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RESEARCH ARTICLE

DEVELOPMENT OF LOWCOST FERMENTATION TECHNOLOGY FOR THE AMYLASE PRODUCTION

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ABSTRACT

In the present study solid state fermentation technique has been employed for the development of low cost fermentation technique for amylase production by using two different species of fungi had been chosen viz. *Aspergillus oryzae, Penicillium expansum.* During the fermentation process the amylase production was highest at 96 hrs for both the fungal species. The effect of temperatures revealed that the amylase production higher during at 30-40°C with the optimum at 40°C for both the fungal species inoculated. The most suitable temperature maintain in a Solid State Fermentation system is in the range 80-40°C. The optimum pH for the fermentation in medium was 7 in the heat neutral range. The amylase becomes denatured below 6. The effect of pH amylase production in Solid State Fermentation of Banana fruit stalk and the skin of the fruit mixture for both the species of fungi, two different species of fungi used, among the two fasted and *Aspergillus oryzae* showed higher activity, enzyme production when compared to *Penicillium expansum,* be suggested that utilization of banana waste; fruit stalk and skin of the fruit mixture as substrate from amylase production in Solid State Fermentation, developed as a low cost fermentation technology for the amylase production.

INTRODUCTION

Microbial amylases for commercial uses about 75 years ago represented a milestone in industrial enzymology. Although Amylases can be obtained from several sources, such as plants & animals, the enzymes from microbial sources generally meet industrial demand. Starch degrading enzymes like amylase have received great deal of attention because of their perceived technological significance and economic benefits. Nowadays, the new potential of using microorganism as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extracellular enzymatic activity in several microorganisms (Buzzini and Marthini, 2002). Microbial amylase have successfully used in starch sacchrification, they also find potential application in a number of industrial processes such as in food, baking, brewing, detergent, textile and paper industries. An extracellular amylase specifically raw starch digesting amylase has found important application in biocerversion of starch and starch based substrates. Bacillus Subtilis produced a-amylase in Submerged Fermentation by utilizing agro-residues. Hence, no studies on banana stalk as well as the skin of the fruit mixture and Sappotta waste are used as substrate for the production of fungal amylase by using SSF technique, Hence the present study attempted to fulfill the above Lacunace.

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MATERIALS AND METHODS

Isolation and Screening and Identification of Amylolytic Fungi

The amylolitic fungi were isolated from litter soil. Starch agar was employed for Isolation. Dilution plating technique was adopted. Ten isolates were made from soil. Spores of the isolates were inoculated in the center of the starch agar, culture were inoculated for 3 days at room temperature. Fungal colonies that appeared showing amylolytic activity were identified by their capacity to hydrolyze starch surrounding their colonies and leave a circular clear zone, addition of a weak Iodine solution over the culture plates revealed the lytic zone clearly.

Fungal species were obtained observed under in microscope by wet mount method and the species were isolated showed the characteristic feature of *Aspergillus Oryzae* and *Pencillium expansum* which was identified using key after fungal biology by Harry and Hadson, Identification the pure culture slants was made with in malt agar and kept refrigerated.

Collection of substrate: Fresh banana fruit stalks (Peduncle) of Musa paradisiace [Variety Poovan Pazham grown in Cuddalore Dt. Tamilnadu) collected from the local market was used as substrate. After removing, the mud and debris adhering to it any by brush knife. They were weighted and ten cut in to small pieces and ground to a fine paste.

Preparation of Inoculum and Inoculation Procedures: A loopful of culture from Malt Agar Slant was inoculated into 50 ml of starch broth and incubated for 3 days at room temperature.

Identification of optimum day: To study the optimum day for the production to enzyme amylase 1ml of young culture was inoculated into 100 ml of starch broth and incubated for different periods. Every 24 hrs the production of amylase enzyme was assayed using Manning and Campbell, (1961) method.

Effect of temperature: To study the temperature on the growth of *Aspergillus oryzae* and *Penicillium expansum* using the methods Manning and Campbell, (1961) method.

Effect of pH: To study the effect of pH on the production of *Aspergillus oryzae* and *Penicillium expansum* 1ml of young culture was inoculated into 50ml starch broth tubes the starch broth tubes were adjusted to pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 using in NaOH and in HCl. The inoculated tubes were incubated and enzyme assay and performed at 630 nm.

Enzyme extraction: The crude enzyme was obtained taking 3 day *Aspergillus oryzae* culture and centrifuged at 10, 000 rpm for 10 minutes and the supernatant was used for further analysis.

Preparation of solid state Fermentation media (SSF): The mineral salt media recommended by Ramesh and Lonsane (1990). For alpha - amylase production under solid state fermentation using banana waste was used in the present study.

Media composition

Na_2HPO_4 $2H_2O$	-	1.1 g
$Na_2H_2PO_4$ $2H_2O$	-	0.6 g
KCl	-	0.3 g
$MgSO_4$	-	0.01 g
Distilled water	-	100 ml

Banana fruit stalk and the skin of the fruit substrate, added to the medium served as the sources of carbon.

Amylase production using banana waste fruit stalk and skin under the Solid state fermentation

10g of banana fruit stalk and skin powder particles were taken.

1. Then they were moistened in 100 ml conical flasks with the prepared minimal medium to an initial moisture content of 65%.
2. It was autoclaved at 121°C for minutes and cooled to about 30°C and inoculated with 10% (V/W) cell suspension.
3. The contents of the flasks were thoroughly mixed to ensure uniform, distribution of the inoculums and left at room temperature in shaker.
4. Samples were removed for every 12 hrs for analysis.
5. The contents were extracted by centrifuging the sample at 10,000 rpm for 10 minutes and the supernatant was used for amylase assay.

Assay of Amylase Enzymes (Manning and Cambell, 1961)

Calorimetrically by measuring the disappearance of broth colour of starch iodide complex.

1. The assay mixture contained 5ml of 1% starch in 0.1ml sodium acetate buffer (pH –5.8), 3 ml of sodium acetate buffer (pH-5.8) 3ml of sodium and 1ml of enzyme was prepared.
2. As the mixture was incubated for 10 minutes at 60°C in a water bath this way arrested by adding 2 ml of HCl.
3. From this assay mixture 0.4ml was introduced into 5ml standard flask.
4. To this 0.1 ml of HCl and 0.2 ml of iodine (3% potassium iodide and 0.3% of iodine in water) added.
5. The volume was made upto 50ml using sodium acetate buffer.
6. The colour developed was measured at 630 nm.
7. The blank was identical to assay mixture but for uninoculated starch media in the place of enzyme addition to iodine was also omitted.

The enzyme activity was calculated the following formula.

$$\frac{ABC - ABD}{ABC} \times \text{mg of starch initially}$$

ABC- Absorbance of assay mixture with inoculated sterile medium in the place of enzyme.
ABD- Absorbency of the assay mixture after reaction.

The enzyme activity is expressed as milligram of starch hydrolysed in 1 minute by 1ml of enzyme preparation = milligram starch hydrolyse min^{-1}, ml^{-1}.

Determination of Reducing Sugar Materials (Benefid, 1955)

1. 1% (W/V) starch was dissolved in phosphate buffer (pH-75, 0.5M) as substrate.
2. 100 ml of suitably diluted enzyme was added to 0.9 ml of substrate solution.
3. Then it is incubated at 50°C for 30 minutes and the reaction was terminated by immediate cooling in ice and addition of 1 ml of 3.5% dinitrosalicylic acid reagents.
4. Absorbance was measured to 540 nm.

One unit of amylalytic activity was defined as the amount of enzyme that catalysed the liberation of 1.0 microgram of reducing sugar equivalent to D-glucose per minute under the optimal assay conditions.

Protein Estimation by Lowry's method

Materials

1. 2% sodium carbonate in 0.1N sodium hydroxide (Reagent A)
2. 0.5% copper sulphate in 1% potassium sodium tartarate (Reagent B)
3. Alkaline copper solution. Mix 50ml of A and 1 ml of B prior to use (Reagent C)
4. Folin ciocalteque reagent (Reagent D)

Protein Solution (Stock solution)

50 mg of Bovine serum albumin (Fraction V) was weighted and dissolved in distilled water and made upto 50ml in a standard flask.

RESULTS

The amylase production by using *Aspergillus oryzae* and *Penicillium expansum* in Solid State Fermentation after every 24 hrs. gradually (Table 1). The effect of temperature and pH of the amylase production activity in SSF for both the species used for the present study (Table - 2 to 3). Among various temperatures studied the amylase production showed an optimum production at 40° for both the culture

Table 1. Amylase production using *Aspergillus oryzae* (mg Starch hydrolysed min/ml) *and Pencillium expansum*(mg) in Solid State Fermentation

Sl. No.	Hours	*Aspergillus oryzae*	*Penicillium expansum*
1.	24	530	300
2.	48	620	510
3.	72	710	590
4.	96	450	680
5.	120	270	320
6.	144	270	330
7.	168	250	210
8.	192	140	150

Table 2. Effects of Temperature an amylase production *Aspergillus oryzae* (mg*)* and *Penicilium expansum* (mg) in Solid State Fermentation

Sl. No.	Temperature (°C)	*Aspergillus oryzae*	*Penicilium expansum*
1.	20°C	190	190
2.	30°C	420	420
3.	40°C	680	680
4.	50°C	360	360
5.	60°C	230	230
6.	70°C	150	150

Table 3. The effect of pH on the amylase production of *Aspergillus oryzae* (mg) and *Penicillium expansum* (mg)

Sl. No.	pH	*Aspergillus oryzae*	*Penicillium expansum*
1.	4.0	250 ± 2.5	210 ± 2.0
2.	4.5	320 ± 2.7	330 ± 2.0
3.	5.0	390 ± 12.4	350 ± 2.1
4.	5.5	480 ± 1.7	490 ± 2.8
5.	6.0	630 ± 1.8	540 ± 2.6
6.	6.5	720 ± 1.4	660 ± 2.5
7.	7.0	690 ± 1.8	680 ± 2.4
8.	7.5	480 ± 1.6	420 ± 2.2
9.	8.0	290 ± 1.7	180 ± 1.8
10.	8.5	120 ± 1.7	100 ± 1.6
11.	9.0	50 ± 1.8	60 ± 106

Table 4. The effect of temperature in determination of Reducing sugars under solid state fermentation by *Aspergillus oryzae* and *Penicillium expansum*

Sl. No.	Temperature (°C)	Amylase
1.	20°C	350
2.	30°C	490
3.	40°C	610
4.	50°C	440
5.	60°C	320
6.	70°C	270
6.	80°C	230

Results showed that the maximum amylase production was recorded after 96 hrs in both the species analysed of the two, the maximum amylase production was recorded in the culture used *Aspergillus oryzae* (690 amylase using starch hydrolysed min-1 ml-1) during 96 hrs. After 96 hrs (4^{th} day) the amylase production was found to decrease followed by a decreasing trend. In general amylase production showed an increasing tend for both the cultures when temperatures were increased from 20 to 40°C the amylase production was found to be decreased for both the cultured species of fungi of the two *Aspergillus oryzae* recorded higher production of amylase when compare to

Penicillium expansum culture. The pH was found to produced more amylase production that alkaline pH with the production being optimum at hear neutral (6.5 to 7) for both the cultures. Nevertheless, among the two, maximum level of amylase production was higher in *Aspergillus oryzae* when compare to *Penicillium expansum* (Table 3). The effect of temperature on the reducing sugars under Solid state fermentationby using two fungal species (Table 4).

In the present study, the decline in enzyme production activity observed after the fourth day (after 96 hrs) of inoculation is due to the denaturation and or decomposition of 2 amylase as a result of interaction with other compounds in the fermented medium. Such reports were also attributed by Ramesh and Lonsane (1990), Shang Shyng and Jan – Yi Wang (1999). In the present study, the temperature was also one of the important factors to induce the amylase production in SSF by using banana fruit stalk and skin of the fruit for

Table 5.The effect of pH for determination of reducing sugars using banana fruit stalk & skin of the fruit under Solid state fermentation by *Aspergillus oryzae* and *Penicillium expansum*

Sl. No.	pH	*Aspergillus* Reducing Sugars mg / ml / min	*Penicillium* Reducing Sugars mg/ml/min.
1.	3.0	250	220
2.	3.5	300	280
3.	4.0	360	340
4.	4.5	410	380
5.	5.0	440	400
6.	5.5	510	460
7.	6.0	530	510
8.	6.5	640	620
9.	7.0	690	530
10.	7.5	540	315
11.	8.0	360	315
12.	8.5	280	240

Table 6. The protein estimation of a fermented banana fruit stalk by using *Aspergillus oryzae* and *Penicillium expansum*

Microorganisms	Protein Con.mg/mVin
Aspergillus niger	600
Penicillium expansum	520

When compare to the two different species. *Aspergillus oryzae* recorded the maximum when compare to *Penicillium expansum* inoculated sample at 40°C. The effect of pH on the reducing sugars under SSF by using two different species (Table 5). When compare to the two different species *Aspergillus oryzae* inoculated sample showed the maximum of 690mg^{-1} ml^{-1} min^{-1}. Table - 6 shows the protein concentration of a fermented banana fruit stalk and fruit skin as substrate by using two different fungal species *Aspergillus oryzae* showed the higher level (600 mg^{-1}-ml^{-1}-min^{-1}) when compare to *Penicillium expansum* inoculated samples.

DISCUSSION

In the present study the amylase production was found to be higher during 96 hrs in solid substrate fermentation by using banana fruit stalk and the skin of the fruit for two different fungal species. Similar observation also made by Pandey (1995) and Alwa et al. (2007). While using other substrate like rice bran, wheat etc. by using single fungal species of *Aspergillus niger* when compare to the production of amylase activity in the present study record a study amount of amylase production. The difference in production probably due to the differences in the substrate used.

both the inoculated fungal species. In the present study it was found that a range of 30-40°C was the most suitable Temperature.

Literature reveals that range Szakacs et al. (2002). Suggested that the usual temperatures maintained in an SSF system is in the range of 30-40°C depending on the growth kinetics of the microorganisms employed for the fermentation purposes. Hence the recent study is also in line with their study. Comparing the two different species of fungi used in the present study. *Aspergillus oryzae* should higher activity/enzyme production, when compared to *Penicilliun expansum*. This is possible due to increase 'amyto' process to hydrolyze starch in *Aspergillus oryzae* inoculated in SSF. Substrates traditionally used in solid state fermentation include rice, wheat, miller, barley, corn and soybean.

In the present study the optimum pH for the fermentation medium was 7 in the near neutral range. This is because the amylase becomes denatured below 6. This confirming the data in the effect of pH on the amylase production in SSF of banana waste fruit stalk and skin of the fruit for both the species of fungi. Similar observation was also reported by several workers (Lonsane et al., 1985; Krishnan and Chandra Sekaran, 1996; Pandey et al., 1999). Recommendation of this present study the methods are ecofriendly and we can use

the tropical fruits waste like sappota and pineapple can use it for the production of Ethanol and citrate as low cost substrate for microbial fermentation. Finally we can protect the environments from pollution.

REFERENCES

Alva, S., Anupama, J., Salva, Chiu, Y.Y., Vyshali, P., Shruthi, M., Yogeeta, S., Bhavya, D., Puri, J., Ruchi, K., Kumudini, B., and Varalakshmi, K.N., 2007. Production and characterization of fungal amylase enzyme isolated from Aspergillus Sp.JG] 12 in solid state culture. African J. Biotechnology, Vol.6(5): 576-581.

Buzzini, P and Martini, A., 2002. Extracellular enzymatic activity profiles in yeast and yeast like strains isolated from tropical environments. J. Applied Microbiology, Vol.93: 1020-1025.

Pandey, A., Ashakumar, L., Selvakumar, P., and Vijayalakshmi, K.S. 1995. Effect of yeast extract on glucoamylase synthesis by *Aspergillus niger* in said state fermentation. Indian J. Microbial., Vol.35(4): 335-338.

Ramesh, M.V., and Lonsane, B.K., 2005. Solid state fermentation for production of higher titres of thermostable alpha-amylase with two peaks for pH optima by *Bacillus licheniformis* M_{27}. Biotechnology Letters. Vol.11: 49-52.

Shang Shyng Yang and Jan-Yi Wange., 1999. Protease any amylase production of *Streptomyces rimosus* in submerged and solid state cultivations. Bot, Bull. Acad. Sin., Vol.40: 250-265.

Szakacs, G., Bogar, B., Szendefy Christor, L., and Tenger, R.P., 2002. Production of alpha amylase and Xylanase by solid substrate fermentation, A.M., symposium on Microbial Biotechnology held at Regional Research Laboratory, Trivandrum.

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