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Research Article

SCREENING OF GLUCOAMYLASE PRODUCING FUNGI FROM THE SOILS OF JABALPUR REGION

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ABSTRACT

The amylolytic soil fungi show potential applications in bakery, alcohol, textile and detergent industries. The present work was designed with aim of isolation of glucoamylase producing fungi from soil near different bakery shops, atta-chakki shops and flour-mill dumped areas of Jabalpur region. Thereafter screening of potent fungal isolates was performed using starch hydrolysis test and solid state fermentation. It was found that out of total 62 fungal isolates obtained from different collection sites. The primary screening of all fungal isolates was done by starch hydrolysis test. Among all the fungal isolates, SSP#1, SSP#12, SSP#13, SSP#14, SSP#16, SSP#17, SSP#22, SSP#26, SSP#38, SSP#42, SSP#47, SSP#49 and SSP#58 were found to show maximum zone of clearance in starch agar media. The maximum zone diameter 27.53 mm was given by SSP#16 with amylase activity index 1.83 and maximum glucoamylase activity 2.68 ±0.52 U/mL/min. The potent fungal isolate SSP#16 was identified as *Aspergillus flavipes* strain. The study shows potential of the local potent fungal isolate for glucoamylase production in industrial sectors for achieving economy.

INTRODUCTION

The soil acts as novel source of glucoamylase producing fungi. The soil inhabits various species of fungus which degrade a wide variety of biological materials. The fungal species are mostly confined near the soil surfaces where aerobic conditions prevail (Sarnraj and Stella 2013). In recent years, several studies are done for the isolation and screening of fungi for enzyme production. The filamentous fungi are used for the production of useful enzymes and biological active secondary metabolites. These fungi had been found to be good source of polysachharide degrading enzymes and exploited for the production of amylolytic (amylase) enzymes.

The vast microbial flora in soil is the major component of earth's ecosystem. The fungi play major and complex role of decomposition in soil is enormously complex. The activities of decomposition can be measured by the secreted amount of enzyme by fungi.

The soil fungi help in degradation of dead matter, releasing vital nutrients thereby plays vital role in proper functioning of the ecosystem. This is done with the utilization of both simple and complex molecules as foods by the secretion of a variety of extracellular enzymes including protease, cellulase, amylase, β-glucosidase and chitinase. Fungi are mostly employed to produce industrially important glucoamylase (Imran *et al,* 2012). Many fungal species are capable of producing glucoamylase under different fermentation conditions and techniques (Norouzian *et al,* 2006). Glucoamylase occur almost exclusively in fungi and the industrial focus has been on glucoamylase from *Aspergillus niger* (Norouzian *et al,* 2006) and *Rhizopus oryzae* (Pandey *et al,* 2000). *Aspergillus awamori* (Norouzian *et al,* 2006) and *Aspergillus oryzae* (Normurodova *et al,* 2016) are also the most intensively considered for commercial production in industries. The aim of present study includes isolation of fungi from different soil samples of Jabalpur region and exploration of fungal isolates for glucoamylase production by starch hydrolysis and solid state fermentation method.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from sites of Jabalpur region rich in starchy waste and organic matter lying on the surface. The collection was done at a depth of 15 cm with the help of 2.5 cm

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diameter stainless steel cork borer pre-sterilized with 95% alcohol. The samples were brought to the laboratory for isolation of fungi and further studies.

Isolation of fungi

Serial dilution plate method (Waksman, 1961) was employed for the isolation of fungi. The soil samples were inoculated to the PDA medium by Spread plate technique following serial dilution, and incubated at 27±1°C for 5 days. The fungal isolates were identified on the basis of the morphological (microscopic and macroscopic) characteristics. These isolates are identified according to the protocol of Aggarwal and Hasija (1980). Macroscopic identification involves observation of all the plates for macroscopic characters of fungi that is for color, hyphae, texture, type of growth of the colony, color/pigmentation at the back side of the slants. Microscopic identification was done by slide culture was calculated as described by Ambasht & Ambasht, 2008. The density of fungal species is equal to total number of individual of particular species divided by the total number of species x 100.

$$\text{Density} = \frac{\text{Total number of individual of particular species}}{\text{Total number of species}} \times 100$$

Screening of efficient soil fungi for glucoamylase production

The primary screening of fungal isolates for glucoamylase production was done by Starch hydrolysis test (Abe *et al*, 1988). Amylase activity index which is clear zone diameter (mm)/colony diameter (mm) was also determined according to Knox *et al,* (2004).

Amylase activity index = clear zone diameter (mm)/colony diameter

The isolates showing positive zone in primary screening were subjected to secondary screening method. This was done by fermentative production of glucoamylase by subjecting the selected fungal isolates to solid state fermentation (Pandey *et al*. 2000). The secondary screening by solid state fermentation was carried out to quantify glucoamylase activity with an outlook of using cheap and readily available agriculture residues. The fermentation media containing five gram rice bran moistened with mineral medium (in the ratio 1:1) acts as a basal medium for enzyme production (Singh *et al.* 2009). The basal medium was taken in 100 mL Erlenmeyer flask and autoclaved at 121ºC for 15 min. The flask was then inoculated by the standard inoculum ($1.5x10^8$ spore/mL) and incubated at 27±1°C for 5 days. The glucoamylase activity was determined using the method given by Cori (1955) and the reducing sugars liberated were determined by Dinitrosalicylic (DNSA) method (Miller *et al.* 1959).

RESULTS

Starchy biomass is the most abundant renewable energy resource and is easily hydrolysable substrate for various industrial applications. The saccharifying glucoamylase is the key enzyme having great demand in starch processing industry. A survey was conducted in the course of present study to collect soil samples from sites of Jabalpur region rich in starchy waste. The collection site was chosen in order to get maximum biodiversity of glucoamylase producing fungi in Jabalpur region. The fungi isolated from different collection sites were assigned code as SSP.

A total 62 fungal isolates were obtained from soil samples as shown in Table 1 wherein 22 fungal isolates were obtained from soil sample of Site 2 whereas sampling from Site 1 yielded 14 fungal isolates. 11 fungal isolates were obtained from soil sample of Site 5, sampling from Site 4 yielded a total of 9 fungal isolates and sampling from Site 3 yielded a total of 8 fungal isolates. The obtained data reveals that Site 2 shows maximum number of fungal isolates and this immense fungal diversity can be used for allocating novel and commercially important enzyme.

Table 1 List of soil fungi isolated from different sites of Jabalpur

S. No.	Collection Site	Isolate Code	Name of the isolated fungi
1.	**Site 1**	SSP#1	*Aspergillus flavus*
		SSP#2	*Mucor* sp.
		SSP#3	*Rhizopus* sp.
		SSP#4	*Aspergillus versicolor*
		SSP#5	*Alternaria alternata*
		SSP#6	*Candida* sp.
		SSP#7	*Aspergillus niger*
		SSP#8	*Aspergillus japonicus*
		SSP#9	*Cephalosporium* sp.
		SSP#10	*Nigrospora* sp.
		SSP#11	*Phoma* sp.
		SSP#12	*Aspergillus terreus*
2.	**Site 2**	SSP#13	Unidentified species 1
		SSP#14	Unidentified species 2
		SSP#15	*Absidia* sp.
		SSP#16	*Aspergillus flavipes*
		SSP#17	*Fusarium* sp.
		SSP#18	*Mucor* sp.
		SSP#19	*Rhizopus* sp.
		SSP#20	*Cladosporium* sp.
		SSP#21	*Curvularia lunata*
		SSP#22	*Aspergillus fumigatus*
		SSP#23	*Alternaria alternata*
		SSP#24	*Candida* sp.
		SSP#25	*Aspergillus niger*
		SSP#26	*Paecilomyces* sp.
		SSP#27	*Nigrospora* sp.
		SSP#28	*Aspergillus terreus*
		SSP#29	*Geotrichum* sp.
		SSP#30	*Trichoderma* sp.
		SSP#31	*Verticillium* sp.
		SSP#32	*Phoma* sp.
3.	**Site 3**	SSP#33	*Aspergillus japonicus*
		SSP#34	*Aspergillus terreus*
		SSP#35	Unidentified species 3
		SSP#36	Unidentified species 4
		SSP#37	*Aspergillus flavus*
		SSP#38	*Mucor* sp.
		SSP#39	*Cladosporium* sp.
		SSP#40	*Pythium* sp.
		SSP#41	*Bipolaris* sp.
		SSP#42	*Aspergillus niger*
		SSP#43	*Cephalosporium* sp.
		SSP#44	*Absidia* sp.
4.	**Site 4**	SSP#45	*Aspergillus flavus*
		SSP#46	*Fusarium* sp.
		SSP#47	*Rhizopus* sp.
		SSP#48	*Curvularia lunata*
		SSP#49	*Alternaria alternata*
		SSP#50	*Aspergillus niger*
5.	**Site 5**	SSP#51	Unidentified species 5
		SSP#52	*Absidia* sp.
		SSP#53	*Mucor* sp.
		SSP#54	*Cladosporium* sp.
		SSP#55	*Curvularia lunata*
		SSP#56	*Pythium* sp.

	SSP#57	*Alternaria alternata*
	SSP#58	*Candida* sp.
	SSP#59	*Aspergillus niger*
	SSP#60	*Aspergillus japonicus*
	SSP#61	*Phoma* sp.
	SSP#62	*Verticillium* sp.

The significant diversity in occurrence of fungal isolates was documented in Table 2. The biodiversity index for each site was calculated using Shannon Weiner species diversity index calculator available online and found to be 2.56 for Site 1, 3.01 for Site 2, 1.96 for Site 3, 2.16 for Site 4 and 2.42 for Site 5. The data shows the species richness in Site 2 is higher than the other sites. The higher value of Shannon's index reveals that Site 2 not only has a greater number of species present, but the individuals in the community are distributed more equitably among these species.

Table 2 Occurrence of fungal isolates in different sites

Fungal isolate	Site 1	Site 2	Site 3	Site 4	Site 5	Total number of fungi	% Density
Absidia sp.	-	1	-	1	1	3	4.83
Aspergillus flavus	1	-	1	1	-	3	4.83
Aspergillus flavipes	-	1	-	-	-	1	1.61
Fusarium sp.	-	1	-	1	-	2	3.22
Mucor sp.	1	1	1	-	1	4	6.45
Rhizopus sp.	1	1	-	1	-	3	4.83
Cladosporium sp.	-	1	1	-	1	3	4.83
Curvularia lunata	-	1	-	1	1	3	4.83
Aspergillus fumigatus	-	1	-	-	-	1	1.61
Pythium sp.	-	-	1	-	1	2	3.22
Aspergillus versicolor	1	-	-	-	-	1	1.61
Alternaria alternata	1	1	-	1	1	4	6.45
Bipolaris sp.	-	-	1	-	-	1	1.61
Candida sp.	1	1	-	-	1	3	4.83
Aspergillus niger	1	1	1	1	1	5	8.06
Paecilomyces sp.	-	1	-	-	-	1	1.61
Aspergillus japonicus	1	1	-	-	1	3	4.83
Nigrospora sp.	1	1	-	-	-	2	3.22
Phoma sp.	1	1	-	-	1	3	4.83
Aspergillus terreus	1	2	-	-	-	3	4.83
Geotrichum sp.	-	1	-	-	-	1	1.61
Trichoderma sp.	-	1	-	-	-	1	1.61
Verticillium sp.	-	1	-	-	1	2	3.22
Cephalosporium sp.	1	-	1	-	-	2	3.22
Unidentified species	2	2	-	1	-	5	8.06
Species richness per site	14	22	7	8	11	62	
Shanon's (H) index	2.56	3.01	1.96	2.16	2.42		

Shanon diversity index (H) for each site is $\sum - (P_i * \ln P_i)$

Screening of efficient soil fungi for glucoamylase production

In the present study, all the fungal isolates were subjected to primary (qualitatively) and secondary (quantitatively) screening to assess amylase production. The colonies selected in the primary screening (showing the highest starch hydrolytic zones) were then tested by secondary screening for the production of glucoamylase using solid state fermentation.

Primary Screening

The primary screening was performed using starch hydrolysis test (Abe *et al*. 1988) which demonstrates the clear zone around the colonies of the fungal isolates indicating positive amylase production. This was detected by clear zones after exposure to iodine solution by the fungal isolates as starch degrading activities. The diameter of clear zone and fungal colonies were evaluated using Hi-Media measuring scale. In case where colony diameters were not circular, the average of the longest and shortest widths was recorded (Khokhar *et al*. 2011). The fungal isolates were able to grow on the screening media (starch agar media) and screened on the basis of the ratio of clearing zone diameter and colony diameter. Table 3 shows primary screening of all fungal isolates by starch hydrolysis test after 48 h, 72 h, 96 h and 120 h incubation. Of all the fungal isolates, SSP#1, SSP#12, SSP#13, SSP#14, SSP#16, SSP#17, SSP#22, SSP#26, SSP#38, SSP#42, SSP#47, SSP#49 and SSP#58 were found to show maximum zone of clearance in starch agar media.

The rest of the isolates showed minimum or no zone of clearance in starch hydrolysis test. An amylase activity index was also determined to select isolate with high amylase activity for further studies. Amylase activity index was determined according to Knox *et al*. (2004). The selected isolates producing excessive amylase were subjected to secondary screening by solid state fermentation.

Table 3 Primary Screening of fungal isolates by Starch hydrolysis test

S. No.	Fungal isolate	Zone of clearance after			
		48 h	72 h	96 h	120 h
1.	SSP#1	++	++	++	++
2.	SSP#2	++	++	++	++
3.	SSP#3	+++	+++	+++	+++
4.	SSP#4	-	-	-	-
5.	SSP#5	++	++	++	++
6.	SSP#6	++	++	++	++
7.	SSP#7	+++	+++	+++	++
8.	SSP#8	+	+	+	+
9.	SSP#9	-	-	-	-
10	SSP#10	-	-	-	-
11.	SSP#11	-	-	-	-
12.	SSP#12	+++	+++	++	++
13.	SSP#13	++	++	++	++
14.	SSP#14	++	++	++	++
15.	SSP#15	-	-	-	-
16.	SSP#16	++++	++++	++++	++++
17.	SSP#17	+++	+++	+++	+++
18.	SSP#18	++	++	++	++
19.	SSP#19	+++	+++	+++	+++
20.	SSP#20	-	-	-	-
21.	SSP#21	-	-	-	-
22.	SSP#22	++	++	++	++
23.	SSP#23	++	++	++	++
24.	SSP#24	++	++	++	++
25.	SSP#25	+++	+++	+++	++
26.	SSP#26	++	++	++	++
27.	SSP#27	-	-	-	-
28.	SSP#28	+++	+++	+++	+++
29.	SSP#29	-	-	-	-
30.	SSP#30	-	-	-	-
31.	SSP#31	-	-	-	-
32.	SSP#32	-	-	-	-
33.	SSP#33	+	+	+	+
34.	SSP#34	+++	+++	+++	+++
35.	SSP#35	-	-	-	-
36.	SSP#36	-	-	-	-
37.	SSP#37	++	++	++	++
38.	SSP#38	++	++	++	++
39.	SSP#39	-	-	-	-
40.	SSP#40	-	-	-	-
41.	SSP#41	-	-	-	-
42.	SSP#42	+++	+++	+++	+++
43.	SSP#43	-	-	-	-
44.	SSP#44	-	-	-	-
45.	SSP#45	++	++	++	++
46.	SSP#46	+++	+++	+++	+++
47.	SSP#47	+++	+++	+++	++
48.	SSP#48	-	-	-	-
49.	SSP#49	++	++	++	++

50.	SSP#50	+++	+++	+++	++
51.	SSP#51	-	-	-	-
52.	SSP#52	-	-	-	-
53.	SSP#53	++	++	++	++
54.	SSP#54	-	-	-	-
55.	SSP#55	-	-	-	-
56.	SSP#56	-	-	-	-
57.	SSP#57	++	++	++	++
58.	SSP#58	++	++	++	++
59.	SSP#59	+++	+++	+++	+++
60.	SSP#60	+	+	+	+
61.	SSP#61	-	-	-	-
62.	SSP#62	-	-	-	-

#Zone of clearance indicated as (-) indicates no zone, (+) below average zone, (++) average zone & (++++) abundant zone

Secondary Screening

The secondary screening involves fermentative production of crude glucoamylase using solid state fermentation in basal fermentation media comprised of substrate (agricultural residues) with mineral media (Singh *et al.* 2009). The glucoamylase activity of the crude extract was determined according to the method given by Cori *et al*. (1955) and reducing sugars liberated were determined by DNSA method (Miller *et al*. 1959). The amount of glucose released by the glucoamylase of isolates was determined with respect to standard graph of glucose. Glucoamylase Activity Unit (U) is defined as amount of glucoamylase which produce 1 micromole of glucose in 1 min under standard assay conditions. The glucoamylase activity was expressed in terms of U/mL/min.

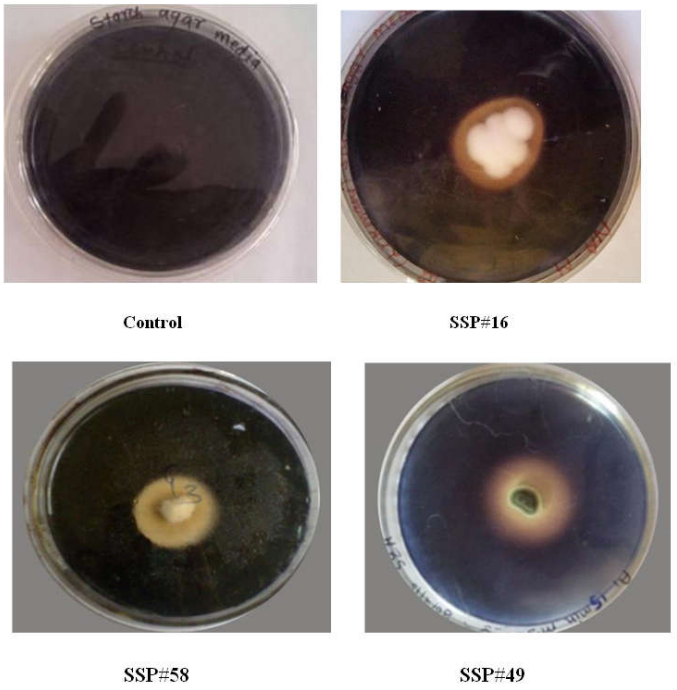
Table 4 and Figure 1 shows screening of selected fungal isolates for glucoamylase production in terms of zone of clearance, amylase activity index and glucoamylase activity. The maximum zone diameter 27.53 mm was given by SSP#16 with amylase activity index 1.83 which is followed by SSP#58 with zone diameter 20.30 mm and amylase activity index 1.42. The least zone diameter of 10.09 mm was shown by SSP#49 with amylase activity index 1.26 and SSP#26 with zone diameter 12 mm and amylase activity index 1.09. The maximum glucoamylase activity was given by SSP#16 with 2.68 ±0.52 U/mL/min which is followed by SSP#58 with glucoamylase activity 1.80 ±0.46 U/mL/min. The least glucoamylase activity 1.04±0.28 U/mL/min was given by SSP#22.

Table 4 Screening of selected fungal isolates for glucoamylase production

S. No.	Fungal isolate	Glucoamylase production			
		Zone inference	Zone diameter (mm)	Amylase activity index	Enzyme Activity (U/mL/min)
1.	SSP#1	++	17.6	1.27	1.60 ±0.82
2.	SSP#12	+++	14.3	1.30	1.15 ±0.39
3.	SSP#13	++	17.8	1.27	1.63 ±0.35
4.	SSP#14	++	19	1.30	1.83 ±0.45
5.	SSP#16	++++	27.53	1.83	2.68 ±0.52
6.	SSP#17	+++	13.33	1.20	1.69 ±0.22
7.	SSP#22	++	13	1.16	1.04 ±0.28
8.	SSP#26	++	12	1.09	1.15 ±0.56
9.	SSP#38	++	14	1.20	1.08 ±0.37
10.	SSP#42	+++	18	1.50	2.31 ±0.67
11.	SSP#47	+++	18	1.50	2.30 ±0.36
12.	SSP#49	++	10.09	1.26	1.36 ±0.72
13.	SSP#58	++	20.3	1.42	1.80 ±0.46

Zone Inference in Starch Hydrolysis indicated as (-) indicates no zone, (+) below average zone, (++) average zone & (++++) abundant zone. # Amylase activity index = clear zone diameter (mm) / colony diameter (mm)

Enzyme activity determined by the DNS assay as mg of glucose equivalents produced per min (Mean ±S.D., n = 3)



a. Primary Screening of fungal isolates

b. Secondary Screening of fungal isolates

Figure 1 Screening of fungal isolates for glucoamylase

Morphological identification of fungal isolates

Identification of soil fungi was done according the protocol of Aggarwal and Hasija (1980) and Domasch (1980). The fungal isolates were identified according to their macroscopic features such as colour, shape and growth of cultured colonies, as well as microscopic characteristics like structure of hyphae, conidia and conidiophores. Obtained data was then compared with the descriptions of fungal species present in the literature (Gilman 1957). In the present research, the fungal species were successfully identified as *Absidia* sp., *Aspergillus flavus*, *Aspergillus flavipes, Fusarium* sp., *Mucor* sp., *Rhizopus* sp., *Cladosporium* sp., *Curvularia lunata*, *Aspergillus fumigatus*, *Pythium* sp., *Aspergillus versicolor*, *Alternaria alternata*, *Bipolaris* sp., *Candida* sp., *Aspergillus niger*, *Paecilomyces* sp., *Aspergillus japonicas, Nigrospora* sp., *Phoma* sp., *Aspergillus terreus, Geotrichum* sp., *Trichoderma* sp., *Verticillium* sp. and some unidentified species. The morphological feature of potent amylolytic fungi SSP#16 is shown in Figure 2. The colonies on PDA were plane, velvety, pale, buff colored, dark pink to brown sclerotia produced, reverse usually in yellow brown to reddish brown shade. The microscopic view reveals conidial heads loosely columnar; conidiophores yellowish, vesicles globose to vertically elongate, sterigmata in two series; conidia globose to subglobose, smooth sclerotia numerous, oval to irregular, hard, pink, turning brown, thus identifying the potent

isolate SSP#16 as *Aspergillus flavipes* strain.

Figure 2 Morphology of potent fungal isolate SSP#16

DISCUSSION

The accessibility of fungal biodiversity in soil for exploration of enzymes in industrial sectors is an area of research interest (Verma *et al.* 2015). In the present study, 62 fungal isolates were obtained from different collection sites and assessed for glucoamylase. Many reports show isolation of fungi from soil samples of different regions for the study of amylase (Varalakshmi *et al*. 2009, Anto *et al*. 2006, Khokhar *et al*. 2011, Lawal *et al*. 2014). Kumar *et al.* (2015) reported isolation of soil mycoflora in agricultural fields at Tekkai Mandal wherein the samples were collected from rhizosphere and rhizoplane regions of different rice fields. Similarly, Abdullah *et al*. (2016) isolated fungi from 5 different regions in explosive institutes and contaminated sites in Riyadh province and reported that the isolated fungi in contaminated soils can be used as bioremediation agents for explosive materials. In another study, Singh *et al*. (2014) reported isolation of fungal strains from soil samples collected from different sites of Jalandhar region of Punjab for the study of amylase activity and the production of fungal amylase was studied using cheap readily available agricultural residues. Ominyi *et al*. (2013) reported 26 fungal isolates showing starch hydrolysis test out of 120 fungi isolated from soil of rice mill industrial areas, corn processing industries, rice milling and dumping ground soil of Nigeria.The variation in diversity index of collection sites indicates that soil harbors pleothra of fungal population which can be used for industrial applications. Jena *et al.* (2015) studied occurrence of culturable soil fungi in Odisha wherein the study reported Shannon-Wiener indices to be 3.12 and 0.9425 respectively in summer and winter seasons.

The study aims at the screening of glucoamylase producing fungi from different collection sites. Similarly, Mishra *et al.* (2010) isolated 15 filamentous fungi from soil of different regions of Rajasthan state and assessed the isolates for amylase producing ability. In a similar observation, Ominyi *et al.* (2013) screened the isolates from soil of rice mill industrial areas, corn processing industries, rice milling and dumping ground soil and reported 26 isolates to show maximum amylase production in starch hydrolysis test of potassium iodide method. Sohail *et al*. (2005) studied screening of microbial population for their ability to possess amylolytic potential and reported that a total of 130 fungal isolates were screened and 21 fungal isolates from soil samples to be amylase producers. *Aspergillus* species was found to be most active amylase producer with frequency occurrence of 16.15% of all the isolates.

The starch hydrolyzing capability of the isolates showed their potential application for bio-ethanol production. The obtained data illustrates occurrence of fungal isolates in soil with high amylolytic potential (Omemu *et al.* 2008). In a recent study, Singh *et al.* (2014) studied amylase production by *Aspergillus fumigates* NTCC1222 for its application in textile industry. The amylase activity of 164.1 U/mL/min was shown by the isolate in secondary screening under solid state fermentation. Bansal *et al*. (2011) examined novel strain of *Aspergillus niger* for industrial enzymes and found clear zone ratio for the production of amylase, pectinase and cellulase.

The present report shows the significance of solid state fermentation involved in secondary screening for use of ago-residues in bioprocess industries. Similarly, Zambare *et al.* (2010) studied solid state fermentation of *Aspergillus oryzae* for glucoamylase production on agro residues by solid state fermentation. The fermentation was done on solid surface of different agro-residues and it was reported that glucoamylase production of 1672-1693 U/gdFs was obtained using wheat bran.

The morphological identification of the fungal isolates was done and the potent soil fungus was identified as *Aspergillus flavipes* strain. Also, the other isolates showing capability of glucoamylase production were identified as *Absidia* sp., *Aspergillus flavus*, *Aspergillus flavipes, Fusarium* sp., *Mucor* sp., *Rhizopus* sp., *Cladosporium* sp., *Curvularia lunata*, *Aspergillus fumigatus*, *Pythium* sp., *Aspergillus versicolor* etc. Similar observation was made by Ominyi *et al.* (2013) and isolated alpha amylase and glucoamylase producing fungi based for bioethanol production. The isolates were identified on the basis of morphological characters as *Aspergillus* sp., *Mucor, and Rhizopus* sp. In another report, Prabarkan *et al*. (2009) reported fungal isolates from soil samples and identified as *Aspergillus fumigatus, Penicillium chrysogenum* and *Verticillium terrestre.* Sohail., (2005) identified fungal isolates on the basis of morphological characters and reported *Aspergillus niger, Aspergillus flavus, Fusarium* sp.*, Alternaria* sp.*, Trichoderma* sp., *Penicillium* sp., *Curvularia* sp. and *Cladosporium* sp.

CONCLUSION

The present study showed that the soil sample from different sites of Jabalpur region is good source of industrially important enzyme glucoamylase. In this study, 62 fungal isolates were obtained from soil samples collected from bakery and flour mill effluents of different areas of Jabalpur (M.P.). The obtained fungal isolates were designated as SSP#1 to SSP#62. A total of 22 fungal isolates were obtained from soil sample of Site 2 whereas sampling from Site 1 yielded 14 fungal isolates, 11 fungal isolates were obtained from soil sample of Site 5, sampling from Site 4 yielded a total of 9 fungal isolates and sampling from Site 3 yielded a total of 8 fungal isolates. The screening for the production of glucoamylase was done for the isolates and among the fungal isolates, SSP#1, SSP#12, SSP#13, SSP#14, SSP#16, SSP#17, SSP#22, SSP#26, SSP#38, SSP#42, SSP#47, SSP#49 and SSP#58 predominated. The

maximum zone diameter 27.53 mm was given by SSP#16 with amylase activity index 1.83 and maximum glucoamylase activity 2.68 ±0.52 U/mL/min. The potent fungal isolate SSP#16 was identified as *Aspergillus flavipes* strain. The present research reports the isolation of glucoamylase producing soil fungi *Aspergillus flavipes* SSP#16 isolated from Jabalpur (M.P) India. The study also emerges as an economical method for glucoamylase production because of easy availability and cost effectiveness of agro-industrial wastes residues used in solid state fermentation (secondary screening). However, the optimization of production parameters and the strain improvement can be done for further studies.

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References

1. Abdullah FM, Al-Dhabban and Bakhali AH. Identification of contaminated soil from isolated fungi in Riyadh province. *Life. Sci. J.,* 2016; 13(2): 51-56.
2. Abe JI, Bergmann FW, Obata K and Hizukuri S. Production of raw starch digesting amylase of *Aspergillus* species K-27. *Appl. Microbiol. Biotechnol.,* 1988; 27(5): 447-450.
3. Aggarwal GP and Hasija SK. Microorganisms in the laboratory. *In:* Laboratory guide of mycology, Microbiology and plant pathology. Ravi printers Jabalpur, M.P. India; 1980: 58.
4. Ambasht RS & Ambasht NK. A Textbook of Plant Ecology. 15th Eds CBS Publishers & Distributors; 2008.
5. Anto H, Trivedi UB and Patel KC. Glucoamylase production by soild-state fermentation using rice flake manufacturing waste products as substrate. *Biores. Technol*., 2006; 97(10): 1161-1166.
6. Bansal N, Tewari R, Gupta JK, Soni R and Soni SK. A novel strain of *Aspergillus niger* producing a cocktail of hydrolytic depolymerising enzymes for the production of second generation biofuels. *Bioresources.*, 2011; 6(1): 552 -569.
7. Cori GT. Amylo-1-6-glucosidase. *Methods in Enzymology*; 1955; 1: 211-214.
8. Domasch KH, Gamas W and Anderson TH. Compendium of Soil Fungi, Academic press, New York; 1980: 559-560.
9. Gillman JC. Manual of soil fungi. 2nd Eds Oxford and IBH Publishing company (Indian reprint); 1957.
10. Imran M, Asad MJ, Gulfraz M, Qureshi R, Gul H, Manzoor N and Choudhary AN. Glucoamylase production from A*spergillus niger* by using solid state fermentation process. *Pak. J. Bot.,* 2012; 44(6): 2103-2110.
11. Jena SK, Tayung T, Rath CC and Parida D. Occurrence of culturable soil fungi in a tropical moist deciduous forest Similipal Biosphere Reserve, Odisha, India. *Braz. J. Microbiol*., 2015, 46(1): 85-96.
12. Khokhar *et al*. 2011, Khokhar I, Mukhtar I and Mushtaq S (2011). Isolation and screening of amylolytic filamentous fungi. *J. Appl. Sci. Environ. Manag,* 15(1): 203 -206.
13. Knox AM, Du-Preez JC and Kilian SG. Starch fermentation characteristics of *Saccharomyces cerevisiae* strains transformed with amylase genes from *Lipomyces kononenkoae* and *Saccharomycopsis fibuligera*. Enzyme. *Microb. Technol.,* 2004; 34(5): 453-460.
14. Kumar PKR, Hemanth G, Shiny NP and Samauel KK. Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal in Srikakulam District. *Int. J. Adv. Pharma. Bio. Chem.*, 2015; 4(2): 484-490.
15. Lawal A, Banjoko K, Olatope AM, Alebiosu SO, Orji FA, Suberu FA, Itoandon YL, Shittu EE, Adelaja KA, Ojo OD, Dike EN and Elemo GN. Production and partial purification of glucoamylase from *Aspergillus niger* isolated from cassava peel soil in Nigeria. *Afr. J. Biotechnol.,* 2014; 13(21): 2154-2158.
16. Miller GL. Use of DNA reagent for the determination of reducing sugars. *Analy. Chem.,* 1959; 31(3): 426-428.
17. Mishra BK and Dadhich SK. Production of amylase and xylanase enzyme from soil fungi of Rajasthan. *J. Adv. Dev. Res.*, 2010; 1(1): 21 -23.
18. Normurodova KT, Muratov GA, Alimdjanova LI and Niyazmetov BA. Purification and characterization of highly active glucoamylase and pre-clinical study of general toxicology of fungus *Aspergillus oryzae* UzMU K-14. *American. J. Biochem*., 2016; 6(4): 91-96
19. Norouzian DA, Akbarzadeh A, Scharer JM and Young MM. Fungal glucoamylase. *Biotechnol. Adva.,* 2006; 24(1): 80-85.
20. Omemu AM, Akpan I, and Bankole MO. Purification and characterization of extracellular amyloglucosidase from *Aspergillus niger* CA-19 by solid state fermentation. *Res. J. Microbiol.,* 2008; 3(3): 129-135.
21. Ominyi MC, Ogbonna JC, Nwoba EG, Nwagi KE and Ukachi R. Isolation and screening of amylase and glucoamylase producing fungi and their application in bioethanol production. *Int. J. Sci. Nat.,* 2013; 4(1): 44-50.
22. Pandey A, Nigam P, Socool CR, Soccol VT, Singh D and Mohan R. Advances in microbial amylases. *Biotechnol. Appl. Biochem.,* 2000; 31(2): 135-152.
23. Prabakaran M, Thennarasu V, Mangala R, Bharathidasan R, Chandrakala N and MohanIndian N. Comparative studies on the enzyme activities of wild and mutant fungal strains isolated from sugarcane field. *J. Sci. Technol.,* 2009; 2(11): 1146-49.
24. Saranraj P and Stella D. Fungal Amylase - A Review. *Int. J. Microbiol. Res.,* 2013; 4(2): 203 211.
25. Singh RK, Kumar S and Surendra K. Production of α-amylase from agricultural by-products by *Humicola lanuginosa* in solid state fermentation. *Curr. Trends. Biotech. Pharma.*, 2009; 3(2): 19-29.
26. Singh S, Singh S, Bali V, Sharma L and Mangla J. Production of Fungal Amylases Using Cheap, Readily Available Agri-residues for Potential Application in Textile Industry. *Biomed. Res. Int.*, 2014: 215748.

27. Sohail M, Ahmad A, Shahzad S and Khan SA. A survey of amylolytic bacteria and fungi from native environmental samples. *Pak. J. Bot.,* 2005; 37(1): 155-161.
28. Varalakshmi KN, Kumudini BS, Nandini BN, Solomon J, Suhas R, Mahesh B and Kavitha AP. Production and characterization of alpha amylase from *Aspergillus niger* JGI 24 isolated in Bangalore. *Polish. J. Microbiol.*, 2009; 58(1): 29- 36.
29. Verma P, Dahayat A, Chandrakar V and Jamaludin. Studies on enzymatic potential fungi isolated from municipal solid waste in Jabalpur. *Int. J. Recent. Scient. Res.,* 2016; 6(8): 5927-5932.
30. Waksman SA. The *Actinomycetes* classification identification and description of genera and species. Baltimore: Williams and Wilkins Company, 1961:327.
31. Zambare V. Solid State fermentation of *Aspergillus oryzae* for glucoamylase production on agro residues. *Int. J. Lifesci.,* 2010; 4: 16-25.

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