INT. J. BIOL. BIOTECH., 19 (4): 397-406, 2022.

COMPARATIVE ANALYSIS OF RAW STARCH DEGRADATION USING *ASPERGILLUS TUBINGENSIS* SY 1

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ABSTRACT

The study was designed to characterize cereal, and halophytic starches, the effect of a fungal amylase on these starches, and their utilization as a source for amylase production. The cereal and halophytic starches were extracted and characterized on the basis of their hydration capacity, moisture content and pH. They were then subjected to fermentation by the fungal strain *A. tubingensis* SY 1. Maximum amylase yields (4.3 IU/ml, 72 hr.) under submerged fermentation (SmF) were obtained by the utilization of potato peels followed by barley bran (3.1 IU/ml, 80 hr.). In solid-state fermentation (SSF), maximum amylase production was observed using potato peels (17.8 IU/ml, 120 hr.) and wheat bran (10.7 IU/ml, 120 hr.). A wide range of substrate specificity was observed with the *A. tubingensis* SY 1 amylase, i.e., the ability to hydrolyze α-1,6 along with the α-1,4 glycosidic bonds. Strong amylase adsorption was observed in barley-bran, wheat-bran, and *Phragmites karka* stem among all the natural plant substrates used. For the scanning electron microscopy, substrates were hydrolyzed by *Aspergillus tubingensis* SY 1 amylase for 24 h which indicated that the enzyme is substrate-specific in action. *Aspergillus tubingensis* SY 1 produced substantial amounts of amylase under both solid-state (SSF) and submerged (SmF) fermentation conditions by consuming natural carbon sources, mainly barley-bran, wheat-bran, potato-peels, and the halophytes *Phragmites karka* and *Typha domingensis*. Potato peels, wheat bran and halophytes, *Typha domingensis* and *Phargmites karka* can prove prospective substrates for enzymatic production of amylases. Thus far, no study has shown the possible production of amylase from these halophytic plants, making them cheap and novel substrates for amylase production.

Keywords: *Aspergillus*, Starch, halophytes, amylase

Abbreviations: Submerged fermentation (SmF), solid-state fermentation (SSF), barley-bran (BB), rice-flour (RF), wheat-bran (WB), potato-peels (PP), maize-flour (MF), *Typha domingensis* shoot (TS), *Phragmites karka* leaves (PK.L), *Phragmites karka* stem (PK.S),3,5-Dinitrosalicylic acid (DNS), Sabouraud's dextrose agar (SDA).

INTRODUCTION

Starch is the principal carbohydrate store in green plants and makes up to seventy percent of the fresh plant material. It is the second most ample carbohydrate on earth, a vital constituent of the human diet extracted from plant roots, tubers, and seeds (Yazid *et al*., 2018; Santana and Meireles, 2014). The shape, nature, and size of starch granules are distinctive of the individual plant with a size ranging from 0.1 to 200 μm in diameter with the granules being angular, spherical, oval, ellipsoidal, or lenticular in shape (Alcázar-Alay and Meireles, 2015; Singh *et al*., 2003, Hoover 2001; Buléon *et al*., 1998).

Starch is used in different industrial processes ranging from food to textile, paper, oil, and pharmaceutical industries that result in scores of starchy wastes. Typically, starchy food wastes like potato-peels, wheat-bran, barley-bran, are present in mammoth amounts worldwide and are discarded without appropriate usage. These nutrient-rich wastes have considerable potential in industries such as in enzyme, bioethanol, biogas (methane), and biohydrogen production (Delabona *et al.*, 2013; Parawira *et al.*, 2005; Pandey *et al*., 2000).

Nature has provided microbes, plants, and animals with enzyme amylases to degrade starch into simple sugars. Factors affecting enzymatic starch degradation include starch source, percentage of amylopectin and amylose, granule size, polymorphic type, crystallinity, hydrolysis conditions, and the type of amylase (Tester *et al*., 2006; Li *et al*., 2004; Hoover and Zhou 2003; Oates 1997). Starches with a porous surface and lower pore diameter are degraded easily compared to those with a smooth surface and higher pore diameter (Yonemoto *et al.*, 2007; Tester *et al*., 2006; Franco and Ciacco, 1992; Franco *et al*., 1988). In recent years, extensive studies were carried out on the action of amylases on the starchy substrates, yet it still deserves attention.

A lucrative amylase production process is currently as significant as in the nineteenth century when the first amylase was identified. An opposite fermentation method, selecting the relevant substrate, and adjusting the

chemical and parameters result in higher amylase yield. Two fermentation methods, namely, submerged (SmF) and solid-state (SSF), are employed for industrial enzyme synthesis owing to their significance. SmF is distinguished due to the simplicity of medium sterilization, synthesis of secondary metabolites, and end product purification. Oppositely, SSF systems are like the natural habitat of microorganisms (De Souza and Magalhães, 2010). SSF requires less capital investment, it has a lower energy requirement, provides superior product recovery, and there is no foam development (Tanyildizi *et al*., 2007; Couto and Sanromán, 2006).

Along with the cultivation of plants for their utilization as food and feed in Pakistan, agricultural waste is also generated in large quantities. This waste can be used as substrate for microbial enzyme production which in turn can hydrolyze the waste generated. The common plant wastes are barley bran, wheat bran, potato peels etc. They are rich in carbon, minerals and nitrogen. Apart from the conventional plant waste as a source of enzyme production and substrate hydrolysis, a number of halophytic plants could be tested to overcome the problem of food vs. fuel conflict. In Pakistan large portion of irrigated area is threatened with the problems of salinity and water logging. A beneficial and inexpensive way is to exploit these salt affected soils by using salt tolerant species as well for enzymatic hydrolysis (Khan and Qaiser, 2006).

The purpose of this work was to assess the physicochemical characteristics of natural starches, to investigate the susceptibility of diverse tuber and root starches to fungal amylase action, and to screen natural substrates (agricultural byproducts) for amylase production from *Aspergillus tubingensis* SY 1 both under SSF and SmF conditions.

MATERIALS AND METHODS

Natural plant substrates

The natural plant substrates used were barley-bran (BB), rice-flour (RF), wheat-bran (WB), potato-peels (PP), maize-flour (MF), *Typha domingensis* shoot (TS) and leaves and stem of *Phragmites karka* (PK.L) and (PK.S). The substrates were dried, powdered, passed through a sieve (100 mesh size) and dried (80^{o}C).

Starch extraction from natural substrates

Plant tissues (0.5 g) were transferred to 5mL of ethanol (80%), incubated for 3 min at 100^{o}C and centrifuged (x3000g, 10 min) to collect the pellet. Extraction with ethanol was repeated two times with pellet, by discarding supernatants. From the final pellet, ethanol was evaporated with the addition of 5 mL of distilled water. The homogenate (500 µL) was transferred to micro-centrifuge tubes, sealed and gelatinized for 10 min, at 100^{o}C (Chow and Landhausser, 2004).

Determination of starch content

Isolated starch from natural substrates (25 µL) was mixed with an equal volume of Na-acetate buffer (50 mM, pH 5.6) followed by adding 1 M Acetic acid (50 µL). Distilled water was used to make up the volume to 2.35 mL, and A_{660} was noted after the addition of 50 µL of the iodine reagent (1% I^{-}:10% KI^{-}: H_20; 1:1:3). A blank was made by substituting the natural plant substrate with distilled water. The starch contents were calculated with a standard starch curve.

Characterization of natural plant substrates

The hydration capacity, moisture content, and pH of the natural plant substrates were determined (Olayemi *et al.*, 2008) as follows:

a) One gram of the starches was mucilaged with distilled water (100 mL) and pH determined.
b) One gram of the extracted starch was weighed, oven-dried (105^{o}C, 1h), weighed, and percentage weight loss on drying was calculated as:
   Final weight / initial weight × 100
c) One gram of the starch powder (*Z*) was mixed in 10mL distilled water for 2h. Following mixing, left to stand for half an hour and centrifuged (10 min. at x 3000*g*). The supernatant was discarded, the powder was weighed (after water uptake - *Y*), and the hydration capacity calculated as;
   Y / Z

Inoculum preparation

Aspergillus tubingensis SY 1 (KX243270) spores were transferred from Sabouraud's dextrose agar (SDA) plates to sterile saline to get a spore suspension (~2 x 10^4 spores/ml). For each experiment, a 500 µl aliquot was used.

Shake-flask (SmF) and solid-state fermentation (SSF) experiments

In shake-flask experiments, the natural substrates or soluble starch were added to the amylase production media [g/100 mL - KCl (0.05); NH_4NO_3 (0.3); KH_2PO_4 (0.1); $FeSO_4.7H_2O$ (0.001); peptone (1.0); $MgSO_4.7H_2O$ (0.05)] at 1% and 0.5% (w/v) concentration, respectively. The fermentation experiments were carried out in a shakobator (150 rpm) at 30°C for five days. The fermentation contents were centrifuged (x6000 *g*, 4°C, 20 min) and CFCS (cell free culture supernatant) was quantified for amylase.

Along with the SmF experiments, SSF was also carried out. The plant substrates (2 g) were hydrated with amylase production medium to achieve a moisture content of 83% and incubated for 10 days (240 h) at 30°C. The enzymes were harvested at 28°C using Na-acetate buffer (8.8 mL, pH 5.9, 50 mM) for 30 min. at 150 rpm in a shaker. The flask contents were filtered by Whattman filter paper No.1 and then centrifuged (4°C, x6000 *g*, 20 min,). The filtrates obtained were used to quantify amylase.

Amylase assay

Soluble starch (0.5% w/v in 50mM Na-acetate buffer, pH 5.6, 25 µL) was mixed with CFCS (25 µL) and incubated (60°C, 30 min). The reaction was halted by adding 1% DNS (150 µL) and boiling for five min. The reaction mixture was cooled on ice for 5 min and A_{500} recorded after the addition of 0.720 mL of distilled water. A reaction blank was prepared by the addition of 1% DNS before enzyme addition (Miller 1959).

One unit of amylase activity is the micromoles of reducing sugar produced by 1 mL of amylase in 1 min. The amount of reducing sugar was calculated using a standard curve of glucose.

Substrate specificity of amylase

The substrate specificity of the enzyme was calculated by replacing starch with sucrose, maltose, raffinose, dextrin, lactose, barley-bran (BB), wheat-bran (WB), rice-flour (RF), maize-flour (MF), *Typha domingensis* (shoot) and *Phragmites karka* [leaf (PK.L) and stem (PK.S)]. Substrate solutions (0.5%) were made in Na-acetate buffer (50 mM, pH 5.6) and CFCS was obtained by growing the fungal strain in enzyme production medium with 0.5% starch.

Amylase adsorption on natural plant substrates

Two ml Cell-free culture supernatant and 0.1 g of soluble starch or natural plant substrates (washed with distilled water and then equilibrated in 50mM Na-acetate buffer, pH 5.6) were mixed for 20 min at 4°C. After centrifugation (10 min, x3000 *g*), amylase activity in the supernatant was quantified. The adsorption rate (AR) was calculated as:

$$AR\ (\%) = [(U-R) / U] \times 100$$

Where:

R - Residual amylase activity in supernatant

U - Untreated enzyme solution (crude extract)

Scanning electron microscopy (SEM) of the soluble starch and natural plant substrates

To check the effects of *A. tubingensis* SY 1 amylase on the substrates, SEM was carried out. CFCS (4 mL) and the substrates (0.5 g; washed with distilled water and then equilibrated with 50 mM Na-acetate buffer (pH 5.6) were mixed and kept at 60°C for varying time intervals. The reaction was halted by adding 1 M NaOH and kept unshaken for 15 min. Amylase activity was tested in the supernatant after centrifugation (x2500 *g*, 10 min.). The pH was lowered back to 5.6 with 1 N HCl, and amylase activity was assessed after centrifugation (x2500 *g*, 10 min) in the supernatant to confirm the reaction stoppage.

Samples were then washed with distilled water thrice to remove the remaining enzyme. The substrates were subjected to dehydration by ethanol, tetra-butanol was added and freeze-dried. The dry powders were observed under SEM using the JSM-6380A JEOL microscope (Tokyo, Japan).

RESULTS AND DISCUSSION

The agro-industrial wastes have substituted the pure and costly raw materials to produce value-added products, for example organic acids, enzymes, ethanol, *etc*. (Pandey *et al*., 2000) worldwide. Pakistan being an agricultural country, has still not followed the modifications in agriculture trends, and a lot of agriculture waste is burnt by the farmers leading to air pollution. These diverse natural plant substrates can be used to highlight their potential for amylase production and their subsequent hydrolysis.

Characterization of natural plant substrates

Natural plant substrates' characterization implies that moisture contents of maize-flour, potato-peels, *Typha domingensis* shoot, and *Phragmites karka* stem were high (Table 1). The larger grain size suggests bigger pores to trap water might cause more significant moisture content. Increased moisture content also represents strong binding of starch granules (Karathanos and Saravacos, 1993). Similarly, the hydration capacity of *Typha domingensis* shoot and *Phragmites karka* stem is significantly high and can absorb twice more water than their weight (Table 1). The substrates *Phragmites karka* (leaf and stem), rice-flour and barley-bran had slightly acidic pH, while other substrates' pH was near neutral (Table 1). This signifies that if the substrates are dispersed in a liquid medium, an acidic or alkaline medium will not result. If the pH balance is not maintained i.e. it is more alkaline or acidic, fungal cells will not be able to multiply and make enzymes.

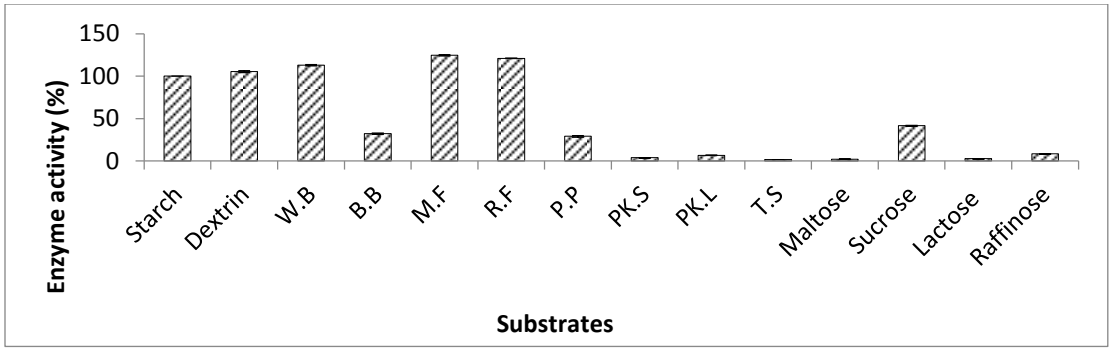


Fig. 1. Substrate specificity of amylolytic enzymes produced by *A. tubingensis* SY 1. For determining the enzyme activity %, soluble starch activity was taken as 100%. WB (wheat-bran), BB (barley-bran), MF (maize-flour), RF (rice-flour), PP (potato-peels), PK.S (*Phragmites karka* stem), PK.L (*Phragmites karka* leaf) and TS (*Typha domingensis* shoot).

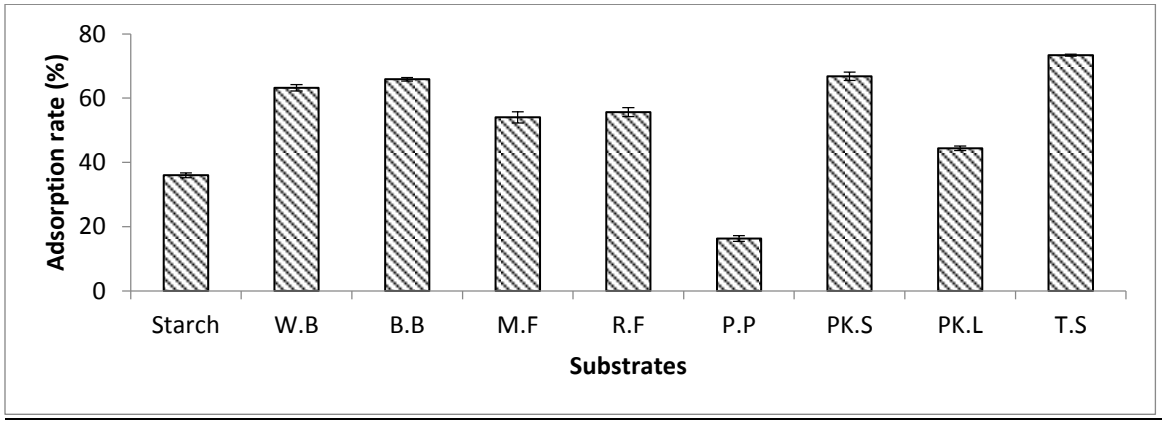
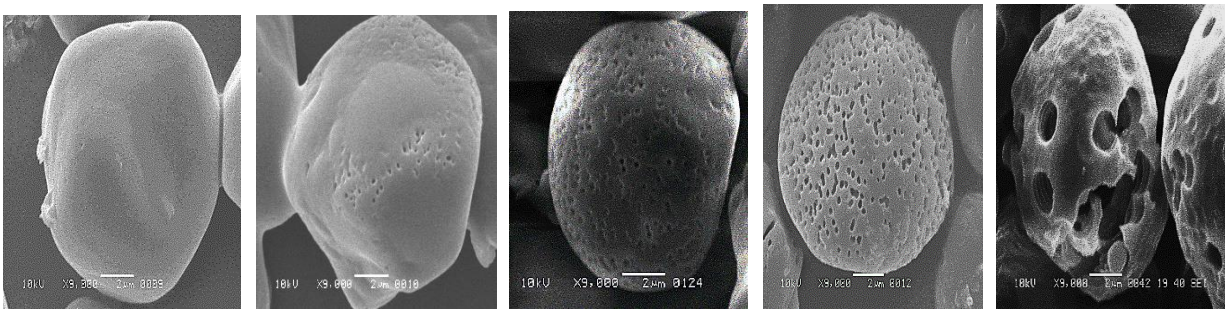


Fig. 2. Adsorption of amylases on natural substrates produced by *A. tubingensis* SY 1. Adsorption rate (%) was determined by taking the enzyme activity before adsorption as 100% (100% of the enzyme activity = 4.207 IU/ml). WB (wheat-bran), BB (barley-bran), MF (maize-flour), RF (rice-flour), PP (potato-peels), PK.S (*Phragmites karka* stem), PK.L (*Phragmites karka* leaf) and TS (*Typha domingensis* shoot).



(a) (b) (c) (d) (e)

Fig. 3. Scanning electron micrographs of the effect of amylase from *A. tubingensis* SY 1 (a) 0 min., (b) 10 min., (c) 20 min., (d) 30 min., (e): 24 h on commercial starch.

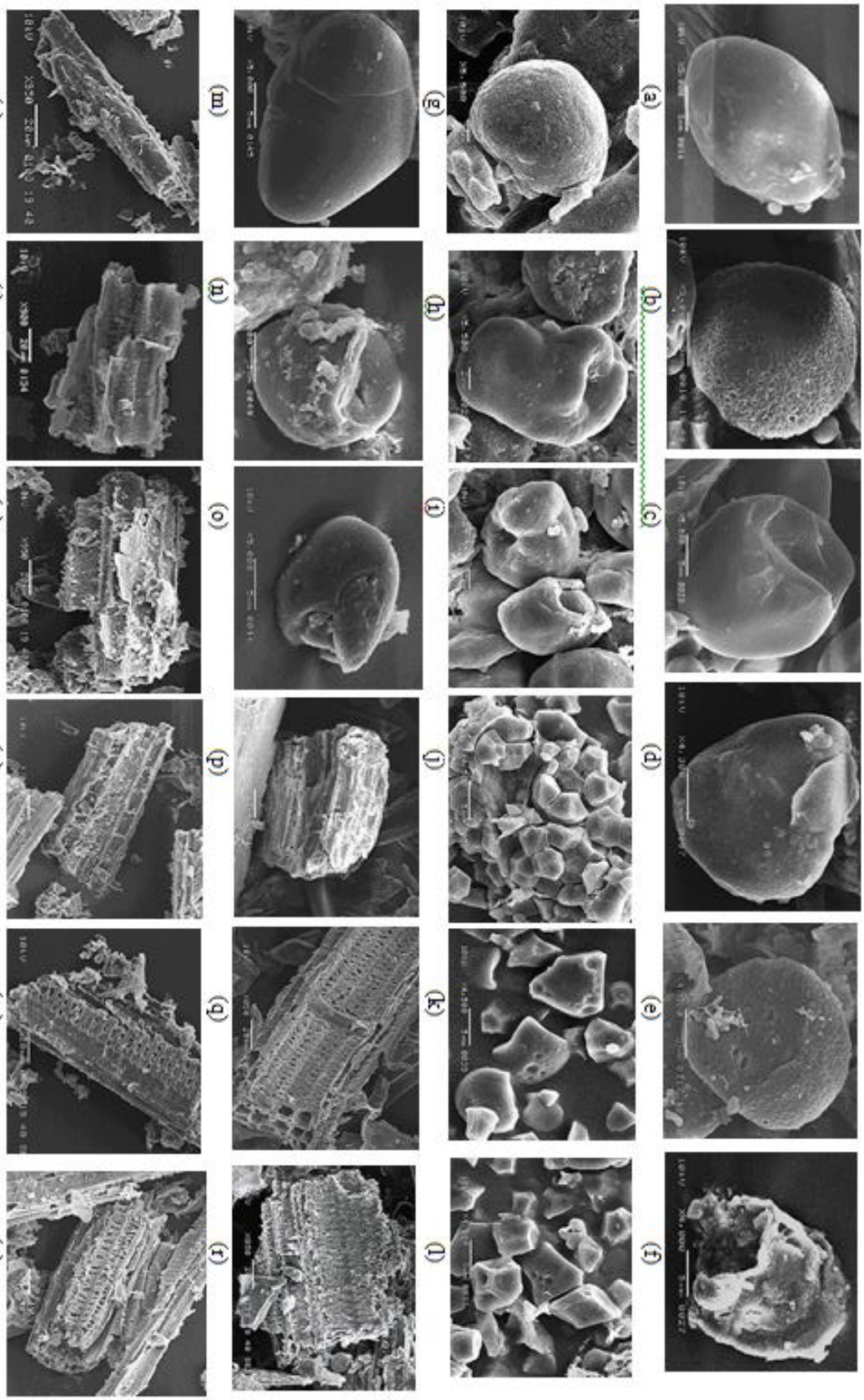
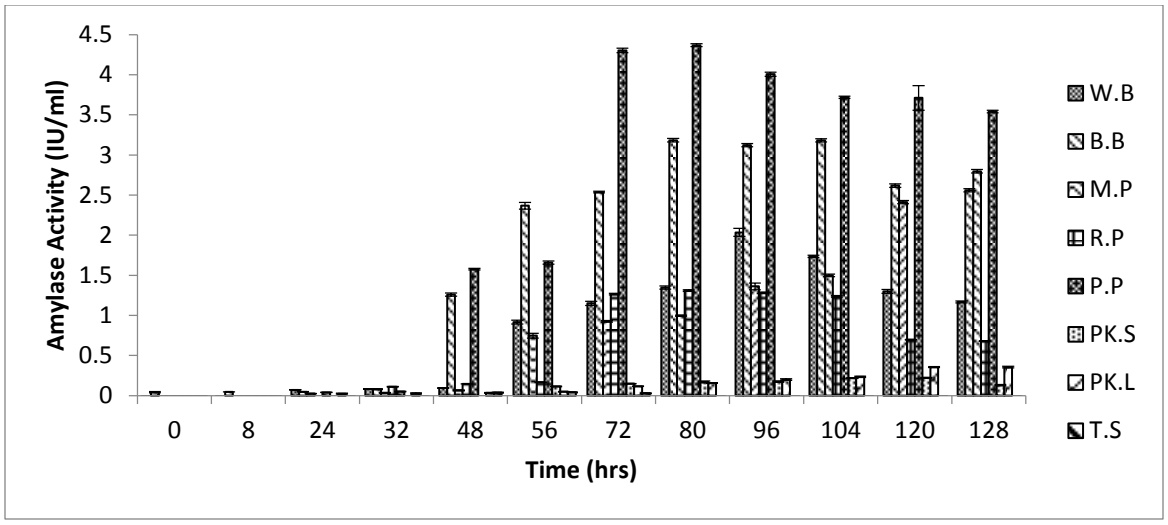
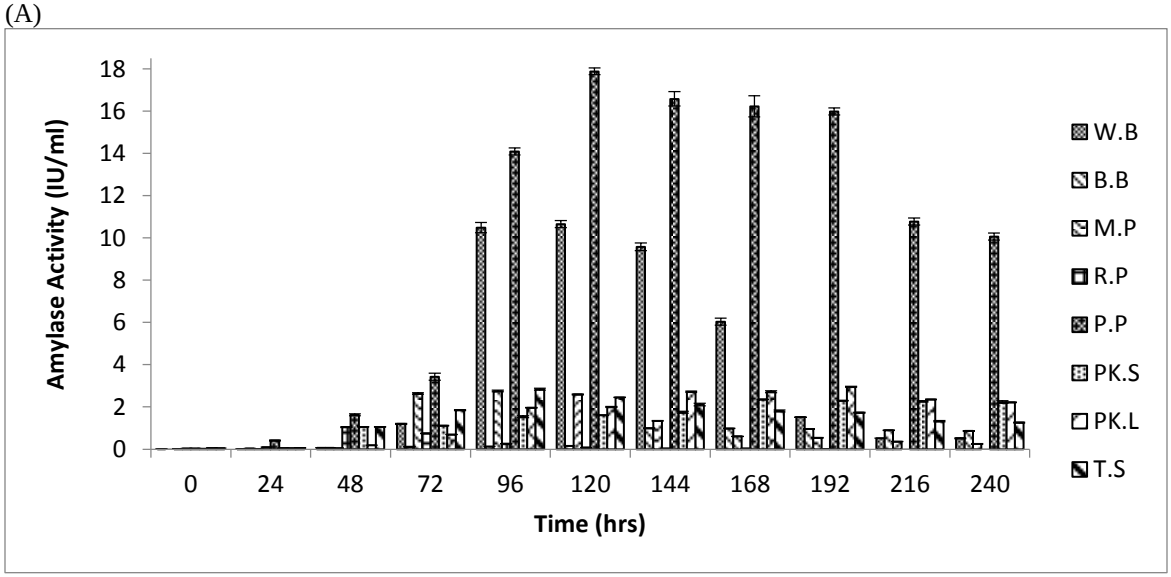


Fig. 4. Scanning electron micrographs of the effect of amylase from *A. tubingensis* SY 1 on wheat-bran (a) 0 min., (b) 30 min., (c) 24 h; barley-bran (d) 0 min., (e) 30 min., (f) 24 h; maize-flour (g) 0 min., (h) 30 min., (i) 24 h; rice-flour (j) 0 min., (k) 30 min., (l) 24 h; potato-peels (m) 0 min., (n) 30 min., (o) 24 h; *Phragmites karka* stem (p) 0 min., (q) 30 min., (r) 24 h; *Phragmites karka* leaf (s) 0 min., (t) 30 min., (u) 24 h; *Typha domingensis* shoot (v) 0 min., (w) 30 min., (x) 24 h.



(A)



(B)

Fig. 5. Amylase production by *A. tubingensis* SY 1 using different natural WB (wheat-bran), BB (barley-bran), MF (maize-flour), RF (rice-flour), PP (potato-peels), PK.S (*Phragmites karka* stem), PK.L (*Phragmites karka* leaf) and TS (*Typha domingensis* shoot) substrates under (A) SmF and (B) SSF conditions.

Table 1. Physico-chemical properties of the natural substrates.

Substrates	**Hydration capacity**	**Moisture content (%)**	**pH**	**Starch content (%)**
Wheat-bran	1.17	92	6.2	0.335
Barley-bran	1.27	92	5.5	0.08
Maize-flour	1.31	94	6.3	0.54
Rice-flour	1.13	89	5.9	0.47
Potato-peels	1.31	94	5.8	0.456
Phragmites karka stem	1.63	94	5.2	0.015
Phragmites karka leaf	1.42	93	5.7	0.01
Typha domingensis shoot	1.58	94	6.8	0.01

Table 2. Volumetric enzyme production (Q_p) and specific productivity ($Y_{p/s}$) from *A. tubingensis* SY 1 by SmF and SSF of natural substrates.

	SmF		**SSF**	
Substrate	**Q_p IU/L/hr.**	**Q_p IU/L/hr.**	**$Y_{p/s}$ IU/mg**	**$Y_{p/s}$ IU/mg**
Wheat-bran	27.26	217.05	0.49	1.34
Barley-bran	64.63	7.95	0.01	3.76
Maize-flour	26.64	37.90	0.08	2.13
Rice-flour	24.99	39.79	0.08	1.77
Potato-peels	89.93	181.92	0.38	5.48
Phragmites karka stem	1.67	16.01	0.10	0.10
Phragmites karka leaf	3.44	17.22	0.08	0.23
Typha domingensis shoot	0.47	38.64	0.18	0.02

Substrate specificity of amylase

The *A. tubingensis* SY 1 amylase had a wide-ranging substrate specificity, displaying the ability to hydrolyze α-1,6 and α-1,4 glycosidic linkages, and it varies with the botanical origin. The hydrolysis of branched alpha glucans such as dextrin, starch, wheat, barley, and rice; were more effective than of sucrose, raffinose, maltose, and lactose (Fig. 1). The cereal starches such as rice and corn are A-type, while potato starch is B-type. The longer double helices and strengthened hydrogen bonds with superior crystalline structures are salient features of B-type starches, thus are less susceptible to amylolysis than A-type starches. Therefore, the hydrolysis rate is affected by the substrate structure, molecular size, and the type of bond (Strąk-Graczyk *et al*., 2019; Okolo *et al*., 2000; Forgarty and Kelly, 1990).

Enzyme adsorption on pure and natural substrates

For hydrolysis to progress, enzyme adsorption is a prerequisite. The results indicated that the amylase strongly adsorbed to *Phragmites karka* stem (66.83%), barley-bran (65.88%), and wheat bran (63.24%), respectively. It was moderately adsorbed to rice- and maize-flours and weakly adsorbed to *Phragmites karka* leaf (PK.L) [Fig. 2]. Apart from the typical natural substrates, a high adsorption rate in *Phragmites karka* stem indicates that this plant can be utilized in the saccharification process.

Topographic changes in pure and natural substrates by *A. tubingensis* SY 1 amylase

Amylase is substrate-specific in action; therefore, it hydrolyzes different substrates differently because of the substrates' structural uniqueness (Strąk-Graczyk and Balcerek, 2020; Das and Kayastha; 2018; Chen *et al*., 2011; Dhital *et al*., 2010). A study by Fannon *et al*. (1992) identified that starchy granules might comprise passages and openings for reagents, enzymes, or water. Scanning electron micrograph showed that the facades of unhydrolyzed starch granules were smooth without scratches, whereas hydrolyzed starches had cracks. After hydrolysis of wheat-bran by the amylase, it was observed that granules were damaged on their exterior, signifying exo-corrosion. The hydrolysis of other substrates by the amylase indicated pores, i.e., endo-corrosion. Due to endo-corrosion, pores are produced through which enzymes can penetrate (Oates, 1997). The pores were not even for all substrates; some substrates were noticeably more susceptible to amylase due to individual substrates' structural and chemical characteristics. With time, the number of cracks or holes increased and became deep and wide (Fig. 3 and 4).

Fermentation experiments with natural plant substrates

Amylase production kinetics was studied under SSF and SmF using the natural substrates. Depending upon the substrate used, amylase production was initiated at different time-intervals. Under SmF, amylase production started at 56 h with *Phargmites karka* (stem), while it initiated at 24 h using wheat-bran. Maximum amylase yields (4.3 IU/mL, 72 h.) under SmF were obtained by the utilization of potato peels which was tailed by barley bran (3.1 IU/mL, 80 h), maize flour (2.8 IU/mL, 128 h), wheat bran (2.0 IU/mL, 96 h) and rice flour (1.3 IU/mL, 80 h) [Fig. 5 (a)]. In SSF experiments for *A. tubingensis* SY 1, the superlative substrate for maximum amylase activity was potato peels (17.8 IU/mL, 120 h) trailed by wheat bran (10.7 IU/mL, 120 h) [Fig. 5 (b)].

The prospective of wheat-bran for amylase production by fungi is well established because of the penetrability and texture which aid mycelial dispersion (Bhargav *et al.*, 2008; Pandey, 2003; Madruga and Camara, 2000). The present study proposes potato-peels as a potential alternative for wheat-bran. Utilization of potato-peel wastes to support hydrolytic enzyme production and microbial growth has also been determined by Parawira *et al.* in 2005. Potato-peels contain 66.8% starch and are superior to wheat-bran in certain mineral contents, water-holding capacity, and total dietary fiber (Al-Weshahy and Rao, 2012; Toma *et al*., 1979).

In conjunction with the conventional plants as a starch source, halophytic plants should be explored to overcome the food *vs.* fuel conflict. These halophytes can be grown on barren land (waterlogged and saline); consequently the productive land for food can be conserved. Studies indicate these high salt-tolerant crops' potential as a realistic choice for developing saline-soils and conserving freshwater (Yensen, 2006; Khan and Qaiser, 2006).

Thus, two halophytic plants *Typha domingensis* and *Phargmites karka* were used as substrates for amylase production. These halophytes were observed as good inducers of amylase under SSF conditions. SSF of PK.L and TS yielded more than 2.7 IU/mL amylase by *A. tubingens*is SY 1. Both halophytes, *Typha domingensis* and *Phargmites karka* can prove prospective substrates for enzymatic production of xylanases, pectinases, endoglucanases *etc*. Thus far, no study has shown the possible production of amylase from these halophytic plants, making them cheap and novel substrates for amylase production.

Amylase production was also assessed in terms of specific productivity ($Y_{p/s}$) and volumetric enzyme production (Q_p) under SSF and SmF conditions. A similar pattern was observed in both SSF and SmF, i.e., maximum Q_p was detected when wheat-bran and potato-peels were used as substrates (Table 2).

Conclusions

It was concluded from this study that

1. *A. tubingensis* SY 1 has an excellent potential to produce substantial amounts of amylase by using natural carbon sources, mainly barley-bran, wheat-bran, potato-peels, and the halophytes *Typha domingensis* and *Phragmites karka*.
2. Amylase titres were high in SSF with maximum volumetric enzyme production (Q_p) in potato-peels (SSF and SmF).

Conflict of interest

Authors have no conflict of interest.

Authors' Contribution:

Saira Yahya: Research idea, performed the experiments and wrote the paper with support from the co-author Shakeel Ahmed Khan: Research idea, supervised the project, in-charge of overall direction and planning.

Acknowledgments

The authors acknowledge the financial support provided by the Higher Education Commission Pakistan.

REFERENCES

Al-Weshahy, A. and V. A. Rao (2012). Potato peel as a source of important phytochemical antioxidant nutraceuticals and their role in human health – A review, pp. 207-224, In: Rao, V.A. (Ed.), *Phytochemicals as nutraceuticals - Global approaches to their role in nutrition and health*. InTech, ISBN: 978-953-51-0203-8.

Alcázar-Alay, S. C. and M. A. A. Meireles (2015). Physicochemical properties, modifications and applications of starches from different botanical sources. *Food Science Technology* (Campinas), 35(2): 215-236.

Bhargav, S., B. P. Panda, M. Ali and S. Javed (2008). Solid-state Fermentation: An Overview. *Chemical and Biochemical Engineering Quartrerly*, 22(1): 49-70.

Buléon, A., P. Colonna, V. Planchot and S. Ball (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules,* 23(2): 85-112.

Chen, Y., S. Huang, Z. Tang, X. Chen and Z. Zhang (2011). Structural changes of cassava starch granules hydrolyzed by a mixture of α-amylase and glucoamylase. *Carbohydrate Polymers*, 85(1): 272-275.

Chow, P. S. and S. M. Landhausser (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology,* 24(10): 1129-1136.

Couto, S. R. and M. A. Sanromán (2006). Application of solid-state fermentation to food industry- A review. *Journal of Food Engineering,* 76(3): 291-302.

Das, R. and A. M. Kayastha (2018). Enzymatic hydrolysis of native granular starches by a new β-amylase from peanut (*Arachis hypogaea*). *Food Chemistry*, 276.

Delabona, P. D., R. Pirota, C. A. Codima, C. R. Tremacoldi, A. Rodrigues and C. S. Farinas (2013). Effect of initial moisture content on two Amazon rainforest Aspergillus strains cultivated on agro-industrial residues: biomass-degrading enzymes production and characterization. *Industrial Crops and Products*, 42(1): 236-242.

De Souza, P. M., De Oliveira and P. Magalhães (2010). Application of microbial α-amylase in industry - A review. *Brazilian Journal of Microbiology*, 41(4): 850-61.

Dhital, S., A. K. Shrestha and M. J. Gidley (2010). Relationship between granule size and in vitro digestibility of maize and potato starches. *Carbohydrate Polymers*, 82(2): 480-488.

Fannon, E., R. J. Hauber and J. N. BeMiller (1992). Surface pores of starch granules. *Cereal Chemistry*, 69: 284-288.

Fogarty, W. M. and C. T. Kelly (1990). Recent advances in microbial amylases. In: Fogarty, W.M. and C.T. Kelly, (Ed.), *Microbial Enzymes and Biotechnology*, (2nd edn.).

Franco, C. M. L. and C. F. Ciacco (1992). Factors that affect the enzymatic degradation of natural starch granules: effect of size of the granules. *Starch*, 44 (11): 422-426.

Franco, C. M. L., C. F. Ciacco, and D. Q. Tavares (1988). Studies on the susceptibility of granular cassava and corn starches to enzymatic attack: part 2 study of the granular structure of starch. *Starch*, 40(1): 29-32.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, 45(3): 253-267.

Hoover, R. and Y. Zhou (2003). In vitro and in vivo hydrolysis of legume starches by α-amylase and resistant starch formation in legumes - A review. *Carbohydrate Polymers*, 54(4): 401-417.

Karathanos, V. T. and G. D. Saravacos (1993). Porosity and Pore Size Distribution of Starch Materials. *Journal of Food Engineering*, 18: 259-280.

Kelly, C. T., D. J. Bolton and W. M. Fogarty (1997). Biphasic production of α-amylase of *Bacillus flavothermus* in batch fermentation. *Biotechnology Letters*, 19: 75-77.

Khan, M. A. and M. Qaiser (2006). Halophytes of Pakistan: Distribution, Ecology, and Economic Importance In: *Sabkha Ecosystems: The South and Central Asian Countries* (Khan, M. A., H. Barth, G. C. Kust and B. Boer, Ed.). Volume II. pp. 129-154.

Li, J. H., T. Vasanthan, R. Hoover and B. G. Rossnage (2004). Starch from hull-less barley: IV. Morphological and structural changes in waxy, normal and high-amylose starch granules during heating. *Food Research International*, 37: 417-428.

Madruga, S. and S. Camara (2000). The chemical composition of multimistura as a food supplement. *Food Biochemistry*, 68: 41-44.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3): 426-428.

Oates, C. G. (1997). Towards an understanding of starch granule structure and hydrolysis. *Trends in Food Science and Technology*, 8(11): 375-382.

Olayemi, O. J., A. R. Oyi and T. S. Allagh (2008). Comparative evaluation of maize, rice and wheat starch powders as pharmaceutical excipients. *Nigerian Journal of Pharmaceutical Sciences*, 7(1): 131-138.

Okolo, B. N., F. S. Ire, L. I. Ezeogu, C. U. Anyanwu, and F. J. C. Odibo (2000). Purification and some properties of a novel raw starch-digesting amylase from *Aspergillus carbonarius*. *Journal of the Science of Food and Agriculture*, 81: 329-336.

Pandey, A., C. R. Soccol and D. Mitchell (2000). New development in solid state fermentation: I.- bioprocess and products. *Process Biochemistry*, 35: 1153-1169.

Pandey, A. (2003). Solid-state fermentation. *Biochemical Engineering Journal*, 13(2-3): 81-84.

Parawira, W., M. Murto, J. S. Read and B. Mattiasson (2005). Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste. *Process Biochemistry*, 40(9): 2945-2952.

Santana, A. L. and M. A. A. Meireles (2014). New starches are the trend for industrial applications: a review. *Food Public Health*, 4(5): 229-241.

Singh, N., J. Singh, L. Kaur, N. S. Sodhi and B. S. Gill (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry*, 81(2): 219-231.

Strąk-Graczyk, E., M. Balcerek, K. Przybylsk and D. Żyżelewicz (2019). Simultaneous saccharification and fermentation of nativerye, wheat and triticale starch. *Journal of the Science of Food and Agriculture*, 99(11): 4904–4912.

Strąk-Graczyk, E. and M. Balcerek (2020). Effect of pre-hydrolysis on simultaneous saccharification and fermentation of native rye starch. *Food and Bioprocess Technology*, 13: 923–936.

Tanyildizi, M. S., D., Ozer and M. Elibol (2007). Production of bacterial α- amylase by *B. amyloliquefaciens* under solid substrate fermentation. *Biochemical Engineering Journal*, 37: 294-297.

Tester, R. F., J. Karkalas and X. Qi (2004). Starch - Composition, fine structure and architecture. *Journal of Cereal Science*, 39(2): 151-165.

Toma, R. B., P. H. Orr, B. D'appolonia, F. R. Dintzis and M. M. Tabekhia (1979). Physical and chemical properties of potato peel as a source of dietary fiber in bread. *Journal of Food Science*, 44(5): 1403-1407.

Yazid, N. M. S., N. Abdullah, N. Muhammad and H. M. Matias-Peralta (2018). Application of starch and starch-based products in food industry. *Journal of Science and Technology*, 10(2): 144–174. https://doi.org/10.30880/jst.2018.10.02.023

Yensen, N. P. (2006). Halophyte uses for the twenty-first century. In: Khan, M. A. and D. J. Weber, (Ed.), *Eco-physiology of High Salinity Tolerant Plants*. Springer, Netherlands. pp. 367-396.

Yonemoto, P. G., M. A. Calori-Domingues and C. M. L. Franco (2007). Efeito do tamanho dos grânulos nas características estruturais e físico-químicas do amido de trigo. *Ciência e Tecnologia de Alimentos*, 27 (4): 761-771.

(*Accepted for publication September 2022*)