism of action: as pectin esterase, pectinase (polygalacturonase), and pectin lyase, and cause the production of galacturonic acid (Patidar et al. 2018). Like many other depolymerizing enzymes, they are usually inducible by the polymer they could degrade. Among them, fungal pectinases have the greatest significance with extensive applications (Patil and Dayanand 2006). The source, substrate, reaction conditions, and reactor design are important factors in upstream pectinase production processing (John et al. 2020). Pectin as the acidic heteropolysaccharide is mainly composed of galacturonic acid which presents the major components of the middle lamella and primary cell wall of plants (Satapathy et al. 2020).

The induction of pectinase production by various organisms from agricultural by-products was described; by *Penicillium fellutanum* from wheat bran (Amin et al. 2021), by *Bacillus pumilus* from a mixture of banana and orange peel (Viayaraghavan et al. 2019), by *Aspergillus niger* DMF 27 and DMF 45 from deseeded sunflower head (Patil and Dayanand 2006), by *Aspergillus niger* from the citrus waste peel (Ahmed et al. 2016), by *Aspergillus sojae* from agricultural and agro-industrial residues (Heerd et al. 2014), by *Aspergillus niger* and *Bacillus gibsoni* from sugar beet pulp (Jacob 2009), by *T. reesei* Rut C-30 from sugar beet pulp (Olsson et al. 2003), and by *Bacillus pumilus* from sugar beet pulp and wheat bran (Tepe and Dursun 2014).

In our previous investigation, for the first time, the exo-polygalacturonase activity of the *Piriformospora indica*, a root endophytic fungus, was reported (Heidarizadeh et al. 2018). There is no report available in the literature related to the application of sugar beet pulp (SBP) for exo-polygalacturonase production by *P. indica*. Hence, the production of exo-polygalacturonase from *P. indica* by SBP as an inducer was optimized by response surface methodology (RSM). Its molecular characteristics were evaluated and in situ analysis carried out.

Materials and methods

Microorganism

The *P. indica* fungus (ATCC-204458) was selected for the production of exo-polygalacturonase and obtained from the Department of Plant Pathology, School of Agriculture, Tarbiat Modares University (Iran).

Culturing media

The *P. indica* was grown mainly on modified Kaefer medium containing 10.0 g/L SBP, 3.0 g/L peptone, 3.0 g/L yeast extract, 1.83 g/L KH_2PO_4, 0.65 g/L $MgSO_4$. $7H_2O$, 2.5 ml/L micro-elements stock solution, 1.0 ml/L vitamin stock solution, 1.0 ml/L of 0.1 M $CaCl_2$ solution, 1.0 ml/L of 0.1 M $FeCl_3$ solution, and 2.5 ml/L of 5.0 g/L $FeSO_4$. $7H_2O$. The microelement stock solution consisted of (g/L): $ZnSO_4$. $7H_2O$, 22.4; H_3BO_3, 11.0; $MnCl_2$. $4H_2O$, 5.0; $CoCl_2$ $6H_2O$, 1.6; $CuSO_4$. $5H_2O$, 1.6; $(NH_4)_6$ Mo_7O_{27}. $4H_2O$, 1.1; Na_2EDTA, 50.0. The vitamin stock solution composed of 0.5 g/L of biotin, 1.0 g/L of p-aminobenzoic acid, 5.0 g/L of nicotinamide, 1.0 g/L of pyridoxal phosphate solution, and 2.5 g/L riboflavin solution (Kumar et al. 2012). Moreover, glucose was replaced with SBP and represented as SBP^+. The fungal stock culture was kept on Kaefer medium supplemented with 15 g/L agar and stored at 4 °C for further studies.

SBP preparation

SBP was prepared from Moghan Agro-Industry and Livestock Co. (Moghan, Iran) and dried at 60 °C for 24 h. The dried SBP was ground using an electric grinder, sieved through the No. 35 mesh sieve (Damavand Sieve Company, Iran), and stored in airtight container. The particles with a mesh sieve adjusted to 500 µm were used for submerged fermentation.

Chemicals

All the chemicals used were purchased from Sigma Aldrich (USA) or HIMEDIA (Mumbai, India) and were of high analytical grade. Polygalacturonic acid from citrus fruit (Sigma) was used as substrate.

Cultivation of fungi and exo-PG production by submerged fermentation

For submerged cultivation of *P. indica*, 10 mm of agar disks was transferred to 250 mL flasks containing 50 mL of modified Kaefer medium supplemented with 10 g/L SBP and incubated in a shaker (200 rpm) at 29 °C. Medium without sugar beet pulp (SBP^-) was used as a control. The active inoculum of 2% grown in modified Kaefer broth for 4 days was used for all experiments (Kumar et al. 2011). The exo-PG activity and fungal growth of the samples were measured.

Measurement of cell fresh and dry weight, growth yield, and specific growth rate

At the end of each incubation time, the culture broth was filtered through Whatman No. 1 paper and growth parameters including; growth yield ($Y_{X/S}$), specific growth rate (μ), and spore yield were determined (Kumar et al. 2011).

Total protein determination

The culture broth was centrifuged at 12,880 rcf at 4 °C for 15 min and the exo-PG activity, pH, and protein content of the cell-free supernatant were determined. Total protein content was determined according to Bradford's method, and bovine serum albumin used as the standard (Bradford 1976).

Exo-polygalacturonase activity

Exo-polygalacturonase activity was evaluated by measuring the released reducing end products, using 3, 5-dinitrosalicylic acid (DNS), and expressed as galacturonic acid equivalent (Miller 1959). The enzymatic reaction mixture included 0.25 ml of cell-free supernatant and 0.75 ml of 1% pectin in 0.2 M phosphate buffer pH 6.5 as substrate. The mixture was incubated at 60 °C for 5 min. One unit (U) was expressed in terms of the enzyme quantity, which would yield 1 µmol galacturonic acid per minute during the standard assay condition.

Identification of the significant variables using experimental design

To maximize enzyme production and understand the role of interacting variables, optimization of the medium constituents was done by central composite design (CCD). Three variables including glucose (A), ammonium sulfate (B), and SBP (C) were selected to find the optimized condition for the production of exo-polygalacturonase and twenty experimental runs with three center points generated including the response surface plot by using the statistical software package Design-Expert 7.0.0 (Stat Ease Inc., Minneapolis, USA). The range and the levels of the variables are given in Table 1. The recommended 20 experiments using different compositions of independent variables are shown in Table 2. Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). The quality of the polynomial model equation was judged statistically by coefficient of determination (R^2), and its statistical significance was determined by F-test.

Table 1 Levels of independent variables used in CCD design

Variables	Units	Symbol code	Levels		
			-1	0	1
Glucose	g/L	A	60	80	100
Ammonium sulfate	g/L	B	6	4	8
Sugar beet pulp (SBP)	g/L	C	10	15	20

Exo-polygalacturonase gel electrophoresis

The fungus was cultured in 250 mL shake flasks with 100 mL of optimized fermentation medium to prepare the exo-polygalacturonase. The fermentation broth was separated by centrifugation at 10,000 g for 15 min at 4 °C, and the cell-free supernatant was saturated with ammonium sulfate to 90% saturation. The saturated solution was left overnight at 4 ºC with gentle agitation, centrifuged at 10,000 g for 20 min at 4 °C, the precipitate solubilized in a minimal amount of 10 mM sodium acetate buffer (pH 5.75) and dialyzed against the same buffer for 24 h at 4 °C. The obtained-dialyzed proteins were used for enzyme characterization. The protein content and enzyme activity were determined as described in the earlier part.

The molecular mass of exo-polygalacturonase was determined by SDS-PAGE (12.5% running gel and 5% stacking gel) (Laemmli 1970). The protein samples were denatured by heating at 100 °C with the sample buffer for 5 min before loading and the gel was stained by the silver staining method (Merril et al. 1981).

Phylogenetic tree simulation

We applied Molecular Evolutionary Genetics Analysis (MEGA X) software as a powerful tool for constructing sequence alignments, gathering phylogenetic histories, and performing molecular evolutionary analysis. This software can be used for comparing DNA and protein sequences. First, we aligned the DNA sequences of more than fifty different strain types of extracellular polygalacturonases and then a phylogenetic tree was constructed for those data by the maximum likelihood method. In this approach, an initial phylogenetic tree was constructed using a neighbor-joining, and its branch lengths are modified to maximize the likelihood of the data set for that tree topology under the desired model of evolution. Then, the NNI (nearest neighbor Interchange) approach was used for creating the variants of the topology. The NNI tries to search for topologies that are in good shape with the data better. The search is repeated until no greater likelihoods are found. Finally, the neighbor-joining tree of different extracellular polygalacturonase strains was constructed after 500 iterations and the bootstrap confidence values

Table 2 CCD design matrix for exo-polygalacturonase production factors and corresponding results

Run	Variables			DW (g/L)	FW (g/L)	pH	Protein (mg/ml)	Observed activity (U/ml)	Specific activity (U/mg)
	A	B	C						
1	60	4	20	62.56	417.85	4.09	0.25	**19.41**	77.64
2	60	6	15	55.5	323.36	3.72	0.18	14.74	81.88
3	80	6	15	62.06	332.6	3.55	0.157	12.33	78.53
4	80	6	10	57.53	205.86	4.7	0.151	10.46	69.27
5	100	4	20	60.85	380.85	3.78	0.196	14.16	72.24
6	80	6	15	47.77	306.98	5.09	0.148	12.13	81.95
7	100	6	15	64.39	245.88	5.76	0.135	10.76	79.70
8	80	8	20	55.94	373.6	4.85	0.433	10.12	23.37
9	80	8	15	55.335	369.56	5.06	0.217	10.67	49.17
10	60	4	10	66.20	188.61	5.29	0.145	14.03	96.75
11	80	4	15	57.11	381.42	4.28	0.148	14.03	94.79
12	80	6	20	67.33	379.35	4.01	0.186	13.28	71.39
13	80	6	15	69.22	336.53	3.88	0.105	13.33	**126.95**
14	60	8	20	53.35	253.26	5.60	0.252	12.35	49
15	100	8	10	52.09	176.76	4.99	0.192	8.07	42.02
16	100	4	10	56.19	251.20	4.69	0.133	9.78	73.53
17	80	6	15	61.75	332.77	4.15	0.19	12.87	67.73
18	80	6	15	73.40	323.33	4.84	0.26	12.54	48.23
19	60	8	10	51.71	266.44	3.6	0.16	10.75	67.19
20	80	6	15	58.99	393.97	5.17	0.11	12.37	111.45

were calculated and shown on the node in Fig. 4. The protein sequences were gathered from Uniprot and GenBank (NCBI) (Chen et al. 2014), (Kumar et al. 2018) and (Verma et al. 1998) (https://academic.oup.com/nar/article/22/22/4673/2400290?login=true).

Results

Growth of fungus

Production of exo-polygalacturonase by *P. indica* was evaluated for 10 days (Fig. 1). The production of exo-polygalacturonase on both mediums reached its maximum rate on day 6th of culture and then decreased. The production of enzyme on SBP^- and SBP^+ medium was determined 2.2 and 3.32 U/ml, respectively. Also, the highest dry and fresh weight on both mediums was detected on day 6 of culture (Fig. 1a and b). As shown in Table 3, the lowest and highest dry cell weight were detected on unmodified Kaefer medium and SBP- and glucose-containing medium, respectively. Furthermore, the highest amount of growth yield and specific growth rate was measured on a medium containing ammonium sulfate, glucose, and SBP, 0.62 and 1.61, respectively.

Optimization of the exo-polygalacturonase production by RSM

Three factors that have the maximum effect on exo-polygalacturonase production were determined by the one-factor-at-a-time method, and the interaction between various factors on polygalacturonase production (glucose, ammonium sulfate, and SBP concentration) was investigated by RSM.

Then, the results were analyzed by standard analysis of variance (ANOVA) and the CCD design was fitted with the second-order polynomial equation:

$$Y\left(\frac{\mathrm{U}}{\mathrm{ml}}\right) = +14.44655 - 0.25632 \times A - 0.050858 \times B + 1.78328 \times C + 0.010096 \times A \times B - 2.36660\mathrm{E} - 003 \times A \times C - 0.094896 \times B \times C + 8.28012\mathrm{E} - 004 \times A^2 - 0.031692 \times B^2 - 0.024311 \times C^2$$

Equation (1) exo-polygalacturonase activity (Y) as a function of glucose (A), ammonium sulfate (B), and sugar beet pulp (C).

The software suggested 20 experiments and the predicted and experimental values for enzyme production are presented in Table 2. The sufficiency of the model was checked using the correlation coefficient (R^2), and the closer the value of R^2 to 1, the better the correlation between the observed and the predicted values. The

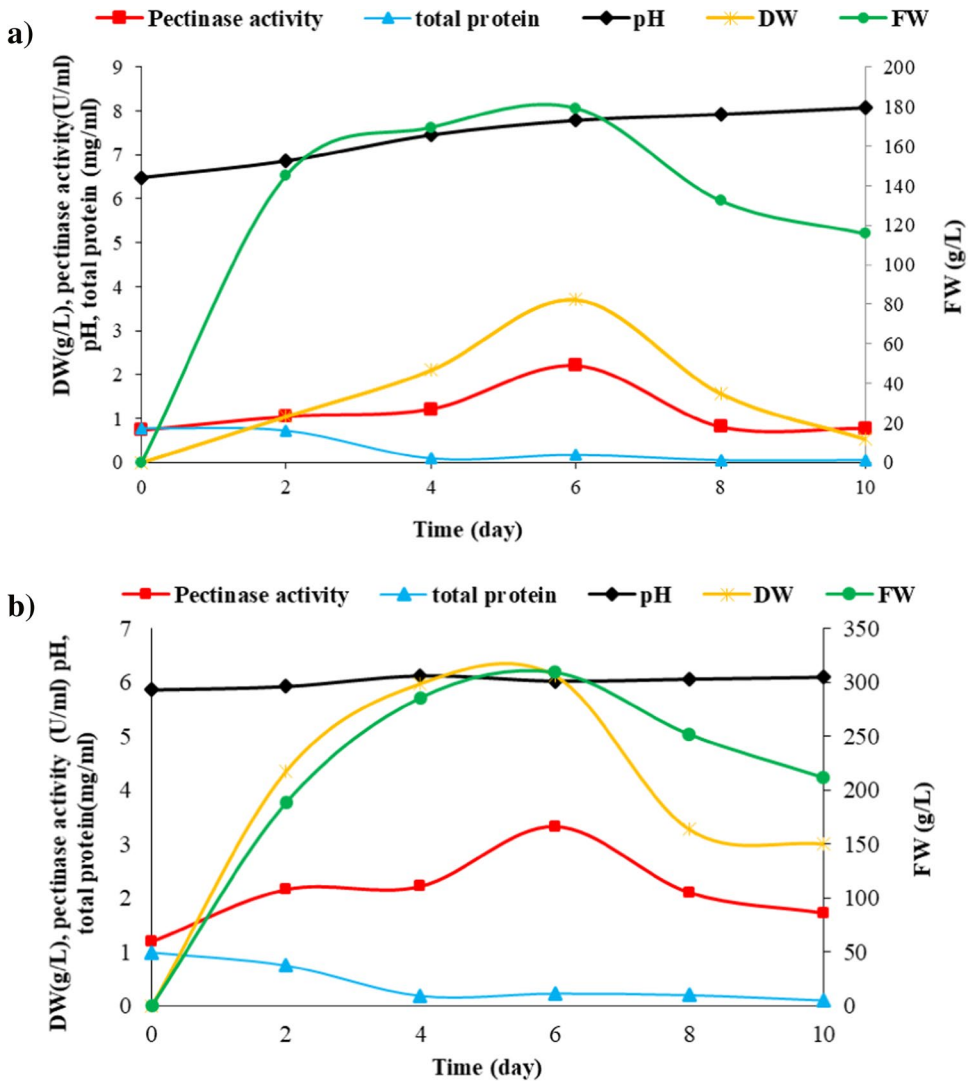

Fig. 1 The effect of SBP on growth parameters and exo-polygalacturonase activity of *P. indica*. Time course profile of *P. indica* on Kaefer medium (**a**) and supplemented with SBP (**b**) in SmF. Data are shown as mean ± SD of three independent experiments in a triplicate lay-out. SBP^+, medium containing SBP; SBP^-, medium without SBP; FW, fresh weight; DW, dry weight

Table 3 Effect of variables on growth of *P. indica*

Culture medium	Maximum DCW (g/L)	$Y_{x/s}$ (g/g)	μ (d^{-1})
Medium	3.7 ± 0.1414		
Medium + SBP	6.125 ± 0.1768	0.61	0.30
Medium + ammonium sulfate	3.95 ± 0.21215	0.28	1.27
Medium + ammonium sulfate + SBP	7.675 ± 0.1768	0.54	0.61
Medium + glucose	15.625 ± 1.096	0.26	0.45
Medium + glucose + SBP	26.45 ± 1.202	0.44	0.54
Medium + ammonium sulfate + glucose + SBP	73.40	0.62	1.61

correlation coefficient (R^2), which shows the relationship between the experimental and predicted responses, was 0.9866; thus, the model could explain more than 98.66% of the variability in the responses (Table 4).

Moreover, R^2 values were in reasonable agreement with adjusted R^2 values of 0.9745 (polygalacturonase production). Values greater than 0.1000 indicate the model terms are not significant. The "Pred *R*-Squared" of 0.9404 is in reasonable agreement with the "Adj *R*-Squared" of 0.9745. Table 4 represents the results of the quadratic response surface model fitting in the form of ANOVA.

The model *F* value is 81.76, which indicates the model's significance. The (*B*) had the highest *F* value of 265 implying that it had the most significant influence on enzyme

Table 4 ANOVA for response surface quadratic model of exo-polygalacturonase production

Source	Sum of squares	df	Mean squares	*F* value	*p* value	
Model	106.59	9	11.84	81.76	< 0.0001	Significant
A	29.23	1	29.23	201.82	< 0.0001	
B	38.49	1	38.49	265.70	< 0.0001	
C	19.13	1	19.13	132.08	< 0.0001	
AB	0.92	1	0.92	6.35	0.0304	
AC	0.32	1	0.32	2.18	0.1705	
BC	6.14	1	6.14	42.37	< 0.0001	
A^2	0.31	1	0.31	2.15	0.1733	
B^2	0.047	1	0.047	0.32	0.5829	
C^2	1.07	1	1.07	7.40	0.0215	
Residual	1.45	10	0.14	-	-	
Lake of fit	0.50	5	0.10	0.53	0.7483	Not significant
Pure error	0.95	5	0.19			
Cor total	108.04	19	-	-	-	

R^2 (0.9866), Adj R^2 (0.9745), Pred R^2 (0.9404), Adeq precision 41.209. *p* values < 0.05 indicate significant differences

activity in comparison to glucose (*A*) and SBP (*C*). Moreover, the lack of fit *F* value was 0.53, which is non-significant relative to the pure error. The model is geared toward perfect fitness.

Interaction between operating factors

According to the ANOVA Table 4, the significance of the independent variables and the interaction between them was determined by *F* values and *p* values. As seen in Table 4, *A*, *B*, *C*, AB, and BC with a very small *p* value ($p < 0.05$) were significant, while AC was insignificant in enzyme production.

Due to the positive linear coefficient of SBP, the enzyme production increased by increasing the SBP concentration within the range. Also, the negative quadratic coefficients of ammonium sulfate and SBP explain the maximum exo-polygalacturonase production at these levels. Subsequently, the enzyme production decreased out of this point. By the coefficients, SBP is determined as the factor which has the most positive impact on enzyme production.

Furthermore, the interaction among variables was confirmed by the 3-D response surface plots used to identify the optimum levels. The interaction between variables was also evaluated in exo-polygalacturonase production.

The 3-D plots represent the interaction between the two factors, while the other factor was fixed at its optimum level for maximum enzyme production (Fig. 2). Figure 2a exhibits the interaction between ammonium sulfate and glucose, revealing an increase in exo-polygalacturonase production by increasing ammonium sulfate and glucose concentrations. The response between glucose and SBP indicated that increasing the SBP content and decreasing glucose concentration led to higher enzyme production (Fig. 2b). The plot for the interaction between SBP and ammonium sulfate (Fig. 2c) represented an increase in enzyme production at the low levels of ammonium sulfate and high levels of SBP.

The maximum enzyme production occurred at high levels of SBP and low levels of glucose and ammonium sulfate.

The effect of all 3 factors on the response was significant ($p > 0.05$), showing the higher and more effective contribution to the enzyme production in SmF (Table 4).

Further, the optimum condition for exo-polygalacturonase production was obtained at 60 g/L of glucose, 4 g/L of ammonium sulfate, and 20 g/L of SBP. At the optimum condition, the enzyme activity increased to 19.41 U/ml which is 5.58-fold more than the unoptimized condition.

The molecular mass of produced exo-polygalacturonase

The supernatant was precipitated by ammonium sulfate. The molecular mass of exo-polygalacturonase was found to be around 60 kDa by SDS-PAGE (Fig. 3).

Simulation results

As shown in Fig. 4, shown nodes the phylogenetic tree gathered from the concentrated rDNA and TEF alignment by applying heuristic ML analysis with the bootstrap values. We compared the phylogenetic relationships of *P. indica* with *Rhizoctonia solani's* extracellular exo-polygalacturonases in the NCBI data bank. In Fig. 5, *Rhizoctonia solani's* and *Thanateporus cucumeris* revealed exo-polygalacturonases

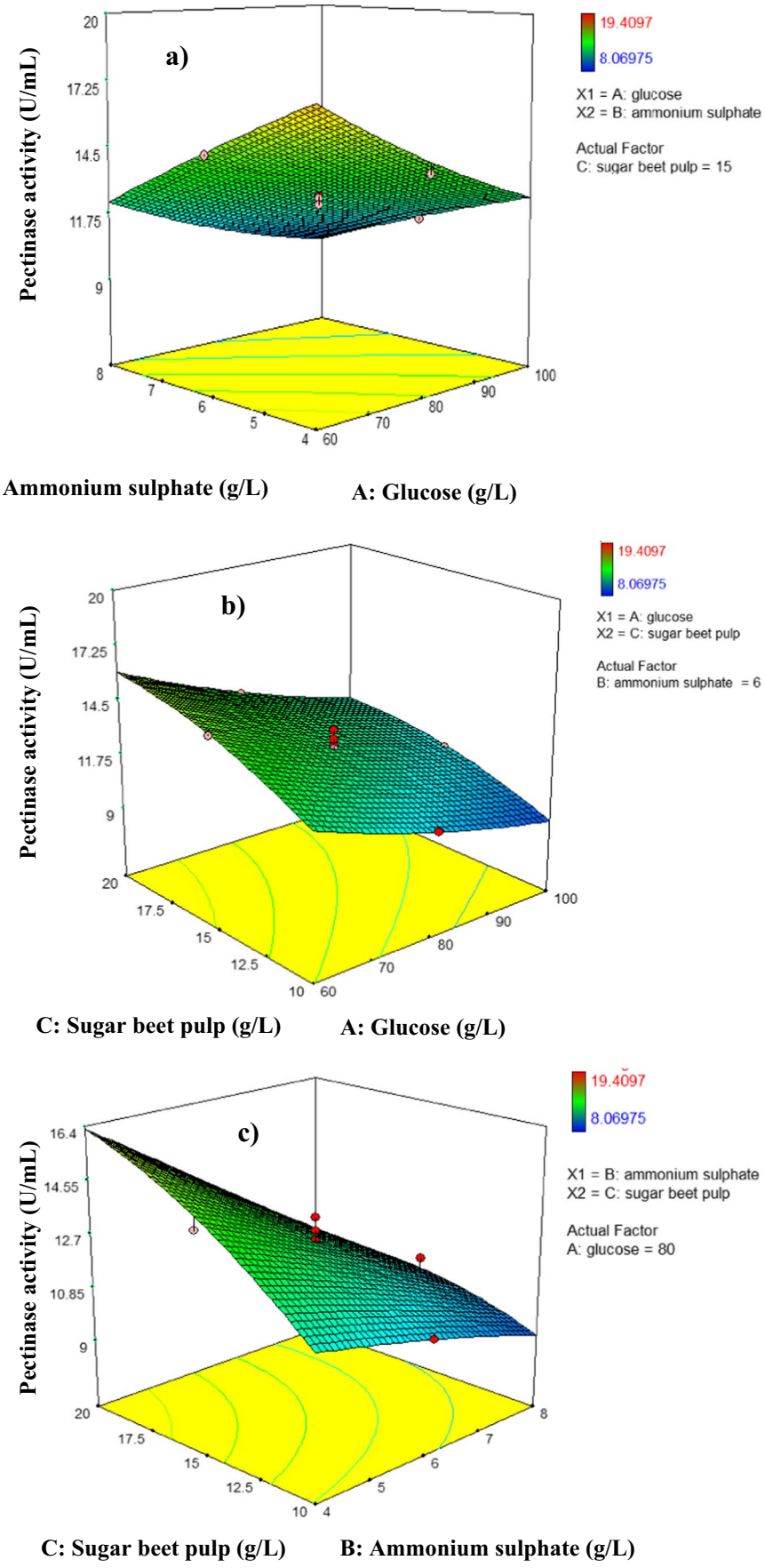

Fig. 2 Response surface plot of exo-polygalacturonase yield under optimal conditions and interaction between variables. (**a**) Interaction between ammonium sulfate and glucose; (**b**) interaction between sugar beet pulp and glucose; and (**c**) interaction between sugar beet pulp and ammonium sulfate

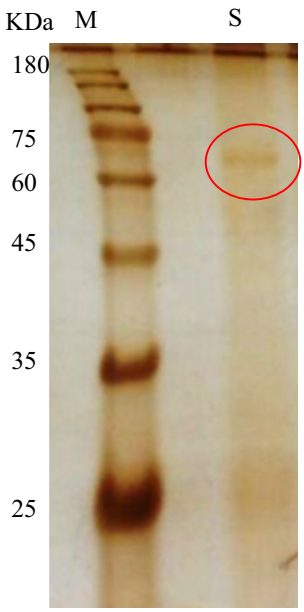

Fig. 3 SDS-PAGE analysis of exo-polygalacturonase. M, protein molecular weight marker; S, the sample

with similar sequences. Also in Fig. 6, we showed the alignment of the predicted amino acids sequences of *P. indica* exo-polygalacturonase with 8 similar sequences.

Discussion

Nowadays, there is a great interest in obtaining pectinase from cheap and suitable substrates like agricultural residues.

Here, the efficiency of *P. indica* in the biodegradation of SBP has been evaluated. Subsequently, the exo-polygalacturonase activity on SBP^+ and SBP^- media has been reported for 10 days (Fig. 1). The exo-polygalacturonase production and fungal growth reached the maximum level simultaneously on both mediums. It is established that the medium's components greatly affect the induction of pectolytic enzymes (Nair and Panda 1997). The utilization of agro-industrial residues, like apple pomace and sugar beet pulp, could further solve the environmental problems of

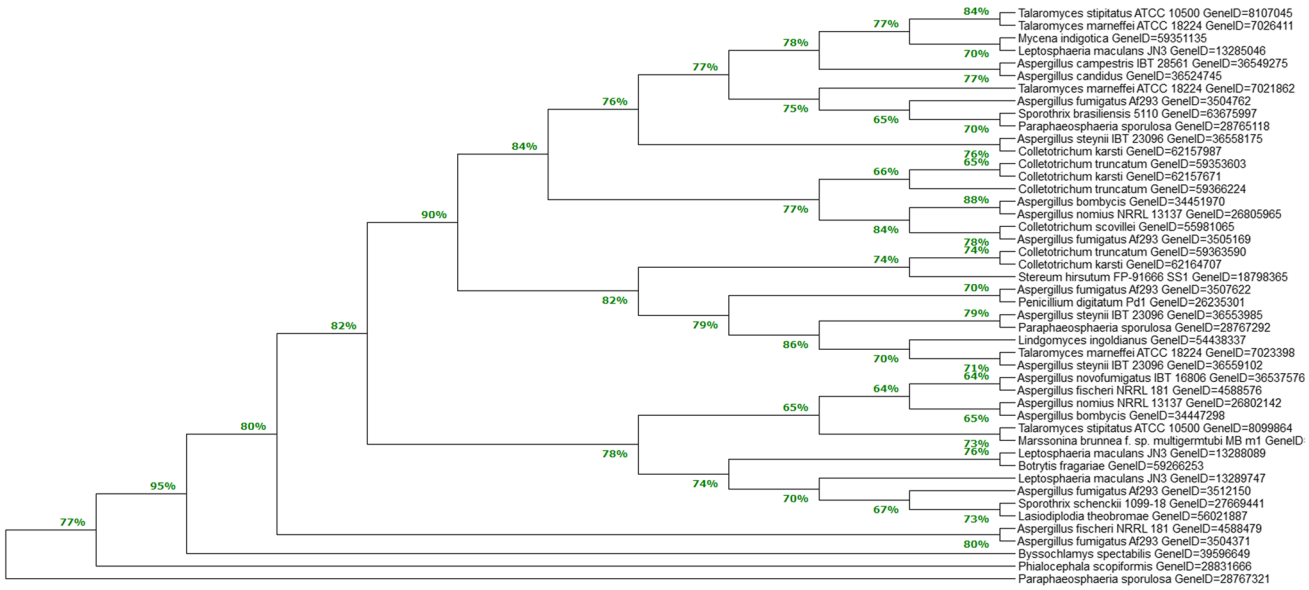

Fig. 4 Phylogenetic tree of the *P. indica* and closely related proteins created using the neighbor-joining method. Bootstrap confidence values (500 repetitions) are shown on nodes. The numbers at each node marked the percentage of supporting bootstrap samples

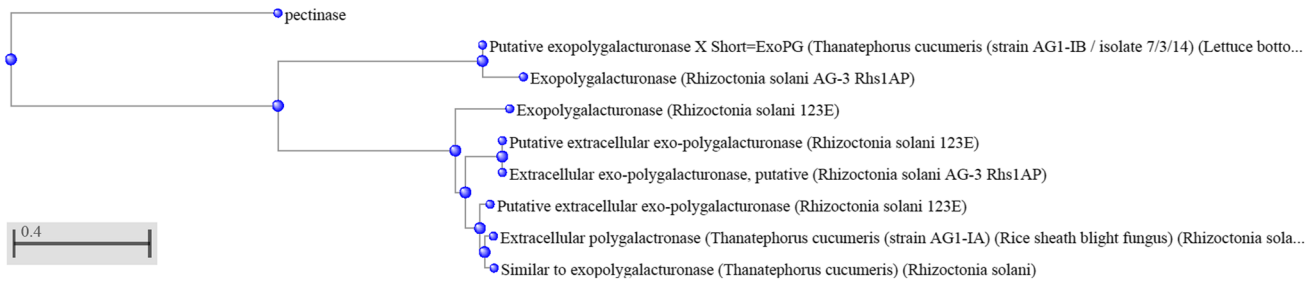

Fig. 5 The phylogenetic tree represents similar DNA sequences to the exo-polygalacturonase gene of *P. indica*. The protein sequences were retrieved from Uniprot and GenBank (NCBI)

Fig. 6 Multiple alignments of the amino acid sequence of *P. indica* exo-polygalacturonase and *Rhizoctonia solani*. The dashes exhibit gaps to improve the alignment. Conserved residues, no gaps, and gaps are represented in red, blue, and gray, respectively. Query_10001: pectinase, Query_10002: extracellular polygalacturonase (*Thanatephorus cucumeris* (strain AG1-IA) (rice sheath blight fungus) (*Rhizoctonia solani*)), Query_10003: putative extracellular exo-polygalacturonase (*Rhizoctonia solani* 123E), Query_10004: putative extracellular exo-polygalacturonase (*Rhizoctonia solani* 123E), Query_10005: extracellular exo-polygalacturonase, putative (*Rhizoctonia solani* AG-3 Rhs1AP), Query_10006: similar to exo-polygalacturonase (*Thanatephorus cucumeris*) (*Rhizoctonia solani*), Query_10007: putative exo-polygalacturonase X Short = ExoPG (*Thanatephorus cucumeris* (strain AG1-IB / isolate 7/3/14) (lettuce bottom rot fungus) (*Rhizoctonia solani*), Query_10008: exo-polygalacturonase (*Rhizoctonia solani* AG-3 Rhs1AP), Query_10009: exo-polygalacturonase (*Rhizoctonia solani* 123E)

the by-products (Heerd et al. 2014). The choice of proper agricultural residue to induce enzyme production depends on cost and availability of the substrates. It is known that the duration of fermentation depends on the medium composition, organism, concentration of nutrients, and physiological conditions (Patil and Dayanand 2006).

SBP has been used as raw material to induce pectinase production by *Aspergillus niger*. Additionally, it has been used as a carbon source and a pectinase inducer to produce extracellular alkaline pectinase, by *Bacillus gibsoni*, under SSF (Jacob 2009). Cultivation of *T. reesei* Rut C-30 on sugar beet pulp (50 g/L), the protein content, pectinase activity, and specific activity reached their maximum value after 60 h of fermentation (0.43 g/l, 0.82 U/ml, and 1.9 U/mg, respectively) (Olsson et al. 2003). The highest enzyme activity by *A. sojae* on 30% sugar beet pulp as an inducer and wheat bran as a medium wetted agent were attained after 8 days (Heerd et al. 2014).

According to our results, the highest activity of exo-pectinase in a medium containing sugar beet pulp was 3.4 U/mL after 96 h of fermentation and following optimization reached 19.41 U/ml.

The greatest endo- and exo-pectinase activity by *A. niger* from sunflower head in SSF (5.1 U/g and 17.1 U/g) and SmF (4.5 U/ml and 16.0 U/ml) were measured on 96 h. According to the different studies about the fermentation time, it exhibited a wide range of 40–120 h and 90–120 h in submerged and solid-state fermentations, respectively (Patil and Dayanand 2006).

The *Colletotrichum* isolated from Argentinian soybean yielded a high amount of the PG (1.08 U/ml) after 7–10 days of incubation and coincided with maximum growth. In a medium involving glucose as a sole carbon source, decreased polygalacturonase production was monitored (Ramos et al. 2010).

Environmental and nutritional factors are two essential factors affecting enzyme production by microorganisms. The pectin and polygalacturonic acid were applied as the only carbon source of the medium which induced the synthesis of pectinolytic enzymes by *A. niger.* There was no pectolytic activity in the medium containing glucose as the only carbon source. Production of pectin-degrading enzymes in the presence of pectin and high glucose concentrations was inhibited, although glucose in low concentrations promoted their production. The observed low pectinolytic activity in media with high glucose concentrations is possibly due to providing the growth needs of organisms by the glucose consumption and causing to decrease the pectin lysis. Furthermore, at low glucose concentrations, high pectolytic activity was observed (Fawole and Odunfa 2003). In agreement with Fawole et al., the highest pectinolytic activity was attained at the lowest glucose concentration (60 g/L).

Pectinolytic activity by *A. niger* on a medium containing pectin, poly galacturonic acid, and glucose at 30 °C for 5 days was 17.2, 13.8, and 0 U/ml, respectively (Fawole and Odunfa 2003).

As it was shown by Aguilar and Huitron (1987), high exogenous glucose and galacturonic acid could be affected endo-PG enzyme production by catabolite repression, whereas glucose did not affect the exo-PG. The glucose concentration above 10% (w/w) in the SSF decreased the activity of endo and exo-PG (Aguilar and Huitrón 1990).

In Solis-Pereyra et al.'s study, exo-PG/gdm and endo-PG/gdm activity by *A. niger* in a medium containing 16% (w/w) citric pectin were 281 U and 152 U, respectively. Moreover, inhibited enzyme production and growth were detected on 20–30% (w/w) pectin concentration (Fontana et al. 2005).

Ammonium sulfate was introduced as the favorable nitrogen source for pectinase production by *A. niger* (Fawole and Odunfa 2003). Our results concur with the observations of Sapunova, who also demonstrated that ammonium salts could act as a stimulator of pectinase production. It has been described that nitrogen limitation decreases the production of polygalacturonase (Thakur et al. 2010).

Bai et al. examined the impact of different nitrogen sources on pectinase induction and great enzyme activity measured with ammonium sulfate, yeast extract, soya peptone, soya pulp, and MGW (Bai et al. 2004).

Patil et al. examined the impact of ammonium phosphate and sulfate on pectinase production by *A. niger* from sunflower head in both SSF and SmF. As their study revealed, ammonium phosphate and sulfate could increase pectinase production in both fermentation conditions. However, this increase was very less with ammonium phosphate than with ammonium sulfate. The maximum production of endo-pectinase and exo-pectinase by DMF 27 was recorded in SmF condition 18.9 U/ml and 30.3 U/ml, respectively (Patil and Dayanand 2006).

As stated in many studies, the average molecular mass of polygalacturonase is 35–80 KDa (John et al. 2020). Different microbial species produced different molecular masses of pectinase enzyme. Different factors such as the substrate, nature of the microorganism, host cell wall, and analytical methods result in different pectinase mass (Oyede 1998). The molecular weight of *P. indica* exo-PG was comparable with other previous reports.

Conclusion

The present study showed that optimized conditions by RSM yielded high exo-polygalacturonase activity by *P. indica* (a root endophytic fungus), and SBP was established as a significant enzyme inducer substrate. In situ analysis confirmed

the similarity of exo-polygalacturonase of *P. indica* with *R. solani's* enzyme. The application of agricultural and agro-industrial wastes could be a great source for enzyme production, because it is economically valuable and decreases environmental pollution. The outcome of the proposed research will open up future pathways for using raw waste materials to produce valuable products with cost-effective and eco-friendly approaches. The outcome of this study will be useful for industries to decrease environmental pollution with cost-effective approaches.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

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Conflict of interests The authors declare no competing interests.

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