ISOLATION AND SCREENING OF AMYLASE PRODUCING FUNGI FROM SOIL AT TAUNGOO UNIVERSITY CAMPUS

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Abstract

Enzymes are the most important substances which are used today in many areas. There are different types of enzymes in the nature. Among these, amylases are one of the most significant and are widely used in the industries. Amylases are widely extracted from the microorganisms, more specifically from the fungi. This present study was aimed to isolate and screen for amylase producing fungi from two different locations of soil at Taungoo University Campus. All together fourteen fungal isolates were obtained from two locations of soil samples. The isolated fungi were preliminary screened for amylolytic activity by growing on potato glucose agar (PGA) using gram's iodine solution. Among fourteen fungal isolates, ten fungal isolates showed positive amylase activity and four isolates showed negative amylase activity after flooding with gram's iodine solution. Soils at Taungoo University Campus can be considered as a valuable natural source of amylase producing fungi.

Keywords - Fungi, enzymes, amylase, isolation, screening, potato glucose agar (PGA)

Introduction

Fungi are the most important microorganisms and play an important role in the decomposition of organic matter due to their degradation abilities. Fungi are important eukaryotic microorganisms and large amounts of extracellular enzymes are produced for the decomposition organic matter (Gomes., 2007). Fungi present commonly in different types of soils, indoor and outdoor air, food, agro wastes and water. Therefore, fungi are found almost everywhere (Asan., 2004). Fungi secrete different types of enzymes extracellularly and have been used for the production of more than 50% of the needed enzymes. Fungal enzymes have been used from ancient times and today in many industries, including baking, brewing, cheese making, antibiotics production, and commodities manufacturing, such as linen and leather. Furthermore, they are also used in paper production, detergent, the textile industry, and in drinks and food technology in products manufacturing ranging from tea and coffee to fruit juice and wine (Hamada, *et al*., 2022)

Amylases are obtained from diverse sources including plants, animals, and microbes (Pandey *et al.,* 2000). Microbial amylases are more useful compared with animals and plants amylases in industrial applications. Amylase are important enzymes employed in the starch processing industries (Alva *et al*., 2007).

Amylase production from fungi is economical because the amylase production rate is higher in fungi as compared to the other microorganisms. Amylolytic enzymes are widely produced by bacteria and fungi. Generally, fungi like *Aspergilllus* species, *Penicillium* species, are widely used for the extraction of amylase (Singh, *et al.,* 2011). Enzyme production can be obtained from several microorganisms especially fungi have gained much attention because of the availability and high productivity (Pandey., 2003). The amylase has been extracted from several fungi, bacteria, yeasts and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in the industrial sectors (Gurung *et al.,* 2013).

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Materials and Methods

Samples collection

Soil sample were collected from the two locations of soil about 15 cm at Taungoo University Campus from June to August 2024. The soil sample was transferred to the sterile plastic bags and brought to the laboratory for further analysis. The soil sample was air-dried for 3 days. After drying, the soil sample was ground and one gram was weighted for serial dilution.

18º57'02.4"N 96º21'51.9"E
(SS-I, Botanical Garden)

18º57'08"N 96º21'51"E
(SS-II, Behind the Library)

Collection of soil sample

Figure 1. Map and locations for collected soil samples

Figure 2. Soil sample was dried and weighted 1 gram for serial dilution

Preparation of soil dilution and microbial culture

Isolation of soil fungi by soil dilution plating was adapted from a method by Pepper and Gerba (2004). One gram of the soil sample was added to 99 ml of sterile water and shaken well to disperse the organism. Next, 1 mL of the suspension was removed using a sterile micropipette and then transferred to a new tube containing 9 ml dilution blank. The dilution series was continued to obtain solutions with dilution factors of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6},10^{-7}, 10^{-8} and10^{-9}. The medium was prepared and sterilized at 121°C for 30 minutes under the pressure of 15 lbs/sq inch. After sterilizing, the media was poured onto the sterilized petri dish for about 20 ml and waited until the medium solidified. Next, 1 ml of each soil dilution was transferred into each respective petri dish containing potato glucose agar plate supplemented with 0.1g/L penicillin using the micropipette and spread onto the plate using the sterile glass spreader. After spreading the diluted soil sample on the plates, the plates were incubated at room temperature for about (25-30°C) for a week.

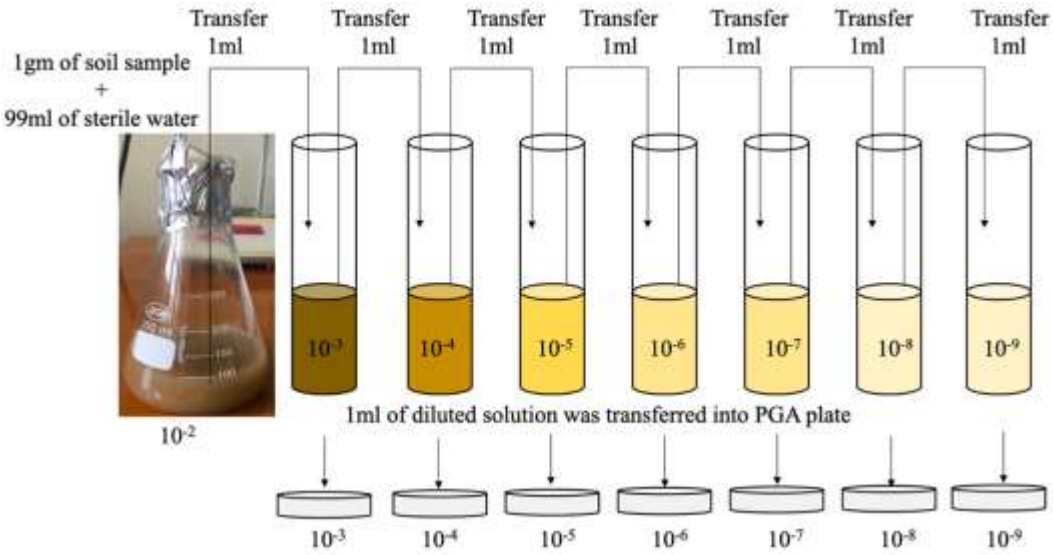


Figure 3. Serial dilution of soil samples

Isolation of fungi

The presence of fungal isolates was observed every day for 7 days. The fungi were then separated based on color and surface morphology of Potato Glucose Agar (PGA) plates. Then, in order to obtain the pure culture, the small amount of spore or hyphal tip from each isolate was then inoculated on new PGA plates supplemented with penicillin to inhibit the growth of bacteria and incubated for 5 days. The pure culture fungi were inoculated on slanted PGA for long term storage.

Macroscopical and microscopical characteristics of Fungi

For macroscopical characteristics of the fungi, colony color, texture, pigmentation are observed. As for microscopical characteristics, hyphae, conidia, fruiting bodies and conidiophores were observed and determined according the reference book of Barnett and Hunter., 1999. The fungal slides were prepared and petri dishes were examined under light microscope with magnification of 10X and 40X objective lens using the microscopic No (27) at the laboratory of department of botany.

Screening of Amylase Producing Activity

Five days old of pure fungal cultures were screened according to the method described by (Ire *et al*., 2020). (2020). The plates were poured with iodine solution and detected the amylase enzyme producing activity. All of the pure isolates obtained were screened for actual and efficient starch degraders. Gram's iodine solution was prepared by adding 1 g iodine crystals to 2 g of potassium iodide solution and 300ml of water and kept in brown bottle and stored at room temperature (James and Chad. 2019). The iodine solution was poured into the plates containing fungi. The production of amylase is indicated by clear visible zone of hydrolysis around the fungal colonies. The fungal colony showing the zone of hydrolysis was selected correspondingly from the potato glucose agar plates for the screening of amylase. The production of amylase by isolated fungi was photographed and recorded.

Results

Isolation of the fungi from soil sample

All together fourteen fungi were isolated from two different locations of soil sample at Taungoo University Campus. Among the cultured (PGA) plates, only fungal growing plates were selected. Seven fungal isolates from soil sample (I) and seven isolates form soil sample (II) were collected. Two locations of soil samples were designated as SS-I and SS-II. All the resulting fungi were designated as isolates with respective numbers.

Macroscopical characteristics of fungal isolates

All together fourteen isolated were obtained from two soil samples. Macroscopical characteristics of seven fungal isolates from soil sample (I) are white, deep olive green, deep green, black, cotton white, pale gray, light green and seven isolates from soil sample (II) are light gray, cream color, green, blue green and light yellow colors. Three fungal isolates produce pigments on agar plates. Macroscopical characteristics and amylase producing activity of isolated fungi are mentioned in Table 1 and 2.

Microscopical characteristics of fungal isolates

For microscopical characteristics, shape of conidia, structure of fungal hyphae and fruiting body were examined under the microscope (400X) magnification. From fungi isolated from soil sample (I), oval shaped conidia are observed from two isolates, three isolates have spherical shaped conidia and the rest of the two isolates showed no formation of conidia. Large zygospore was observed from isolate No. 6. Two isolates such as No. 2 and 3 have hyphae with septum and aseptate hyphae were observed from the rest of five isolates. Two fungal isolates such as No. 3 and 6 showed brush-like fruiting bodies and one isolate of No. 4 has fruiting body with head at the tip of conidiophore. Fruiting body cannot be observed from the rest of four isolates.

From fungi isolated from soil sample (II), spherical shaped conidia are observed from all seven fungal isolates. Only one isolate has hyphae with septum and aseptate hyphae were observed from the rest of six isolates. Isolates No. 11 and 14 showed brush-like fruiting bodies and isolates No. 8 and 12 has fruiting body with head at the tip of conidiophore. Fruiting body cannot be observed from the rest of three isolates. Microscopical characteristics are also described in Table 3 and 4 respectively.

Amylase activity on agar plate

Among fourteen fungal isolates, four isolates (No.3, 4, 5 and 7) from SS-I and six isolates (No.9, 10, 11, 12, 13, and 14) from SS-II showed positive amylase producing activity by detecting the clear zone around the fungal colony after flooding with the iodine solution. These fungal isolates may be the promising fungal lines for amylase production. Three fungal isolates (No.1, 2, and 6) from SS-I and one isolate (No.8) do not exhibit amylase activity. Totally ten fungal isolates showed amylase activity.

Table 1. Macroscopical characteristics and amylase activity of seven fungal isolates from soil sample (I)

Isolates	Colony Morphology	Macroscopical characteristics of Fungal colony	Amylase activity on agar plate
1		Colonies characters are white and the surface of the colony looks chalky in appearance.	 Negative
2		Colonies characters are deep olive green in color and the surface of the colony shows compact mycelial growth in appearance.	 Negative
3		Colonies characters are deep green in color and white in periphery, the surface of the colony looks compact and powdery in appearance.	 Positive
4		This isolate has a rapid growth rate. The colonies are black and white in periphery. The surface texture of the colony shows powdery in appearance.	 Positive
5		Colonies characters are white cotton in color and the surface of the colony looks fluffy, velvety and rapid growth in appearance.	 Positive
6		Colonies characters are pale gray in color and the surface of the colony shows compact with white mycelial growth in periphery.	 Negative
7		Colonies characters are light green in color and the surface of the colony shows white peripheral mycelial growth in appearance.	 Positive

Table 2. Macroscopical characteristics and amylase activity of seven fungal isolates from soil sample (II)

Isolates	Colony Morphology	Macroscopical characteristics of Fungal colony	Amylase activity on agar plate
8		Colonies characters are light gray and white in periphery, the surface of the colony looks fluffy in appearance. Pigments was produced.	 Negative
9		Colonies characters are creamy in color and the surface of the colony looks compact in appearance. Pigments was produced.	 Positive
10		Colonies characters are creamy in color and the surface of the colony shows white compact mycelial growth with lobate in appearance.	 Positive
11		Colonies characters are green and the surface of the colony shows powdery and white in periphery. Pigments was produced.	 Positive
12		Colonies characters are blue green and white in periphery. The surface of the colony looks fluffy and powdery in appearance.	 Positive
13		Colonies characters are light yellow in color mycelia and black powdery at the middle and the surface of the colony looks fluffy and pale yellow mycelial growth in periphery in appearance.	 Positive
14		Colonies characters are green in color and the surface of the colony shows compact and lobate margin in appearance.	 Positive

Table 3. Microscopical characteristics of seven fungal isolates from soil sample (I)

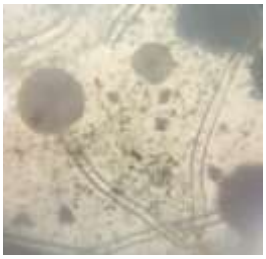
Isolates	Colony Morphology	Microscopical characteristics of Fungal colony
1		The conidia of this fungal isolate are oval shape. Conidiophore and fruiting and conidiophore cannot be observed. The fungal hyphae are hyaline and have no septum.
2		Conidia are oval shape. Conidiophore and fruiting cannot be observed. The fungal hyphae are hyaline and have septum.
3		Conidia are small circular in shape and chain pattern. Conidiophore show in branch pattern and fruiting body with brush like structure. The fungal hyphae have septum.
4		The microscopic observation shows the presence of hyaline hyphae, conidiophores, phialides and conidia. The conidiophores were originated from the basal foot cell and terminated in a vesicle head. Conidia are spherical shape with rough surface and are located over the phialides. The fungal hyphae have no septum.
5		No conidia formation was formed. The hyphae are thin, hyaline and have no septum.
6		Conidia are small circular in shape and the fungal hyphae have no septum. Fruiting body of fungi show brush-like structure. Zygospores were also observed.
7		No conidia are observed and the fungal hyphae have no septum.

Table 4. Microscopical characteristics of seven fungal isolates from soil sample (II)

Isolates	Colony Morphology	Macroscopical characteristics of Fungal colony
8		Conidia are spherical in shape and the fungal hyphae have no septum. Conidiophore with head and fruiting body were observed.
9		Conidia are spherical in shape and the fungal hyphae have no septum. Conidiophore and fruiting body were not observed.
10		Conidia are spherical chain in shape and the fungal hyphae have no septum. Conidiophore and fruiting body were not observed.
11		Conidia are one-celled small spherical in shape, chain pattern and also appear green in color. Conidiophore show in branch pattern and fruiting body with brush-like structure. The fungal hyphae are hyaline and have no septum.
12		Conidia are spherical in shape and the fungal hyphae have no septum. Conidiophore with vesicle head and fruiting body are observed.
13		Conidia are spherical in shape and the fungal hyphae have septum. Cytoplasmic inclusion of hyaline hyphae were visualized. Conidiophore and fruiting body were not observed.
14		Conidia are small spherical in shape and chain pattern. Conidiophore and fruiting body with brush like structure are observed. The fungal hyphae have no septum.

Discussion and Conclusion

In this study, amylase producing fungi were isolated from the soil sample and screened for the production of amylase enzyme. The sub-cultured fungal colonies were studied on the basis of their colonial characteristics and examined for the amylase activity test. For the sake of fungi production of amylase, the fungal colonies were sub-cultured in potato glucose agar media and

incubated at room temperature and then gram's iodine was flooded on the media and zone of clearance was noticed which indicated the production of amylase. Totally fourteen fungi from soil were isolated and all of the fungal colony were viewed under microscope and found to be a variety of fungal isolates based on their microscopical characteristics.

According to macroscopical characteristics, the fungi isolated from SS-I showed seven different colors, chalky white, deep olive green, deep green, black, cotton white, pale gray and light green. From SS-II, colors of the fungal colony are light gray, two creamy colors, blue green, light yellow and two green colors.

For microscopical characteristics of SS-I, hyphae with septum are observed from two isolates No. 2 and No. 3 and the rest of five isolates have no septum. Brush-like fruiting bodies are observed from isolates No. 3 and No. 6. Only isolate No. 4 have fruiting body with head. Zygospores are observed from isolate No.6 and sexual state of fungal hyphae also occurred. Three fungi produce spherical shaped conidia, two isolates produce oval shaped conidia and the rest of the two fungi have no conidia. Seven different colony morphology are observed from SS-II and fruiting body with head are found in two isolates No. 8 and No. 12. Brush-like fruiting body are also observed from two isolates such as No. 11 and No. 14. Six fungal lines possess aseptate hyphae and only one have hyphae with septum. All of them bear spherical shaped conidia.

Among seven isolates from SS-I, four isolates showed positive amylase activity and three showed negative amylase activity. From SS-II, six fungal lines showed positive amylase activity and only one showed negative amylase activity. The fungi isolated from SS-II showed more amylase activity and can be selected as promising fungal lines for further study. Based on the results of macroscopical and microscopical characteristics, the isolated fungi may be possible different species. In the present investigation, totally fourteen fungi were isolated from different locations of soil samples at Taungoo University Campus. The results showed significant variants in morphological characteristics thus it is speculated that the soil harbors diverse fungal populations. From plate assay method, ten isolates showed amylolytic potential and four fungal isolates showed negative amylase activity.

This study can share the knowledge of the importance of different types of fungi that produced amylase enzymes applied in the industrial field. This study showed for the production of expensive amylase enzyme from the cheap sources of soil sample. This can be concluded that some soils at Taungoo University Campus can be considered as a valuable natural source of amylase producing fungi. These promising fungal isolates were further selected and screened for the production of the amylase in liquid medium in the future study. However, further studies are required for identifying and classifying soil fungi.

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