Bioconversion of Agro-residues to Make Extracellular Polysaccharides in Solid State Fermentation *via* *Trichoderma hamatum* Using Response Surface Methodology: Antioxidant and α-Glucosidase Inhibitor Activity

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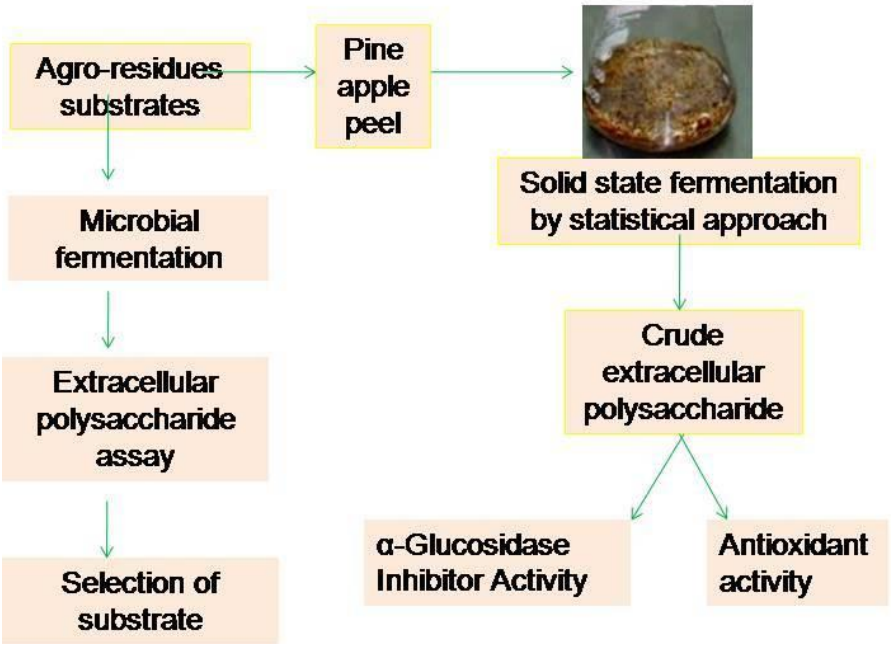
GRAPHICAL ABSTRACT



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Agro-residues, including banana peel, pineapple peel, mango peel, apple peel, and coconut husk, were utilized to produce extracellular polysaccharide (EPS) *via* solid state fermentation(SSF) by the fungus, *Trichoderma hamatum*. EPS production was highest in pineapple peel (84.2 ± 0.4 mg/g), followed by mango peel (70.3 ± 0.41 mg/g) ($p<0.01$). The SSF bioprocess was optimized *via* a two-level full factorial design and response surface methodology. The effects of five selected variables on EPS biosynthesis, namely, the concentrations of glucose (10 to 30%), ammonium sulphate (0.1 to 1%), yeast extract (0.5 to 2%), $MgSO_4$ (0.01 to 0.1%), and medium pH (4.5 to 6.5), were analyzed *via* a full factorial design (FFD). The EPS production ranged widely from 15.3 to 576.2 mg/g substrate. Three significant variables affecting EPS production were assessed in central composite design (CCD) to optimize concentrations of $MgSO_4$, and glucose, and the pH. The designed CCD model was fitted to the quadratic model and was significant ($p<0.0001$). For 50 µL of EPS, the scavenging ratio was 43.4 ± 4.1% at a concentration of 200 µL (78.5 ± 6.9 µL) ($p<0.01$). The extracted EPS exhibited an alpha-glucosidase inhibitory effect ($p<0.001$). Solid-state fermentation allows the utilization of low-cost biomass for EPS production and the application of *T. hamatum* EPS as a natural antioxidant and α-glucosidase inhibitor.

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Keywords: Pineapple peel; Solid-state fermentation; Central composite design; Extracellular polysaccharide; Antioxidant; α-Glucosidase inhibitor

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INTRODUCTION

Agro-industrial residues, which are sometimes called agro-wastes, are generally disposed into the environment and constitute an environmental risk. This has affected the environment and led to the generation of greenhouse gases and affect environmental and human health. Agro-residues, such as seeds, peels, and stones from vegetables and fruits, contain various phytochemical compounds, and these compounds can be utilised for the production of various products (Marraiki *et al.* 2020; Sathya *et al.* 2023). The culture of

microbes on types of agro-residues to produce biochemical compounds in solid state fermentation (SSF) has increased in recent years as an alternative approach to costly synthetic media (Sathya *et al.* 2024; Vijayaraghavan *et al.* 2024). Microorganisms, especially fungi, utilise agro-residues and produce enzymes, extracellular polysaccharides, bioactive compounds, organic acids, and nutritional supplements. The lignocellulosic biomass is composed of lignin, hemicelluloses, and celluloses, which can be utilised by microbes to produce various products. These residues, especially renewable biomasses, are desirable alternative sources for the production of pharmaceuticals, biofuels, and functional compounds (Vijayaraghavan *et al.* 2014; Arokiyaraj *et al.* 2024). *Trichoderma* is one of the largest groups of fungi. It is a member of the Ascomycota. Asexual reproduction occurs *via* conidia. It has been exploited to produce various biomolecules, and has been applied in textiles, paper, agriculture, and biofuels because of its ability to produce biomolecules (Chaverri *et al.* 2003; Giraldo *et al.* 2007).

To date, only a few studies have optimized bioprocesses to produce EPS from *Trichoderma* sp. using response surface methodology. The extracellular polysaccharide-producing strains, such as *Trichoderma erinaceum* DG-312 (Ji-Hoon and won 2005), *Trichoderma* sp. KK19L1 (Li *et al.* 2017), and *Trichoderma harzianum* (Saravanakumar *et al.* 2021), were previously reported. Fungi produce polysaccharides, primarily extracellular polysaccharides (EPSs),which present novel therapeutic characteristics such as antioxidant, antimicrobial, and anticancer properties. EPSs are applied in various processes in various industries, including, the food, cosmeceutical, and pharmaceutical industries (Farraj *et al.* 2024; Rajaselvam *et al.* 2024). Considering the significance and application of EPS for biotechnological processes, EPS can be produced either by submerged fermentation or solid-state fermentation. The process conditions, such as the medium, temperature, and pH, are very significant factors influencing fungal growth and product production (Biji *et al.* 2016; El-Sheikh *et al.* 2020). In the fermentation process, the optimization of bioprocess conditions is a significant approach for improving product yield; hence statistical approach is used to optimize the medium components. Response surface methodology is an important statistical tool for predicting the optimum response of medium components (Al-Farraj *et al.* 2020; Kalaiyarasi *et al.* 2020). In this study, the agro-residues banana peel, pineapple peel, mango peel, apple peel, and coconut husk were used for EPS production. The antioxidant, and α-glucosidase inhibitor properties of EPS were determined.

EXPERIMENTAL

Isolation of Fungi from Sugarcane Bagasse

The sugarcane bagasse sample (0.5 g) was ground using a pestle and mortar, and it was serially diluted with sterile double-distilled water. The diluted sample was spread on potato dextrose agar (PDA) medium and incubated at 28 °C for 4 days. The morphologically dissimilar colonies were preserved on PDA slants at 4 °C and the strain was subcultured every four months. Fungi isolated from sugarcane bagasse were used for extracellular polysaccharide production.

Inoculum

A total of 58 fungal strains were isolated from sugarcane bagasse, and 11 isolates (EP2, EP5, EP13, EP15, EP20, EP28, EP31, EP39, EP48, EP54, and EP58) were selected

for EPS production. These 11 isolates grew well on PDA medium within 96 h of incubation. The isolated 11 strains were cultivated in Sabouraud's broth medium composed of peptone (10 g/L), glucose (40 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), KH_2PO_4 (1.0 g/L), $FeSO_4 \cdot 7H_2O$ (0.05 g/L), and were incubated in an orbital shaker incubator at 175 rpm at 28 °C for 5 days. The seed cultures (EP2, EP5, EP13, EP15, EP20, EP28, EP31, EP39, EP48, EP54, and EP58) were inoculated into a basic mineral salt medium (g/L) composed of dipotassium phosphate 0.700, ammonium chloride 0.800, disodium EDTA 0.0092, magnesium sulphate. heptahydrate 0.010, calcium sulphate, dihydrate 0.002, ferrous sulphate heptahydrate 0.007, zinc sulphate, heptahydrate 0.0001, boric acid 0.0001, cobalt nitrate 0.00001, sodium molybdate dihydrate 0.00001, manganese sulphate, quadrahydrate 0.00002, and copper sulphate pentahydrate 0.0005. In the Erlenmeyer flask, 100 mL culture medium was added and was inoculated with 2% inoculum. The mixture was cultured in an incubator for 10 days at 28 °C.

Extraction of Extracellular Polysaccharide and Assay

The broth culture was centrifuged at 5000×g for 10 min, and the cell-free extract was retained. The supernatant was concentrated *via* a speed vacuum at 40 °C using a rotary evaporator. It was further mixed with 95% ethanol (double volume) and incubated for 24 h at 28°C. The mixture was subsequently centrifuged at 5000×g for 10 min and resuspended in a small volume of ethanol (0.5 mL). It was deproteinized using Sevag reagent (chloroform-n-butanol at 4:1, v/v), decolourized with 10% (v/v) $H_2O_2$for 2 h under stirring, and dialyzed for 4 h. The sample was concentrated with ethanol (95%) and lyophilized. The final yield was weighed, and the sample was considered EPS. The carbohydrate content of each sample was assayed using the anthrone-sulfuric acid method. Approximately 100 µL of sample was hydrolyzed with acid and incubated with anthrone reagent. The absorbance of each sample was read at 620 nm. Glucose (10to100 µg/mL) was used as a reference. The amount of EPS produced was determined by the carbohydrate content (µg) of the EPS/L production medium.

Solid State Fermentation (SSF)

Approximately 5 g of agro-residue (banana peel, pineapple peel, mango peel, apple peel, or coconut husk) was transferred to a 100-mL Erlenmeyer flask, and the moisture content was adjusted to 65%. The substrates were sterilized for 30 min for 1 h at 121 °C. The maximum polysaccharide-producing fungal strain (EP20) was subsequently inoculated into the medium and incubated for 8 days. After 8 days of incubation, the extracellular polysaccharide was extracted and assayed.

Screening of Variables to Produce EPS

The effects of $MgSO_4$, glucose, yeast extract, and ammonium sulphate on EPS production were determined. Pineapple peel (5 g) was mixed with sodium phosphate buffer (3 mL) at pH 6.0. To optimize the $MgSO_4$ concentration, 0.02 to 0.1% $MgSO_4$was added to the substrate. Similarly, the culture medium was supplemented with glucose (20 to 80%), yeast extract (0.25 to 1.25%), and ammonium sulphate (0.2 to 1%) at various concentrations and the optimum concentrations were analyzed.

Screening of Variables for Extracellular Polysaccharide Production

A two-level full factorial design (FFD) was used to screen the significant variables affecting EPS production in SSF. Pineapple peel was used as a solid substrate until

otherwise stated. The FFD was used to screen the medium components influencing EPS production. The selected major medium supplements were, glucose (10 to 30%), ammonium sulphate (0.1 to 1%), yeast extract (0.5 to 2%), medium pH (4.5 to 6.5), and $MgSO_4$ (0.01 to 0.1%), and the FFD experiment was designed using the Design Expert software (Version 8.1, Stat-Ease, Inc., Minneapolis, MN, USA). The selected factors were set at low and high levels and are illustrated in Table 1. The FFD matrix design comprises 32 experiments for five variables.

Table 1. Low and High Values of the Selected Variables for Screening Experiments in a Two-Level Full Factorial Design

Factor	Name	Units	Low Actual	High Actual
A	Yeast extract	%	0.5	2
B	pH		4.5	6.5
C	$MgSO_4$	%	0.01	0.1
D	Glucose	%	10	30
E	Ammonium sulphate	%	0.1	1

Central Composite Design (CCD) and Response Surface Methodology (RSM)

Three significant variables affecting EPS production were assessed *via* CCD design to optimize the concentrations of $MgSO_4$, glucose and the pH of the culture medium. A total of 20 experiments were performed based on the Design-Expert matrix. In recent years, CCD has been widely used to optimize culture medium components and environmental factors for product development. This method is considered as precise and has proved to be an efficient method for designing, predicting, and optimizing variables. The selected variables were screened at five different levels (−1.682, −1, 0, +1, +1.682) as depicted in Table 2.

Table 2. Variables and Levels in Central Composite Design and Response Surface Methodology

Factor	Name	Units	Low Coded	High Coded	Mean
A	pH		-1	1	5.75
B	$MgSO_4$	(%)	-1	1	0.06
C	Glucose	%	-1	1	12.39

Antioxidant Activities

The free radical scavenging ratio of EPS to1-diphenyl-2-picryl-hydrazyl (DPPH) was tested as described previously (Shimada *et al.* 1992). The DPPH was prepared in ethanol at 0.2 mmol/L concentration. A total of a 2 mL of DPPH solution was mixed with EPS at various concentrations (50 to 250 µL) and incubated at 30 ± 1 °C for 30 min. After 30 min of incubation, the optical density of the sample was evaluated at 517 nm against the reagent blank. The free radical scavenging activity was calculated *via* Eq.1,

$$\text{Free radical scavenging ratio} = (1 - A_1/A_0) \times 100 \quad (1)$$

where A_0 is the absorbance of the blank and $A_1$is the absorbance for the test condition.

Determination of Alpha-Glucosidase Inhibition Activity

The α-glucosidase inhibitory activity of EPS was determined. Alpha-glucosidase was prepared at a 1 U/mL concentration in 0.1 M PBS (pH 6.5) and EPS was prepared at various concentrations (2, 4, 6, 8, and 10 mg/mL). Approximately 50 µL of the diluted sample was mixed with 100 µL of the diluted alpha-glucosidase solution and incubated for 10 min at 28 ± 1 °C. After 10 min incubation, 50 µL of 4-nitrophenyl-β-D-glucopyranoside (5 mM) was added and the mixture was incubated for 5 min at 28 ± 1 °C. The absorbance of each sample was read at 405 nm before and after the final incubation. Acarbose (0.01 mg/mL) was used as the positive control and the results are expressed as follows,

$$\alpha\text{-glucosidase inhibition } (\%) = \left(1 - \Delta A_{sample} / \Delta A_{control}\right) \times 100 \quad (2)$$

where A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control.

Statistical Analysis

The experimental procedures were done three times for each condition, and the final results are expressed as mean ± standard deviation. A p-value<0.05 was considered a statistically significant result.

RESULTS AND DISCUSSION

Screening of Extracellular Polysaccharide-Producing Fungi

The fungal strains (EP2, EP5, EP13, EP15, EP20, EP28, EP31, EP39, EP48, EP54, and EP58) screened from the sugarcane bagasse produced extracellular polysaccharides. The isolated strains were cultivated in a Sabouraud's broth medium and the yield varied on the basis of the isolate. Compared with the other isolates, EP20 produced a significant amount of EPS (51.2 ± 1.6 mg/mL) ($p<0.01$) (Fig. 1). Fungi and bacteria produce EPS for industrial applications. Considering the significance of EPS, the isolated fungi were screened for EPS production. Microbial EPS are the subject of research in various fields in science and technology.

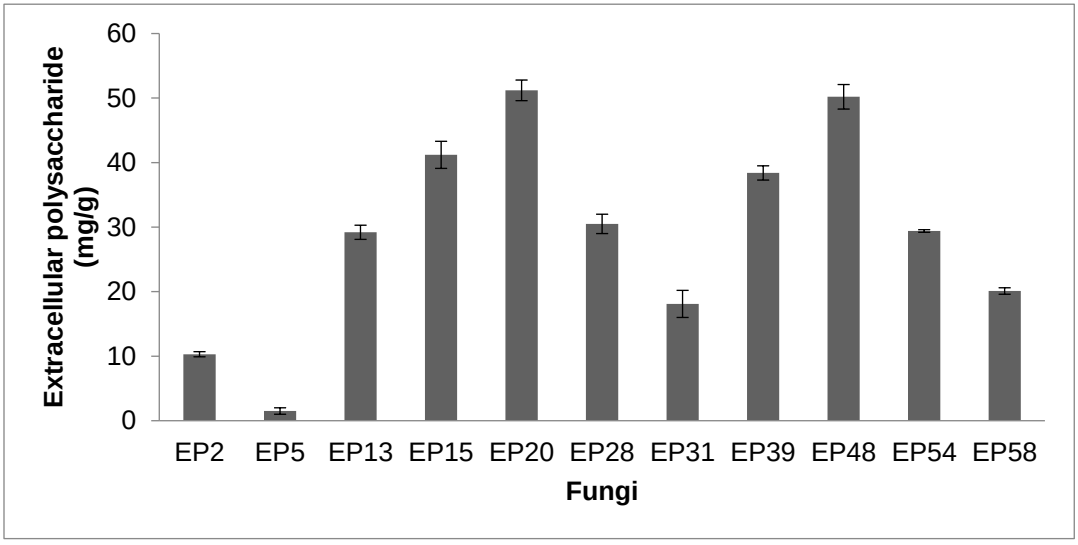


Fig. 1. Extracellular polysaccharide-producing fungi isolated from sugarcane bagasse

The major research direction is focused on determining variables responsible for synthesis, optimization, and application of EPSs in various fields. The bioprocess of EPS derivation involves selecting suitable fungi, culture (solid state fermentation or submerged fermentation) and suitable extraction methods (Osińska-Jaroszuk *et al.* 2015).

Agro-residues as a Cheap Substrate for Extracellular Polysaccharide Production

To screen agro-residues to produce EPS, various biomass, including banana peel, pineapple peel, mango peel, apple peel, and coconut husk were used. The fungus *T. hamatum* EP20 utilized these substrates and produced EPS in SSF. Moreover, the amount of EPS produced varied among substrates (Fig. 2). The EPS production was high in the pineapple peel (84.2 ± 0.4 mg/g), followed by mango peel (70.3 ± 0.41 mg/g). Among the biomass, coconut husk showed the least EPS production (12.1 ± 0.2 mg/g) ($p<0.01$). Fungi from the Ascomycota and Basidiomycota groups presented potent biological and industrial properties. The selected *T. hamatum* EP20 was an Ascomycota group fungus that may be highly applicable in industry. Similarly, fungi from the Basidiomycetes group utilize solid substrates, especially broadbean seed capsules, cane bagasse, and the strain *Kosakonia cowanii* LT-1 which utilize a mixture of these two substrates and produce EPS (Montoya *et al.* 2013). Moreover, the amount of EPS produced by the strain *K. cowanii* LT-1 was lower than that in this study. EPS producing basidiomycetes species, including *Lentinula edodes*, *T. versicolor*, and *Pleurotus ostreatus* were used,and they utilized lignocellulosic residues such as coconut fiber, oak sawdust, corn bran, coffee husks, and soybean oil (Sánchez and Montoya 2020). The variation in the biomass composition positively regulated EPS production in SSF. In this study, *T. hamatum* EP20 utilized peels and coconut husk, and EPS production varied with substrate. The lignocellulose biomass is considered the substrate for the production of extracellular polysaccharides. Lignocelluloses are composed of lignin, hemicelluloses, and cellulose, among which sugar polymers account for a large proportion of the biomass. Microorganisms utilise these sugars for the conversion of polysaccharides (Manivasagan *et al*. 2013). Thus, agro-residues, preferably lignocellulose biomass can be utilized for polysaccharide production.

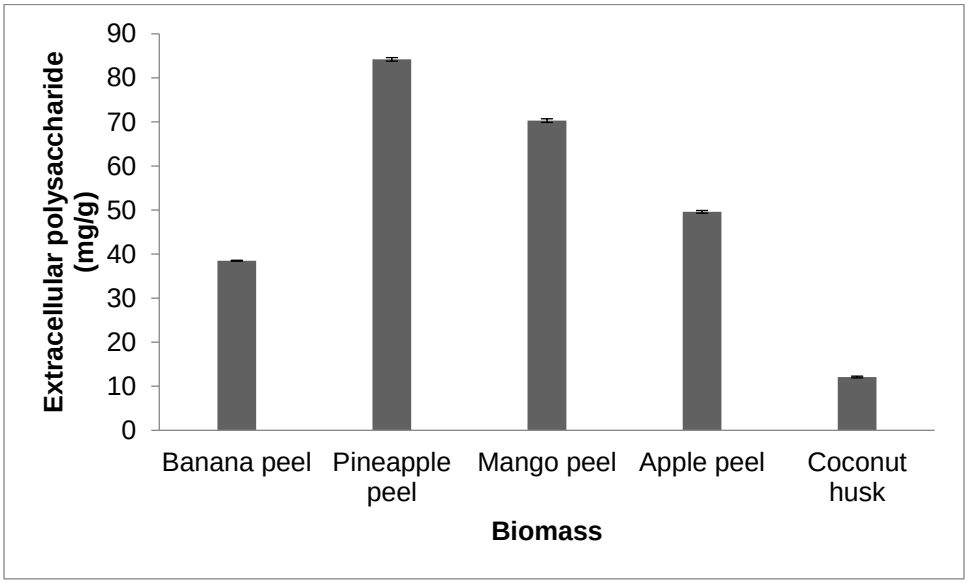
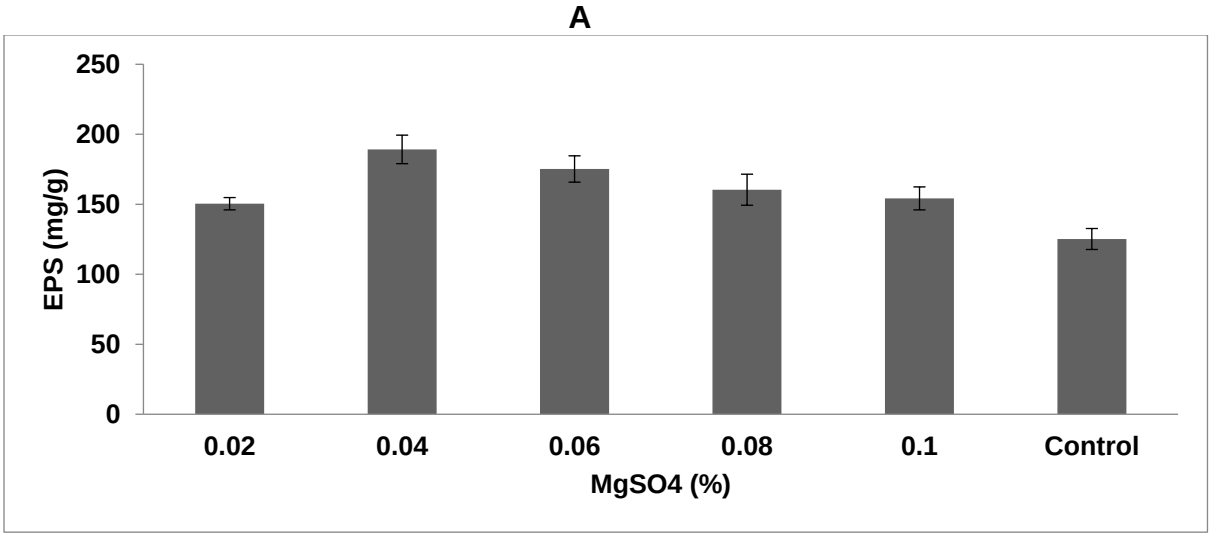


Fig. 2. Agro-residues to produce extracellular polysaccharides in solid state fermentation

Preliminary Screening of Nutrient Factors for Improved Production Extracellular Polysaccharide

In EPS production, nutrient factors ($MgSO_4$, glucose, and ammonium sulphate) significantly influenced product yield. $MgSO_4$ was tested at various concentrations, and maximum EPS production was achieved at 0.04% $MgSO_4$ concentration (189.2 ± 10.2 mg/g) (Fig. 3a). The effects of glucose concentration on EPS production were tested (Fig. 3b). Higher EPS production (184.2 ± 7.4 mg/g substrate) was obtained at 60% glucose concentration. Furthermore, most microorganisms have been reported to utilize sucrose or glucose to produce extracellular polysaccharides (Zhang *et al.* 2020);therefore glucose was selected as the preferred carbon source. In this study, EPS production improved at increasing concentrations of glucose up to 60% level. The effects of the yeast extract concentrations on EPS production were tested (Fig. 3c). The higher EPS production (154.3 ± 1.7 mg/g substrate) was obtained at 0.75% yeast extract. Various ammonium sulphate concentrations were tested in the present study to determine their effects on EPS production and maximum EPS production was obtained at 0.6% ammonium sulphate (182.5 ± 4.5 mg/g substrate) (Fig. 3d). Several nitrogen sources have been screened for EPS production; however, the yield varies with nitrogen source and organism. The supplemented nitrogen sources improved the biosynthesis of polysaccharides, and this finding was similar to results of Cui *et al.* (2017), in which available nitrogen sources improved the growth of bacteria and EPS production. The optimum carbon/nitrogen ratio is critical for EPS production; thus optimization of process parameter is warranted for the improvement of EPS yield. In the current study, EPS production was affected at higher ammonium sulphate concentrations, and these results are in accordance with the previous reports. Lo *et al.* (2007) reported the inhibitory effect of nitrogen sources on EPS yield in *P. acidipropionici* culture, whereas EPS production was improved in *S. thermophilus*. This previous study revealed that the requirements of nitrogen sources vary among organisms. Hence, optimization of culture conditions for any new isolate is needed to analyse nutrient requirements to improve product yield.



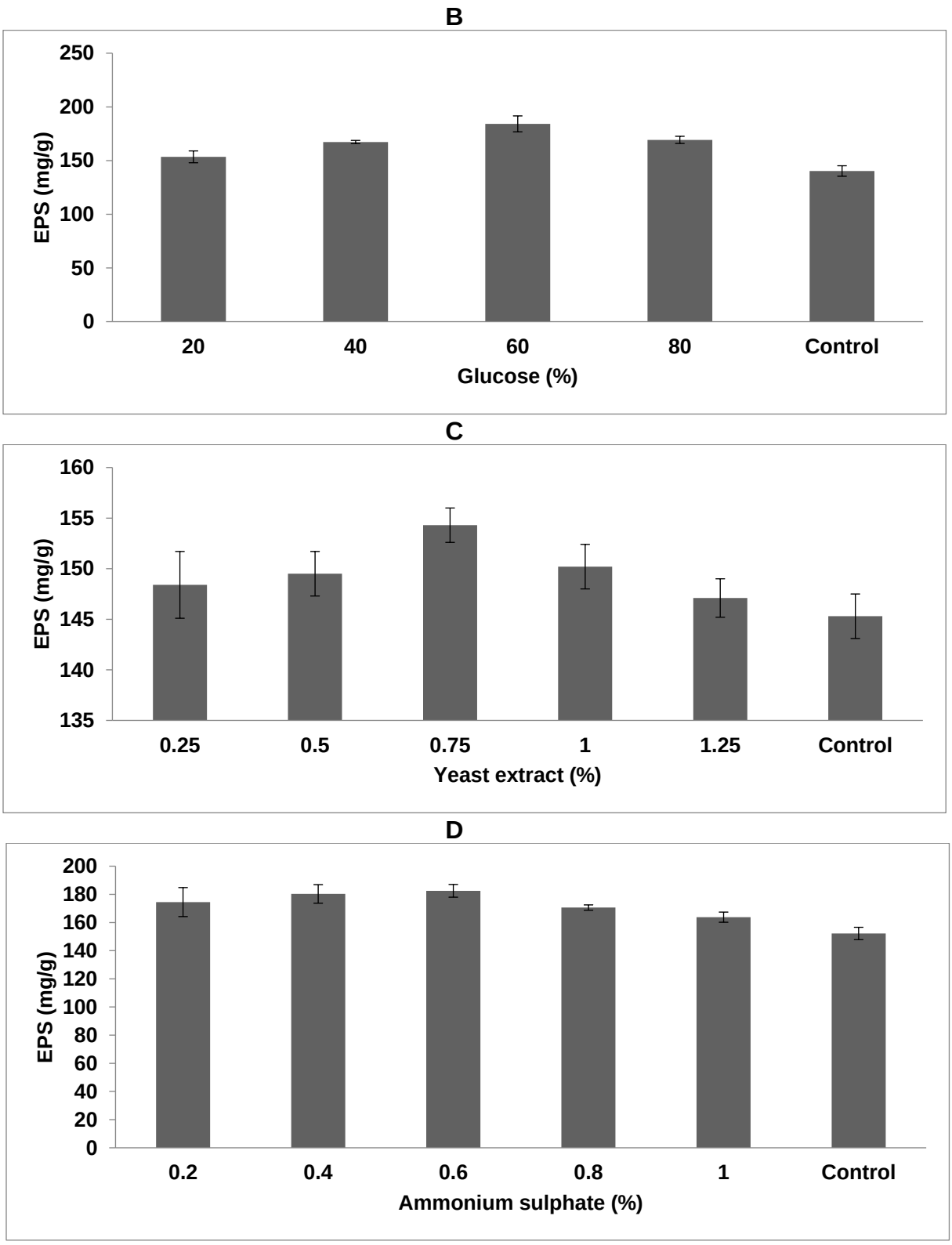


Fig. 3. Effects of $MgSO_4$ (A), Glucose (B),Yeast extract (C), and Ammonium sulphate (D) on EPS production. Pineapple peel (5 g) was mixed with various concentrations of $MgSO_4$, glucose, yeast extract, and ammonium sulphate. Then, it was inoculated with 2% inoculums and incubated for 8 days at 32 ± 1 °C.

Two-Level Full Factorial Design and Analyses

A full factorial design enables the determination of the main effect of each factor, and it allows screening of the interactive effect of factors using low and high levels. The effects of the five variables on EPS biosynthesis, *i.e.*, the concentrations of glucose (10to30%), ammonium sulphate (0.1to1%), yeast extract (0.5to2%), $MgSO_4$(0.01to0.1%), and the pH of the medium (4.5to6.5) were analysed *via* FFD. The response of variables (EPS production) varied widely from 15.3 to 576.2 mg/g substrate (Table 3).

Table 3. Two Level Full Factorial Experimental Design and Extracellular Polysaccharides Production

Run	Yeast Extract (%)	pH	$MgSO_4$ (%)	Glucose (%)	Ammonium Sulphate (%)	EPS (mg/g)
1	0.5	4.5	0.01	10	1	30.4
2	2	6.5	0.1	10	0.1	120.1
3	0.5	4.5	0.01	30	0.1	230.5
4	0.5	6.5	0.1	10	1	120.8
5	2	6.5	0.1	30	0.1	576.2
6	2	4.5	0.1	30	1	220.3
7	0.5	6.5	0.1	30	0.1	210.5
8	0.5	6.5	0.01	30	0.1	150.4
9	2	6.5	0.1	30	1	518.5
10	0.5	4.5	0.1	30	1	46.3
11	2	6.5	0.01	30	1	316.2
12	2	4.5	0.1	10	0.1	150.1
13	0.5	4.5	0.01	30	1	30.6
14	0.5	4.5	0.1	30	0.1	239.3
15	2	6.5	0.1	10	1	238.5
16	0.5	4.5	0.1	10	1	119.5
17	0.5	6.5	0.01	30	1	249.3
18	0.5	4.5	0.01	10	0.1	28.3
19	2	6.5	0.01	10	0.1	240.5
20	0.5	6.5	0.1	10	0.1	120.5
21	0.5	6.5	0.01	10	1	182.4
22	2	4.5	0.01	30	0.1	120.5
23	2	4.5	0.1	10	1	118.5
24	2	4.5	0.01	10	1	26.4
25	2	4.5	0.01	30	1	180.4
26	2	4.5	0.01	10	0.1	15.3
27	2	4.5	0.1	30	0.1	140.5
28	0.5	6.5	0.01	10	0.1	240.5
29	0.5	4.5	0.1	10	0.1	150.2
30	2	6.5	0.01	30	0.1	60.2
31	0.5	6.5	0.1	30	1	398.4
32	2	6.5	0.01	10	1	175.2

The variation in EPS yield revealed the significance of the screening method for analysing the interactive effect between the selected variables. The analysis of variance of the full factorial experiments is depicted in Table 4. The concentrations of $MgSO_4$, yeast extract, and glucose significantly influenced EPS production and the p-values were0.01, 0.00032, and 0.002, respectively. The F-test analysis revealed that pH significantly influenced EPS production (p=0.0003). Balasubramanian *et al.* (2019) screened medium components to produce EPS by *Aspergillus* sp. The supplementation with gelatine, maltose, and divalent ions influenced EPS production in SSF. The EPS yield ranged between 3.18 and 11.65 mg/g substrate, and the yield obtained in the present study was greater than that reported previously. The two-level full factorial design and Plackett–Burman design are frequently used to screen process variables before optimising the concentrations of variables or factors involved in bioprocesses (Al-Ansari *et al.* 2020; Marraiki *et al.* 2020). In the present study, a two-level full factorial design was employed to screen the selected variables to determine the optimum response and the Plackett–Burman design was employed to screen a large number of variables. The two-level factorial design was best suited to screen a small number of variables, because a large number of experimental runs are needed.

Table 4. Analysis of Variance of the Two Level Full Factorial Design Experiments to Produce Extracellular Polysaccharide

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F
Model	481922.8413	20	24096.14	4.685351	0.0058
A-Yeast extract	51092.31	1	51092.31	13.092	0.0032
B-pH	134045.4753	1	134045.5	26.06434	0.0003
C-$MgSO_4$	45836.35031	1	45836.35	8.912604	0.0124
D-Glucose	81093.71281	1	81093.71	15.76819	0.0022
E-Ammonium sulphate	991.2378125	1	991.2378	0.19274	0.6691
AB	7071.577813	1	7071.578	1.375026	0.2657
AC	14659.00031	1	14659	2.850355	0.1195
AD	7365.945313	1	7365.945	1.432264	0.2566
AE	9908.800313	1	9908.8	1.926707	0.1926
BC	866.3203125	1	866.3203	0.168451	0.6894
BD	6947.257813	1	6947.258	1.350853	0.2697
BE	19144.35281	1	19144.35	3.722505	0.0799
CD	20640.04031	1	20640.04	4.013332	0.0704
DE	2550.765313	1	2550.765	0.495981	0.4959
ABD	1502.890313	1	1502.89	0.292228	0.5996
ABE	8394.840313	1	8394.84	1.632327	0.2277
ADE	3302.812813	1	3302.813	0.642212	0.4399
BCD	72171.50281	1	72171.5	14.03332	0.0032
BDE	15046.78781	1	15046.79	2.925758	0.1152
ABDE	16375.97531	1	16375.98	3.184211	0.1019
Residual	56571.55094	11	5142.868		
Cor Total	538494.3922	31			

Five variables were selected, and it comprised a total of 32 experimental runs; hence, this method was selected. Manivasagan *et al.* (2013) screened variables, including pH, NaCl concentration, fructose, tryptone, glucose, and yeast extract *via* Plackett-Burman design for EPS production by *Streptomyces violaceus* MM72, and central composite design was preferred for the determination of optimum response. In the present study, central composite design was utilized to determine the optimum medium components for EPS production in the SSF.

Central Composite Design and Response Surface Methodology

Based on a two-level full factorial experimental design, three variables (pH, $MgSO_4$, and glucose) were selected for optimizing the variable concentrations. The middle value of the variables was selected based on two-level full factorial design experiments. The CCD model analyzes the effects of variables at five different levels.The experiment was designed *via* Design-Expert software. A total of 20 experimental runs were used to determine the optimum response, and the results of CCD are summarized in Table 5.

Table 5. Matrix of CCD and Extracellular Polysaccharide

Run	pH	$MgSO_4$ (%)	Glucose (%)	EPS (mg/g)
1	5	0.02	45	192
2	5	0.02	15	102.1
3	5.75	0.06	30	495.8
4	5	0.1	45	502
5	5.75	-0.007272	30	108.5
6	5.75	0.06	4.77310754	139.2
7	6.5	0.1	15	158
8	5.75	0.06	30	556.2
9	5.75	0.06	55.2268925	396.4
10	4.488655	0.06	30	308
11	5.75	0.1272717	30	403
12	6.5	0.02	45	405
13	5.75	0.06	30	498.2
14	5.75	0.06	30	494.1
15	5.75	0.06	30	501.2
16	6.5	0.1	45	470.5
17	6.5	0.02	15	112.4
18	5.75	0.06	30	427.5
19	5	0.1	15	385.3
20	7.011345	0.06	30	342

The table highlights the significant impact of glucose and $MgSO_4$ on EPS production when *T. hamatum* EP20 was employed in SSF. The supplemented $MgSO_4$ and glucose on pineapple peel substrate significantly improved EPS production to a greater extent than pH variance. In the current study, EPS yield positively correlated with $MgSO_4$and glucose concentrations, revealing the significant role of metal ions and glucose

in the production of EPS in fungi. The carbon sources and mineral ions in the culture medium regulated fungal growth and EPS yield (Lin and Chen 2007; Vijayaraghavan *et al*. 2016). The production of EPS ranged from 102.1to556.2 mg/g. The variation in yield reflected the significance of optimization methods on EPS production. The designed CCD model was fitted to a quadratic model, and the ANOVA results revealed that the model was statistically significant (F-test value 40.25, p-value <0.0001). The lack of fit of the model was insignificant, and an insignificant lack of fit was good for this model. The determination coefficient R^2 value was 0.97, and the adjusted R^2 value of 0.949 revealed that most of the total variation in EPS yield was attributable to the screened factors and that only approximately 3% of the total variation could not be explained by the designed model. The lack-of fit of this designed CCD model was 0.767, which confirmed the validity of the designed model (Table 6). The present findings revealed the significance of the response surface model in determining the optimum response of the variables. Response surface methodology has been frequently employed in the optimization of EPS production using agro-residues. Similarly, an orthogonal design has been employed to optimize EPS production by *Cordyceps militaris* in solid state fermentation using rice as a culture medium. The supplementation of peptone, glycerin, fructose, corn bran, and $MgCl_2$ improved the EPS yield in fermented rice (Xu *et al.* 2019). This previous result was in agreement with the current study and supported the role of sugars and $MgCl_2$ in EPS production.

Table 6. Analysis of Variance of the CCD

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F
Model	454019.9	9	50446.65	40.25438	< 0.0001
A-pH	34.41963	1	34.41963	0.027465	0.8717
B-$MgSO_4$	105369.2	1	105369.2	84.08031	< 0.0001
C-Glucose	113362.5	1	113362.5	90.45871	< 0.0001
AB	272.6113	1	272.6113	0.217533	0.6509
AC	19850.28	1	19850.28	15.83972	0.0026
BC	29052.55	1	29052.55	23.18276	0.0007
A^2	9132.4	1	9132.4	1.1083	0.3171
B^2	93020.13	1	93020.13	74.22629	< 0.0001
C^2	83416.36	1	83416.36	66.56287	< 0.0001
Residual	12531.97	10	1253.197		
Lack of Fit	4181.646	5	836.3292	0.500777	0.7670
Pure Error	8350.32	5	1670.064		
Cor Total	466551.8	19			

Response Surface Graphs

The 3D response surface graph is a graphical representation of the fitted quadratic polynomial equation. The 3D response surface graph allows visualization of the relationship between the experimental levels and the responses of each factor, and the interactions between the variables from the elliptical or circular nature of the plots. The

response surface plot in Fig. S1A shows the impact of $MgSO_4$ and pH on EPS yield and their interactions when the glucose level was considered zero. An elliptic surface plot is shown in Fig. S1B, revealing an interaction between glucose and pH for EPS production when the $MgSO_4$ level was considered zero. Figure S1C reveals the interactive effect of glucose and $MgSO_4$ on the EPS yield when the pH was middle level. When the concentration of $MgSO_4$ and the pH of the culture medium increased, the EPS yield gradually increased and then decreased. When the concentration of glucose in the medium was increased, EPS yield was augmented and then slowly decreased. When the concentrations of glucose and $MgSO_4$ increased, EPS yield increased and then decreased. A mathematical model was used to predict the corresponding optimum response to achieve the maximum EPS yield.

The final equation in terms of coded factors was as follows:

$$EPS=+494.82+1.59A+87.84B+91.11C-60.26AB+49.81AC+ 5.84BC+5.86\ A^2+80.34B^2+76.08C^2 \quad (3)$$

The optimum medium values calculated *via* Design Expert software were: pH 5.77, 0.078% $MgSO_4$, and 38.1% glucose. Response surface methodology was used previously to determine the optimum response of EPS production. However, the variables and their ranges vary widely on the basis of fermentation type and organism. Liu and Wang (2007) utilised a three-level Box–Behnken factorial design for *Agaricus blazei* and the optimized medium components were, 26.3 g/L glucose, 6.84 g/L yeast extract, and 6.62 g/L peptone. In the present study, the requirement of glucose was slightly greater (38.1%) than that of *Agaricus blazei* for EPS production. In addition, extracellular polysaccharide production was optimized earlier *via* response surface methodology in *Ganoderma formosanum*. The optimum medium components were 49.2 g/L glucose, an initial pH of 5.3, and 4.9 g/L of yeast extract. The optimized medium improved EPS production and the yield was 1.4-fold greater than that of the unoptimized medium (Hsu *et al.* 2017). However, in the present study, yeast extract had a weaker effect on EPS production; hence, it was not considered for the CCD model. With the respect to other factors, glucose significantly improved EPS production in the current study, and pH had less of an impact on EPS production. These findings were consistent with the results obtained for *Ganoderma formosanum*. In one study, Ragavan and Das (2019) analyzed the optimum culture conditions to improve EPS production *via* response surface methodology in *Lipomyces starkeyi* VIT-MN03. The selected variables such as sucrose content, pH and incubation period positively influenced EPS production compared with the incubation temperature. The probiotic yeast *L. starkeyi* VIT-MN03 produced EPS which was sixfold greater than that of unoptimized medium. Earlier investigations and the present study revealed variations in nutrient requirements and optimal environmental conditions among microorganisms for their growth and EPS yield.

Validation of the Experimental Design

The predicted optimum response *via* the regression polynomial equation was 561 mg/g, and the corresponding variable concentrations were, pH 5.77, $MgSO_4$ (0.0781%), and 38.1% glucose. To validate the predicted response, experiments were performed to evaluate the predicted optimum response. Under the experimental conditions, the EPS yield was 544 mg/g, which was close to the predicted response (611 mg/g). The present findings

revealed that the designed model was adequate and satisfactory for reflecting the significance of the central composite model.

Antioxidant Activity

The free radical scavenging ratio of the crude EPS was tested at various concentrations. At 50 µLof EPS, the scavenging ratio was 43.4 ± 4.1% and reached the maximum activity at 200 µLof EPS (78.5 ± 6.9%). The results revealed that the antioxidant activity was significantly increased with increasing concentration ($p<0.01$) (Fig. 4). The crude extract obtained in this study showed potent antioxidant activity, which was greater than that reported in previous reports. The antioxidant properties of EPS were reported previously. Chen *et al.* (2010) used *Rhodella reticulate* crude extract which exhibited superoxide anion radical scavenging activity and was greater than that of α-tocopherol (Chen *et al.* 2010). In addition, EPS isolated from *Morchella esculenta* showed hydroxyl radical scavenging activity and moderate 1,1-diphenyl-2-picryldydrazyl radical scavenging activity (Fu *et al.* 2013). The dose-dependent antioxidant activity of EPS assayed in the present study was comparable to the antioxidant activity of EPS from *Fomitopsis pinicola* (Hao *et al.* 2016) and *Aspergillus terreus* (Wang *et al.* 2013).

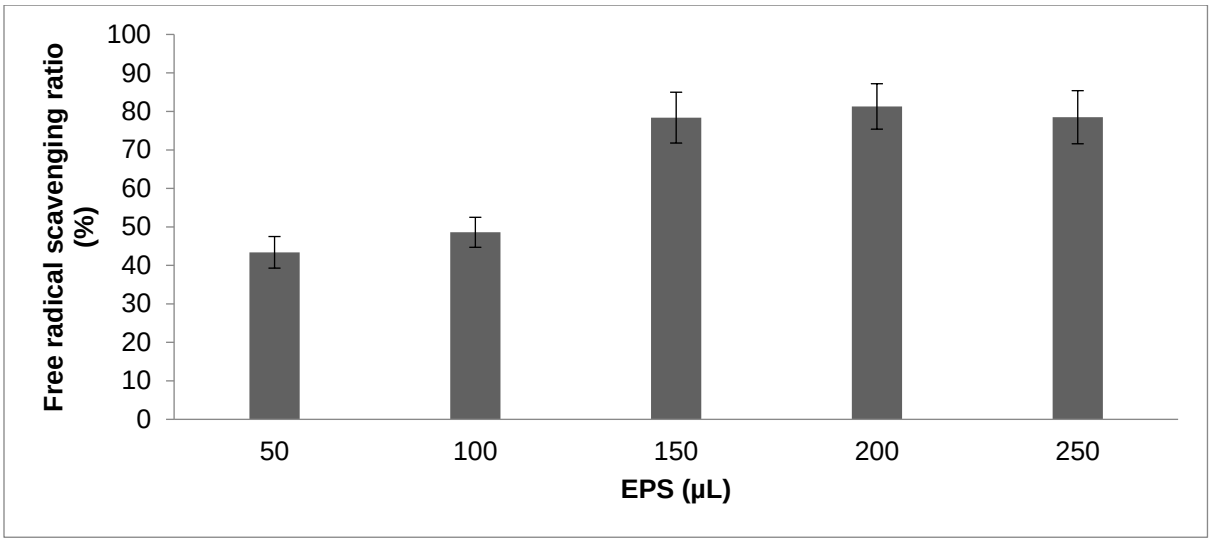


Fig. 4. Effect of EPS concentration on free radical scavenging ratio. To determine the antioxidant activity, EPS was tested at various concentrations (50 to 250 µL) and incubated for 30 min. The amount of free radical scavenging activity was expressed as % free radical scavenging ratio.

Alpha-Glucosidase Inhibitory Effects of EPS

Alpha-glucosidase is an important enzyme that catalyzes carbohydrates digestion in the small intestine, and the alpha-glucosidase inhibitory effect of EPS can delay the enzymatic breakdown of starch, maintaining blood glucose at low levels. Hence, alpha glucosidase inhibitors are important factors for type II diabetes treatment. As shown in Fig. 5, the amount of EPS that inhibited alpha-glucosidase increased with increasing EPS concentration ($p<0.001$). At a concentration of 10 mg/mL concentration, the glucosidase inhibitory effect was 56.9±2.5%. Acarbose exhibited 91.4 ± 1.3% activity at 0.01mg/mL concentration. α-Glucosidase is a main target in the management of diabetic cases. Therefore, interest has been growing in the analysis of α-glucosidase inhibitor activity among extracellular polysaccharide samples from various sources has increased. The EPS isolated from *Lachnum* YM406 exhibited potential α-glucosidase inhibitor activity (Jing *et*

al. 2016). The EPS isolated from *Annona squamosa* showed α-glucosidase inhibitor activity. The purified EPS increased the activity of α-glucosidase inhibitors with an IC_{50} of 0.667 mg/mL (Gu *et al.* 2021). Hang *et al.* (2020) reported α-glucosidase inhibitory activities of the fungus *Ophiocordyceps sinensis* and the isolated EPS was considered for clinical applications. The EPS isolated from *Hypsizygus marmoreus* and *Aconitum coreanum* were associated with α-glucosidase inhibitor activity. *In vivo* analysis revealed that the administration of EPS delayed sugar absorption and was useful in the management of hyperglycemic patients (Song *et al.* 2020a,b).

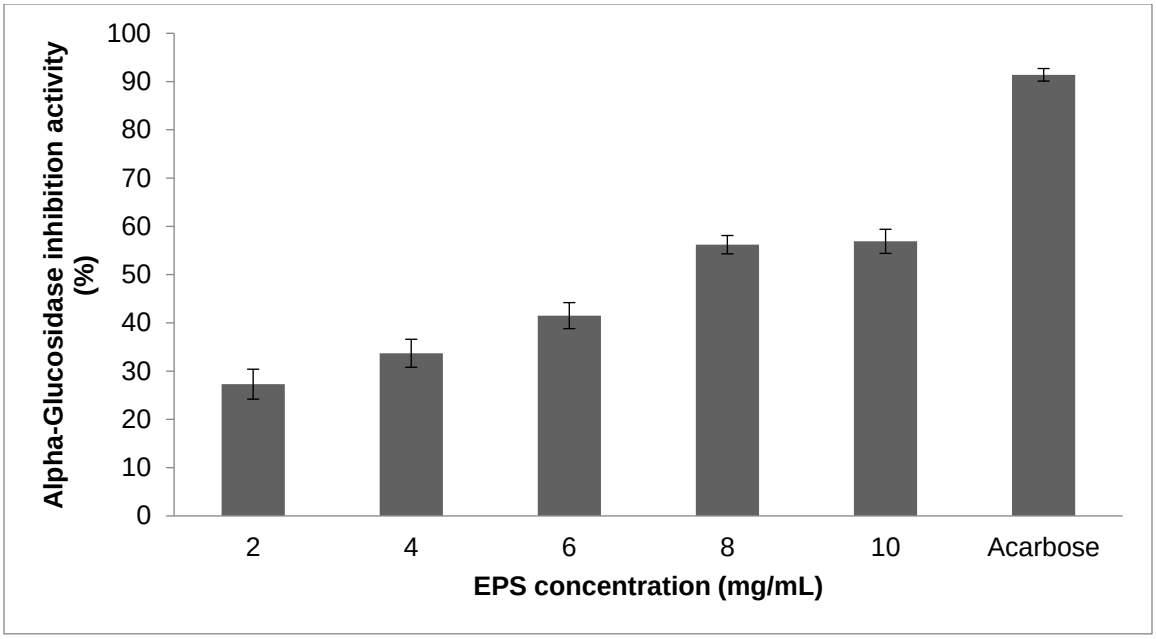


Fig. 5. Alpha-glucosidase inhibitory effect of crude extracellular polysaccharide at various concentrations

CONCLUSIONS

1. The lignocellulosic biomasssuch as banana peel, pineapple peel, mango peel, apple peel, and coconut husk were utilized to produce extracellular polysaccharides in solid-state fermentation. Compared with other lignocellulosic biomasses, the isolated fungus, *Trichoderma hamatum* significantly increased extracellular polysaccharides (EPS) production in pineapple peel substrates.
2. The two-level full factorial design was more suitablefor screening the medium components and physical parameters to improve the EPS yield. The central composite design was effective, and the EPS yield improved >2 fold compared with that of the unoptimized medium.
3. The EPS exhibited free radical antioxidant activity in a dose-dependent manner.EPS had an alpha glucosidase inhibitory effect and the inhibitory effect increased with increasing EPS concentration ($p<0.001$).

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REFERENCES CITED

Al Farraj, D. A., Kumar, T. S. J., Vijayaraghavan, P., Elshikh, M. S., Alkufeidy, R. M., Alkubaisi, N. A., and Alshammari, M. K. (2020). "Enhanced production, purification and biochemical characterization of therapeutic potential fibrinolytic enzyme from a new *Bacillus flexus* from marine environment," *Journal of King Saud University-Science* 32(7), 3174-3180.DOI: 10.1016/j.jksus.2020.09.004

Al-Ansari, M., Kalaiyarasi, M., Almalki, M. A., and Vijayaraghavan, P. (2020). "Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from *Streptomyces* sp. AS11 isolated from the marine environment," *Journal of King Saud University-Science* 32(3), 1993-1998. DOI: 10.1016/j.jksus.2020.02.005

Arokiyaraj, S., Elshikh, M. S., Alarjani, K. M., Alghmdi, M. A., and Vijayaraghavan, P. (2024). "Valorization of rubber tree sawdust biomass for the production of laccase-lignin peroxidase and bioconversion of sawdust," *Biomass Conversion and Biorefinery* 2024, 1-14. DOI: 10.1007/s13399-024-05322-w

Balasubramanian, B., Ilavenil, S., Al-Dhabi, N. A., Agastian, P., and Choi, K. C. (2019). "Isolation and characterization of *Aspergillus* sp. for the production of extracellular polysaccharides by response surface methodology," *Saudi Journal of Biological Sciences* 26(3), 449-454. DOI: 10.1016/j.sjbs.2018.10.015

Biji, G. D., Arun, A., Muthulakshmi, E., Vijayaraghavan, P., Arasu, M. V., and Al-Dhabi, N. A. (2016). "Bio-prospecting of cuttle fish waste and cow dung for the production of fibrinolytic enzyme from *Bacillus cereus* IND5 in solid state fermentation," *3 Biotech* 6, article 231. DOI: 10.1007/s13205-016-0553-0

Chaverri, P., Castlebury, L. A., Samuels, G. J., and Geiser, D. M. (2003). "Multilocus phylogenetic structure within the *Trichoderma harzianum*/*Hypocrealixii* complex," *Molecular Phylogenetics and Evolution* 27(2), 302-313. DOI: 10.1016/S1055-7903(02)00400-1

Chen, B., You, W., Huang, J., Yu, Y., and Chen, W. (2010). "Isolation and antioxidant property of the extracellular polysaccharide from *Rhodella reticulate*," *World Journal of Microbiology and Biotechnology* 26, 833-840. DOI: 10.1007/s11274-009-0240-y

Cui, Y.W., Shi, Y.P., and Gong, X.Y. (2017). "Effects of C/N in the substrate on the simultaneous production of polyhydroxyalkanoates and extracellular polymeric substances by *Haloferaxmediterraneivia*kinetic model analysis," *RSC Advances* 7(31), 18953-18961. DOI: 10.1039/C7RA02131C

El-Sheikh, M. A., Rajaselvam, J., Abdel-Salam, E. M., Vijayaraghavan, P., Alatar, A. A., and Biji, G. D. (2020). "*Paecilomyces* sp. ZB is a cell factory for the production of gibberellic acid using a cheap substrate in solid state fermentation," *Saudi Journal of Biological Sciences* 27(9), 2431-2438. DOI: 10.1016/j.sjbs.2020.06.040

Farraj, D. A. A., Gawwad, M. R. A., Elshikh, M. S., Arokiyaraj, S., and Vijayaraghavan, P. (2024). "Biodegradation of 17β-estradiol by drug-resistant *Stenotrophomonas maltophilia* MN08 and *Pseudomonas aeruginosa* KL10 isolated from the sediment

sample," *Environmental Quality Management* 34(1), article e22183. DOI: 10.1002/tqem.22183

Fu, L., Wang, Y., Wang, J., Yang, Y., and Hao, L. (2013). "Evaluation of the antioxidant activity of extracellular polysaccharides from *Morchella esculenta*," *Food and Function* 4(6), 871-879. DOI: 10.1039/C3FO60033E

Giraldo, L. A., Tejido, M. L., Ranilla, M. J., and Carro, M. D. (2007). "Effects of exogenous cellulase supplementation on microbial growth and ruminal fermentation of a high-forage diet in Rusitec fermenters," *Journal of Animal Science* 85(8), 1962–1970. DOI: 10.2527/jas.2006-318

Gu, S., Sun, H., Zhang, X., Huang, F., Pan, L., and Zhu, Z. (2021). "Structural characterization and inhibitions on α-glucosidase and α-amylase of alkali-extracted water-soluble polysaccharide from *Annona squamosa* residue," *International Journal of Biological Macromolecules* 166, 730-740. DOI: 10.1016/j.ijbiomac.2020.10.230

Hang, L. T. T., Duy, B. L., Chau, H. B., Hiep, D. M., and Thang, N. T. (2020). "Study of antioxidant and α-glucosidase inhibitory activities of exopolysaccharide and extracts from caterpillar fungus *Ophiocordyceps sinensis* cultured in olive oil-supplemented broth," *Vietnam Journal of Chemistry* 58(4), 482-487. DOI: 10.1002/vjch.201900171

Hao, L., Sheng, Z., Lu, J., Tao, R., and Jia, S. (2016). "Characterization and antioxidant activities of extracellular and intracellular polysaccharides from *Fomitopsis pinicola*," *Carbohydrate Polymers* 141, 54-59. DOI: 10.1016/j.carbpol.2015.11.048

Hsu, K. D., Wu, S. P., Lin, S. P., Lum, C. C., and Cheng, K. C. (2017). "Enhanced active extracellular polysaccharide production from *Ganoderma formosanum* using computational modelling," *Journal of Food and Drug Analysis* 25(4), 804-811. DOI: 10.1016/j.jfda.2016.12.006

Ji-Hoon, J. O. O., and Jong-Won, Y. U. N. (2005). "Structural and molecular characterization of extracellular polysaccharides produced by a new fungal strain, *Trichoderma erinaceum* DG-312," *Journal of Microbiology and Biotechnology* 15(6), 1250-1257.

Jing, L., Zong, S., Li, J., Surhio, M. M., and Ye, M. (2016). "Purification, structural features and inhibition activity on α-glucosidase of a novel polysaccharide from *Lachnum*YM406," *Process Biochemistry* 51(10), 1706-1713. DOI: 10.1016/j.procbio.2016.08.007

Kalaiyarasi, M., Ahmad, P., and Vijayaraghavan, P. (2020). "Enhanced production antibiotics using green gram husk medium by *Streptomyces* sp. SD1 using response surface methodology," *Journal of King Saud University-Science* 32(3), 2134-2141. DOI: 10.1016/j.jksus.2020.02.014

Li, H., Yu, H., and Zhu, H. (2017). "Structure studies of the extracellular polysaccharide from *Trichoderma* sp. KK19L1 and its antitumor effect *via* cell cycle arrest and apoptosis," *Applied Biochemistry and Biotechnology* 182(1), 128-141. DOI: 10.1007/s12010-016-2315-1

Lin, E. S., and Chen, Y. H. (2007). "Factors affecting mycelial biomass and exopolysaccharide production in submerged cultivation of *Antrodiacinnamomea* using complex media," *Bioresource Technology* 98(13), 2511-2517. DOI: 10.1016/j.biortech.2006.09.008

Liu, G. Q., and Wang, X. L. (2007). "Optimization of critical medium components using response surface methodology for biomass and extracellular polysaccharide

production by *Agaricus blazei*," *Applied Microbiology and Biotechnology* 74, 78-83. DOI: 10.1007/s00253-006-0661-6

Lo, Y. M., Argin-Soysal, S., and Hsu, C. H. (2007). "Bioconversion of whey lactose into microbial exopolysaccharides," in: *Bioprocessing for Value-Added Products from Renewable Resources, New Technologies and Applications*, S. T. Young (ed.), Elsevier Science, Amsterdam, Netherlands, pp. 559-583.DOI: 10.1016/B978-044452114-9/50023-2

Manivasagan, P., Sivasankar, P., Venkatesan, J., Senthilkumar, K., Sivakumar, K., and Kim, S. K. (2013). "Production and characterization of an extracellular polysaccharide from *Streptomyces violaceus* MM72," *International Journal of Biological Macromolecules* 59, 29-38. DOI: 10.1016/j.ijbiomac.2013.04.012

Marraiki, N., Vijayaraghavan, P., Elgorban, A. M., Dhas, D. D., Al-Rashed, S., and Yassin, M. T. (2020). "Low cost feedstock for the production of endoglucanase in solid state fermentation by *Trichoderma hamatum* NGL1 using response surface methodology and saccharification efficacy," *Journal of King Saud University-Science* 32(2), 1718-1724. DOI: 10.1016/j.jksus.2020.01.008

Montoya, S., Sánchez, Ó. J., and Levin, L. (2013). "Polysaccharide production by submerged and solid-state cultures from several medicinal higher Basidiomycetes," *International Journal of Medicinal Mushrooms* 15(1), 71-79. DOI: 10.1615/IntJMedMushr.v15.i1.80

Osińska-Jaroszuk, M., Jarosz-Wilkołazka, A., Jaroszuk-Ściseł, J. *et al.* (2015). "Extracellular polysaccharides from *Ascomycota* and *Basidiomycota*: Production conditions, biochemical characteristics, and biological properties," *World Journal of Microbiology and Biotechnology* 31, 1823-1844 (2015). DOI: 10.1007/s11274-015-1937-8

Ragavan, M. L., and Das, N. (2019). "Optimization of exopolysaccharide production by probiotic yeast *Lipomycesstarkeyi* VIT-MN03 using response surface methodology and its applications," *Annals of Microbiology* 69, 515-530. DOI: 10.1007/s13213-019-1440-9

Rajaselvam, J., Kadaikunnan, S., Alharbi, N. S., Khaled, J. M., and Vijayaraghavan, P. (2024). "Biodegradation of endocrine disrupting compounds from the wastewater by the immobilized indigenous bacteria," *Environmental Quality Management* Early View, Available Online. DOI: 10.1002/tqem.22252

Sánchez, Ó. J., and Montoya, S. (2020). "Assessment of polysaccharide and biomass production from three white-rot fungi by solid-state fermentation using wood and agro-industrial residues: A kinetic approach," *Forests* 11(10), article 1055. DOI: 10.3390/f11101055

Saravanakumar, K., Park, S., Sathiyaseelan, A., Mariadoss, A. V. A., Park, S., Kim, S. J., and Wang, M. H. (2021). "Isolation of polysaccharides from *Trichoderma harzianum* with antioxidant, anticancer, and enzyme inhibition properties," *Antioxidants* 10(9), article 1372. DOI: 10.3390/antiox10091372

Sathya, R., Arasu, M. V., Al-Dhabi, N. A., Vijayaraghavan, P., Ilavenil, S., and Rejiniemon, T. S. (2023). "Towards sustainable wastewater treatment by biological methods–A challenges and advantages of recent technologies," *Urban Climate* 47, article 101378. DOI: 10.1016/j.uclim.2022.101378

Sathya, T. A., Alarjani, K. M., Elshikh, M. S., Flanetraj, S. R., and Ponnuswamy, V. (2024). "Co-composting of green leaves and kitchen waste: Characterization of

organic amendments, microbial activity and analysis of defense enzymes in plants," *Biomass Conversion and Biorefinery* 2024, 1-12. DOI: 10.1007/s13399-024-05608-z

Shimada, K., Fujikawa, K., Yahara, K., and Nakamura, T. (1992). "Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion," *Journal of Agricultural and Food Chemistry* 40(6), 945-948. DOI: 10.1021/jf00018a005

Song, J., Wu, Y., Ma, X., Feng, L., Wang, Z., Jiang, G., and Tong, H. (2020b). "Structural characterization and α-glycosidase inhibitory activity of a novel polysaccharide fraction from *Aconitum coreanum*," *Carbohydrate Polymers* 230, article 115586. DOI: 10.1016/j.carbpol.2019.115586

Song, Q., Teng, A. G., and Zhu, Z. (2020a). "Chemical structure and inhibition on α-glucosidase of a novel polysaccharide from *Hypsizygusmarmoreus*," *Journal of Molecular Structure* 1211, article 128110. DOI: 10.1016/j.molstruc.2020.128110

Vijayaraghavan, P., Lazarus, S., and Vincent, S. G. P. (2014). "De-hairing protease production by an isolated *Bacillus cereus* strain AT under solid-state fermentation using cow dung: Biosynthesis and properties," *Saudi Journal of Biological Sciences* 21(1), 27-34. DOI: 10.1016/j.sjbs.2013.04.010

Vijayaraghavan, P., Veeramanikandan, V., Pradeep, B. V., Pothiraj, C., Alarjani, K. M., Al Farraj, D. A., Nguyen, V.H., and Balaji, P. (2024). "Enhanced aerobic naphthalene degradation utilizing indigenous microbial flora as a biocatalyst in oil-contaminated wastewater," *Topics in Catalysis*2024, 1-11. DOI: 10.1007/s11244-024-01953-5

Vijayaraghavan, P., Vincent, S. G. P., Arasu, M. V., and Al-Dhabi, N. A. (2016). "Bioconversion of agro-industrial wastes for the production of fibrinolytic enzyme from *Bacillus halodurans* IND18: Purification and biochemical characterization," *Electronic Journal of Biotechnology* 20, 1-8. DOI: 10.1016/j.ejbt.2016.01.002

Wang, C., Mao, W., Chen, Z., Zhu, W., Chen, Y., Zhao, C., Zhao, C., Li, N., Yan, M., *et al.* (2013). "Purification, structural characterization and antioxidant property of an extracellular polysaccharide from *Aspergillus terreus*," *Process Biochemistry* 48(9), 1395-1401. DOI: 10.1016/j.procbio.2013.06.029

Xu, L., Wang, F., Zhang, Z., and Terry, N. (2019). "Optimization of polysaccharide production from *Cordyceps militaris* by solid-state fermentation on rice and its antioxidant activities," *Foods* 8(11), article 590. DOI: 10.3390/foods8110590

Zhang, X., Esmail, G. A., Alzeer, A. F., Arasu, M. V., Vijayaraghavan, P., Choi, K. C., and Al-Dhabi, N. A. (2020). "Probiotic characteristics of *Lactobacillus* strains isolated from cheese and their antibacterial properties against gastrointestinal tract pathogens," *Saudi Journal of Biological Sciences* 27(12), 3505-3513. DOI: 10.1016/j.sjbs.2020.10.022

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DOI: 10.15376/biores.19.4.8368-8387

APPENDIX

Supplementary Information

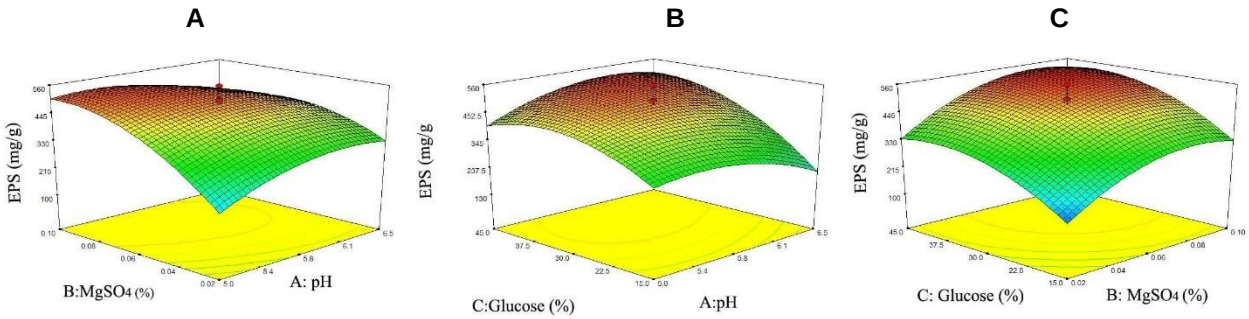

Fig. S1. The 3D-response surface plots of extracellular polysaccharide yield (mg/g) and the tested factors: A: $MgSO_4$ and Ph; B: Glucose and pH; and C: Glucose and $MgSO_4$