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Amylase Production by some Local Fungal Isolates Using some Agricultural by -Products Via Solid State Fermentation .

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ABSTRACT

The potential of eight local fungal isolates for amylase production using some agricultural by -productes (wheat bran ,corn bran ,rice bran and potato waste was investigated.With all wastes the optimum incubation period for enzyme production was at 7 days . The maximum production of amylase by *Penicillium* sp .(1) was 183.34 ,119.24 ,55.98 and 46.51 IU/ g .after 7 days of incubation when wheat bran , corn bran, potato waste and rice bran was used ,respectively. After 5 days of incubation ,at 28°C the production was 267.92 ,80.19 ,34.22 and 24.34 IU/ g .The maximum yield of enzyme 268.96 IU/ g by *Penicillium* sp . (2) was obtained with wheat bran after 5 days of incubation ,while with corn bran 75.48 IU/ g ,potato waste 29.76 IU/ g and 36.79 IU/ g rice bran . The maximum yield (398.47 IU/ g) was obtained by *Penicillium chrysogenum* with wheat bran after 7 days of incubation followed by corn bran (163.54 IU/ g) after 9 days ,rice bran (125.24 IU/ g) and potato waste (98.87 IU/ g) after 9 days of incubation . *Penicillium georgiense* achieved the highest values of enzyme (407.65 IU/ g) after 7 days and 347.52 IU/ g after 5 days of incubation ,at 28°C when wheat bran was used as cultivation medium. Using wheat bran , rice bran ,corn bran and potato waste, enzyme production by *Penicillium* sp.(3) at the optimum time of incubation at 28°C was 320.49,82.68 ,134.85 and 97.98 IU/ g , respectively. The maximum production of enzyme by *Verticillium* sp. was 258.6 IU/ g in case of using wheat bran ,while it was 102.29 IU/ g ,103.20 and 96.50 IU/ g with rice bran ,corn bran and potato waste ,respectively .By using wheat bran higher capacity of enzyme production by *Aspergillus westerdijkiae* (387.45 IU/ g) was obtained after the 7th day of incubation at 28°C,but after the 5th day of incubation the production was 234.64 IU/ g .The production after 7 days was 157.52 ,102.49 and 90.37 IU/ g , when corn bran, potato waste and rice bran was used ,while the production after 5 days of incubation at 28°C were 101.32 IU/ g ,84.42 IU/ g and 57.12 IU/ g respectively . The highest value of enzyme (194.57 IU/ g) was obtained by *Alternaria* sp. after the 7th day of incubation when wheat bran is used as cultivation medium .From the results it could be concluded that wheat bran proved to be the best waste for enzyme production ,followed by corn bran ,rice bran and potato waste .

Keywords :Amylase ,Agricultural wastes ,Solid state fermentation .

INTRODUCTION

Amylases are important enzymes that hydrolyze polysaccharides such as starch into simple sugars (Suganthi *et al*. (2011) .Amylases are resemble 25% of the world enzyme requirements. It is used in many industries such as foods , detergents , pharmaceuticals , textile and paper industries .Amylases are classified into three subtypes α, β and γ amylases . They act on α, 1-4 glycosidic bonds in starchy materials. It can be divided into endo amylases and exo amylases .the endo amylases catalyze hydrolysis in random manner producing chains of linear and branched oligosaccharides with various lengths. Meanwhile , exoamylases act on the non-reducing end resulting in successively shorter end products (Gupta *et al*., 2003). All α amylases (EC 3.2.1.1) hydrolyze starch into glucose (monosaccharide) and maltose (disaccharide) .

Amylases can be obtained from plants, animals and microorganism, but microbial production is favored because of their advantages over plant and animal amylases it have high production in short time with low cost. Also, it have stability at different extreme conditions as well as less harmful .(Pandey *et al.,* 2000) production of amylase by fungi have the advantage of being secreted extra cellulary. Also, fungi have the ability to penetrate hard substrates facilitates the hydrolysis process. In addition, in solid state fermentation, fungi are highly suitable. (Subash *et al.,* 2017)

Submerged fermentation and solid state fermentation (SSF) are the two fermentation process for enzyme production . Solid state fermentation has a lot of advantages over submerged fermentation .These advantages are simple technique ,low capital investment , less water output and low energy requirement .The using of agricultural and industrial wastes in solid state fermentation is considered an important advantage because it reduce the production cost and solve the problems of pollution . In fermentation studies , it is very important to select isolates that have high capacity of enzyme production .(Tunga and Tunga 2003)

The aim of this work is to produce amylase by local fungal isolates using some agricultural by -productes via SSF in order to minimize enzyme cost production and environmental pollution .

MATERIALS AND METHODS

Media used :

Basal fermentation media .It is used for enzyme production ,it consists of 5gm of agricultural by -product and 5ml of distilled water .Medium was sterilized at 121°C for 20 min .

Potato dextrose agar (PDA) (Murray *et al*., 1995).It is used for maintenance of fungal isolates , it consists of :Potatoes (sliced) 200.0g , dextrose 20.0g , agar 20.0g and distilled water 1000ml.
pH was adjusted to 6.5 .

Potato dextrose starch agar medium (PDSA) (Chimata *et al*., 2011):Potatoes (sliced) 200.0g , dextros 10.0g , soluble starch 10.0g , agar 20.0g and distilled water 1000ml . pH was adjusted to 6.5 .

Screening offungal isolates for starch hydrolysis :

The fungal isolates were carried out using Potato dextrose starch agar medium (PDSA) of Chimata *et al*., (2011) .Fungal isolates were identified by the Regional Center of Mycology and Biotechnology ,Al Azhar University ,Cairo ,Egypt .It was grown at 30°C and stored at 4°C.

Extraction of crude enzyme :

After each fermentation period , 50ml of distilled water were added to each fermented flask .Flasks were spanned on a rotary shaker at 200 rotation per min .After filtration through cheese cloth ,the suspension was centrifuged at 8000 rpm at 4 °C for twenty min . The resultant filtration is considered crude enzyme solution , its activity was assayed by DNS method (Miller ,1959) .

Crude enzyme activity determination :

It was determined using DNS method of Miller ,(1959) .To test tube ,0.1 ml of crude enzyme was reacted with 0.9 ml of starch buffered (0.4% starch) under standard reaction conditions .one ml of DNS was added to each test tube to stop the reaction .Tubes were boiled for 5 min and cooled in ice water .The color produced was measured at 540 nm . standard curve was made using glucose .One unit (IU) of alpha amylase activity was defined as the number of micromoles of reducing sugars released per minute by the total amount of enzyme extracted from 1.0g dry weight of the agricultural by –product used .

One unit (U) of enzyme was defined as the amount of micromoles of reducing sugars released by 1 ml of enzyme solution under the assay conditions .

RESULTS AND DISCUSSION

Data in Table 1 show that amylase production by *Penicillium* sp .(1) as affected by the source of agricultural by –product .Wheat bran proved to be the best waste for enzyme production ,followed by corn bran ,rice bran and potato waste .With the all wastes , the optimum incubation period for enzyme production was at 7 days . The productivity increase by increasing incubation time, but after 7 days ,further increase in incubation time decreased the enzyme production . After 7 days of incubation ,the maximum production of amylase by *Penicillium* sp .(1) was 183.34 ,119.24 ,55.98 and 46.51 IU/ g when wheat bran , corn bran , potato waste and rice bran was used ,respectively , while after 5 days of incubation the production was 267.92 ,80.19 ,34.22 and 24.34 IU/ g .

Table 1. Effect of agricultural by -product and incubation time on amylase production by *Penicillium* sp.(1):

Agric.by -product	Enzyme activity (IU/g substrate),days			
	3	5	7	9
Wheat bran	84.33	267.92	183.34	74.26
Rice bran	17.89	34.22	46.51	57.60
Corn bran	30.62	80.19	119.24	148.65
Potato waste	14.13	24.34	55.98	23.3

Fermentation medium consists of 5gm agricultural by -product ,5ml tap water , incubation performed at 28 °C .

Data in Table(2) demonstrated the effect of using agricultural by -productes and incubation time on amylase production by *Penicillium* sp .(2) . The maximum yield of enzyme 268.96 IU/ g was obtained with wheat bran after 5 days of incubation ,while with corn bran 75.48 IU/ g ,potato waste 29.76 IU/ g and 36.79 IU/ g rice bran, the maximum yield was obtained after 9 days of incubation .

Table 2. Effect of agricultural by -product and incubation time on amylase produced by *Penicillium* sp .(2)

Agric. by -product	Enzyme activity (IU/g substrate),days			
	3	5	7	9
Wheat bran	147.69	268.96	237.41	164.82
Rice bran	25.65	36.79	58.62	78.42
Corn bran	48.15	75.48	117.54	151.04
Potato waste	19.55	29.76	65.74	97.40

Fermentation medium consists of 5gm agricultural by -product ,5ml tap water incubation performed at 28 °C .

Data in Table(3) showed the production of amylase by *Penicillium chrysogenum* as affected by using the different agricultural by -product . The maximum yield (398.47 IU/ g) was obtained with wheat bran after 7 days of incubation followed by corn bran (163.54 IU/ g) after 9 days ,rice bran (125.24 IU/ g) and potato waste (98.87 IU/ g) after 9 days of incubation at28 °C .

Table 3. Effect of agricultural by -product and incubation time on amylase production by *Penicillium chrysogenum*

Agric. by -product	Enzyme activity (IU/g substrate), days			
	3	5	7	9
Wheat bran	191.37	284.81	398.47	81.13
Rice bran	79.27	95.51	125.24	101.03
Corn bran	51.75	89.87	136.75	163.54
Potato waste	44.33	71.42	91.36	98.87

Fermentation medium consists of 5gm agricultural by -product ,5ml tap water incubation performed at 28 °C .

Results of *Penicillium sp* .are in agreement with those obtained by Ertan *et al.*,(2006) ,who reported that wheat bran was the best substrate for α-amylase production by *Penicillium chrysogenume* .In contrary Vijayaraghavan *et al*. (2011) found that banana peel was the best substrates for amylase production by *Penicillium* sp.

Data in Table 4 showed the effect of agricultural by -product and incubation time on amylase production by *Penicillium georgiense* . *Penicillium georgiense* produced high amount of amylase after 7 days of incubation at 28°C with the different agricultural by -product used , *Penicillium georgiense* achieved the highest values of enzyme (407.65 IU/ g) after 7 days and 347.52 IU/ g after 5 days of incubation when wheat bran was used as agricultural by -product . Results clearly show that after the 7th day of incubation *Penicillium georgiense* produced the highest value of enzyme comparing to the other fungi .

Table 4. Effect of agricultural by –product and incubation time on amylase produced by *Penicillium georgiense* .

Agric. by -product	Enzyme activity (IU/g substrate), days			
	3	5	7	9
Wheat bran	205.03	347.52	407.65	117.64
Rice bran	69.91	101.76	129.11	133.57
Corn bran	65.42	97.40	157.84	173.21
Potato waste	64.17	114.24	154.75	123.54

Fermentation medium consists of 5gm agricultural by -product ,5ml tap water, incubation performed at 28 °C .

Data in Table 5 showed that comparing to the other wastes ,wheat bran was the best source for enzyme production by *Penicillium sp 3*. The optimum fermentation

period for enzyme production was 7 days with wheat bran ,while with rice bran ,corn bran and potato waste it was 9 ,7 and 9 days ,respectively .Using wheat bran , rice bran ,corn bran and potato waste enzyme production by *Penicillium sp*(3) at the optimum time of incubation was 320.49 ,82.68 ,134.85 and 97.98 IU/ g , respectively.

Table 5. Effect of agricultural by –product and incubation time on amylase produced by *Penicillium* sp. (3).

Agric. by -product	Enzyme activity (IU/g substrate), days			
	3	5	7	9
Wheat bran	124.89	260.75	320.49	141.55
Rice bran	13.45	26.57	46.95	82.68
Corn bran	56.31	82.47	134.85	129.65
Potato waste	23.14	36.21	57.68	97.98

Fermentation medium consists of 5gm agricultural by -producte ,5ml tap water , incubation performed at 28 °C

Results on *Penicillium* sp.isolates are in agreement with results obtained by Arora *et al*.,(2017),who found that the maximum production of glucoamylase by *Penicillium* sp, using rice bran ,wheat bran and banana peel as agro industrial wastes ,was obtained after 7 days of incubation .On the other hand ,they found that rice bran proved to be the best waste for enzyme production by *Penicillium* sp, followed by wheat bran and banana peel .

Data in Table 6 :demonstrated that the optimum time of incubation for enzyme production by *Verticillium* sp. was after the 5th day ,7th day ,7th day when wheat bran , rice bran ,corn bran and potato waste were used, respectively. The maximum production of enzyme was 258.6 IU/ g in case of using wheat bran ,while it was 102.29 IU/ g ,103.20 and 96.50 IU/ g with rice bran ,corn bran and potato waste, respectively .

Table 6. Effect of agricultural by –product and incubation time on amylase production by *Verticillium sp*.

Agric. by -product	Enzyme activity (IU/g substrate) days,			
	3	5	7	9
Wheat bran	174.33	258.67	148.72	76.29
Rice bran	33.79	65.06	102.29	52.91
Corn bran	38.43	64.50	103.20	96.32
Potato waste	36.69	81.96	96.5	62.56

Fermentation medium consists of 5gm agricultural by -product ,5ml tap water, incubation performed at 28 °C .

Data in Table 7 revealed the effect of agricultural by -product and time of incubation on amylase production by *Aspergillus westerdijkiae* . By using wheat bran higher capacity of enzyme production by *Aspergillus westerdijkiae* (387.45 IU/ g) was obtained after the 7th day of incubation ,but after the 5th day of incubation the production was 234.64 IU/ g .Among the other tested fungi, *Aspergillus westerdijkiae* gave the highest value of enzyme production of 229.14 IU/G after 9 days of incubation with wheat bran was followed by corn bran, potato waste ,and rice bran the production at 7 days was 157.52 ,102.49 and 90.37 IU/ g , respectively, while the production after 5 days of incubation was 101.32 IU/ g ,84.42 IU/ g and 57.12 IU/ g respectively .

Table 7. Effect of agricultural by -product and incubation time on amylase production by *Aspergillus westerdijkia*

Agricultural by -product	Enzyme activity (IU/g substrate), days			
	3	5	7	9
Wheat bran	142.87	234.64	387.45	229.14
Rice bran	66.75	57.12	90.37	110.04
Corn bran	55.24	101.32	157.52	110.24
Potato waste	66.10	84.42	102.49	96.09

Fermentation medium consists of 5gm agricultural by -producte ,5ml tap water, incubation performed at 28 °C .

Results are in agreement with those reported by Manivannan *et al* ., (2015) who found that the highest amount of amylase produced by *Aspergillus flavus* when wheat bran is used as a substrate ,while the lowest amount was obtained with rice bran. Also similar results were obtained by Khan and Yadav (2011) who found that wheat bran was the best substrate for amylase production by *Aspergillus niger* . On the other hand , Ajikumar *et al*. (2014) found that the highest activity of amylase produced by *Aspergillus* sp was obtained when rice bran was the substrate and corn bran and wheat bran showed low amylase activity .

Data in Table 8: showed the production of amylase by *Alternaria* sp. as affected by agricultural by -product and incubation time . The highest value of enzyme (194.57 IU/ g) was obtained at the 7th day of incubation when wheat bran is used as agricultural by -product. Using rice bran the maximum enzyme production (103.31 IU/ g) was noticed at the 9th day , while it was 88.25 IU/ g and 74.22 IU/ g after the 9th day with corn bran and potato waste, respectively .In contrary , Abd El Aty and Mostafa .,(2015) obtained the maximum yield of amylase from *Alternaria alternata* with potato shells followed by wheat bran .

Table 8. Effect of agricultural by -product and incubation time on amylase produced by *Alternaria* sp.

Agricultural by -product	Enzyme activity (IU/g substrate),days			
	3	5	7	9
Wheat bran	82.45	144.49	194.57	133.95
Rice bran	58.28	87.57	97.69	103.31
Corn bran	14.71	39.32	77.81	88.25
Potato waste	69.22	80.38	103.21	74.22

Fermentation medium consists of 5gm agricultural by -product ,5ml tap water , incubation performed at 28 °C

Generally, the results show that amylase production varies from microbe to another , and from agricultural by -product to another . Subash *et al.,*(2017) found that the level of amylase production varies from one microbe to another , even among the same species and strain .Also , it could be concluded from the results that wheat bran could effectively use as a substrate for amylases production through SSF .

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انتاج الأميليز بواسطة بعض العزلات الفطرية المحلية باستخدام بعض المخلفات الزراعية عن طريق التخمر الصلب .

عبدالله العوضي إبراهيم سليم[1] ، عزه عبدالرحمن مصطفى [2] و مي عاطف محمود عيد .

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تم دراسة إمكانية استخدام بعض المخلفات الزراعية لإنتاج الأميليز بواسطة بعض السلالات الفطرية المحلية المحللة للنشا .مع كل المخلفات (نخالة القمح ونخالة الأرز ونخالة الذرة ومخلفات البطاطس كانت فترة التحضين المثلى لإنتاج الإنزيم هي 7 أيام. وكان الحد الأقصى لإنتاج الأميليز بواسطة. (1). *Penicillium* sp IU/ g 46.51 and 55.98, 119.24, 183.34 بعد 7 أيام من التحضين عندما تم استخدام نخالة القمح ونخالة الذرة ومخلفات البطاطس ونخالة الأرز على التوالي، في حين بعد 5 أيام من التحضين كان الإنتاج 267.92 و 80.19 و 34.22 و 24.34 وحدة دولية / جم من المخلف المستخدم. تم الحصول على أقصى عائد للإنزيم 268.96 وحدة دولية /جم بواسطة (2). *Penicillium* sp مع نخالة القمح بعد 5 أيام من التحضين، بينما مع نخالة الذرة كان 75.48 وحدة دولية / جم، ومخلفات البطاطس 29.76 وحدة دولية / جرام و 36.79 وحدة دولية / جم نخالة الأرز. تم الحصول على أعلى عائد (398.47 وحدة دولية / جم) من قبل *Penicillium chrysogenum* مع نخالة القمح بعد 7 أيام من التحضين تليها نخالة الذرة (163.54 وحدة دولية / جم) في 9 أيام ونخالة الأرز (125.24 وحدة دولية / جم) ومخلفات البطاطس (98.87 وحدة دولية / جم) بعد 9 أيام من التحضين. حققت *Penicillium georgiense* أعلى قيم الإنزيم (407.65 وحدة دولية / جم) بعد 7 أيام و 347.52 وحدة دولية / جم بعد 5 أيام من التحضين عندما كانت نخالة القمح تستخدم كوسط للنمو الفطري . باستخدام نخالة القمح ونخالة الأرز ونخالة الذرة ومخلفات البطاطس كان إنتاج إنزيم من(3). *PenicillIUm* sp في الوقت الأمثل من التحضين 82.68،320.49، 134.85 و 97.98 وحدة دولية / جم ، على التوالي. وقد بلغ الإنتاج الأقصى للإنزيم بواسطة *Verticillium sp* 258.6 وحدة دولية / جم في حالة استخدام نخالة القمح بينما كان 102.29 وحدة دولية / جم و 103.20 و 96.50 وحدة دولية / جم مع نخالة الأرز ونخالة الذرة ومخلفات البطاطس على التوالي . باستخدام نخالة القمح كانت اعلى انتاجيه للإنزيم بواسطة *Aspergillus westerdijkiae*(387.45 وحدة دولية / جم) في اليوم السابع من التحضين، ولكن في اليوم الخامس من التحضين كان الإنتاج 234.64 وحدة دولية / جم. وكان الإنتاج بعد 7 أيام 157.52 و 102.49 و 90.37 وحدة دولية / جم ، عند استخدام نخالة الذرة ومخلفات البطاطس ونخالة الأرز، بينما كان الإنتاج بعد 5 أيام من التحضين 101.32 وحدة دولية / جم، 84.42 وحدة دولية / جرام و 57.12 وحدة دولية / جرام على التوالي. تم الحصول على أعلى قيمة للانزيم (194.57 وحدة دولية / جم) من قبل *Alternaria sp* في اليوم السابع من التحضين عند استخدام نخالة القمح كوسط غذائي للفطر . من النتائج يمكن استنتاج أن نخالة القمح أثبتت أنها أفضل المخلفات لإنتاج الإنزيم ، تليها نخالة الذرة ونخالة الأرز ومخلفات البطاطس .