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Research Article

PRODUCTION OF ENZYMES BY *ASPERGILLUS NIGER* BY USING SURFACE AND SUBMERGED FERMENTATION USING WASTE AGRO-INDUSTRIAL SUBSTRATE

MULLA M.A.W., SHAIKH B., PATHAN H.A.* AND WAVHAL S.D.
Department of Biotechnology, Ismail Yusuf College, Mumbai, 400060, University of Mumbai, Mumbai, Maharashtra, India
*Corresponding Author: Email - hinaa_123@yahoo.com

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Abstract- The aim of this project is to carryout to improve the production of enzyme by *Aspergillus niger*. The effect of alternative carbon source in czapek-dox medium as inducer of beta-galactosidase, beta-fructofuranosidase and keratinase activity. *Aspergillus niger* was grown in czapek Dox medium with orange peel as inducer for enzyme and czapek medium with human hair and chick feather as a substrate for keratinase production. The organism was grown in surface and submerged condition. The enzymes were extracted and purified and activity was determined by spectrophotometer analysis for beta-galactosidase, beta-fructofuranosidase and keratinase. Thus the alternative carbon source as orange peel, human hair and chick feather exhibit great potential as inducer for the production of generating significant amount of beta-galactosidase , beta-fructofuranosidase and keratinase using waste agro industrial substrate.

Keywords- *Aspergillus niger, Czapek dox medium, Beta-galactosidase, Beta-fructofuranosidase and keratinase*

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Introduction

Aspergillus niger

Aspergillus niger is a filamentous ascomycete fungus that is ubiquitous in the environment. *A. niger* is most widely known for its role as a citric acid producer with production of citric acid at over one million metric tons annually, as a common member of the microbial communities found in soils, *A. niger* plays a significant role in the global carbon cycle. This organism produces hydrolytic and oxidative enzymes involved in the breakdown of plant lignocellulose. A variety of these enzymes from *A. niger* are important in the biotechnology industry.

Galactocidase

Beta-Galactosidase, commonly known as lactase, is an enzyme responsible to hydrolyze lactose. It has wide applications in food-processing industries. The presence of excessive lactose in intestine typically leads to tissue dehydration and reduced calcium absorption due to low acidity that causes diarrhea, flatulence, and cramp. Absorption of lactose requires the activity of lactase enzyme, found in small intestine that functions by splitting the bond linking the two sugars (mono saccharides). The deficiency of this enzyme in intestine leads to lactose in tolerance, and the people suffering from it are unable to consume milk and dairy products. Furthermore, the ability of this enzyme to produce a colored product during a chemical reaction has gained its importance in molecular biology.

Fructofuranosidase (Invertase)

Enzymes are complex generally globular proteins found in living cells acting as a bio-catalyst facilitating metabolic reactions in an organism's body. This Enzyme is catalytic nature is responsible for the functioning. In industrial application, non-toxic and biodegradable can result in high quality and quantity products, fewer by-products and simpler purification procedures. Also enzymes can be obtained from different microorganisms and that too in large amount without using any chemical resistant approaches

Production of Keratinase

Keratinase Production in Submerged Fermentation

Microbial keratinases are generally extracellular, when grown on keratinous substrates and intracellular keratinases have also been reported. In most cases, keratin serves as the inducer. Majority of reports on keratinase production are under submerged shaking/static conditions, the synthesis of keratinase due to catabolite repression, while complex sugars like starch have shown to enhance the synthesis of keratinases of biochemistry was centred on yeast fermentations and processes for conversion of starch to sugar.

Application of enzymes

1) Food industry
2) Production of hydrolyzed milk products
3) Fructofuranosidase as invertase
4) Sucrose of invertase: kinetics of invertase enzyme
5) Animal feed production
6) Production of nitrogen fertilizer /biofertilizer

Procedure

1. Prepare a czapek medium (500ml) for both
2. Submerge fermentation(250ml) Surface fermentation(250ml)
3. Add orange peel and inoculate *Aspergillus niger*
4. Incubate at room temperature for five days.
5. After five days of fermentation
6. Fungi were grown in static condition on shaker.
7. Submerge fermentation and surface fermentation
8. Filtered and obtain cells and suspension filtered and obtain cells and after obtaining the cells allow it to after obtaining the cell allow it to freeze
9. freeze for 24 hrs
10. Break the ice with the help of distilled break the ice with the help of distilled
11. Water

12. Centrifuge it at 5000rpm for 5 min. centrifuge it at 5000rpm for 5minAfter centrifuge, obtain the supernatant after centrifuge, obtain the supernatant
13. The filtrate was dialyzed overnight at 4°C. the filtrate was dialyzed overnight

Enzymatic Assay

1) **B-Fructofuranosidase Activity**
2) fructofuranosidase was determine by analyzing reducing sugar that is released after incubation of the properly diluted enzyme with 0.2M sucrose in 50mM sodium acetate buffer (Ph4.0)
3) Keep it for water bath for 10min at 60°C.
4) Add Dinitrosalicylic acid which helps in reducing the amount of sugar
5) An enzyme activity was determined which was measured using
6) **B-Galactosidase Activity**
7) This activity was performed by mixing 100µl of approximately diluted enzyme with 500µl of the synthetic substrate *i.e.* β-D-galactopyranoside (ONPGal;3m M).
8) Dissolve it in 50mM sodium citrate buffer (pH 4.5).
9) Incubate the mixture at 40°C for 10 mins *i.e.* boiling water bath.
10) After incubation the reaction was stopped by the addition of 2ml of 0.2 M Na_2CO_3.
11) And this enzyme activity was determined from the amount of released O-nitro phenol, which was measured using a spectrophotometer at 410 nm.
12) **Keratin Degradation**
13) Take a human hair and feather meal (2.5g) .Wash it from TritonX100 and warm water.
14) Prepare a czapek medium (250ml).In this add 2.5g of human hair and feather meal.
15) Inoculate with *Aspergillus niger*.
16) Keep it in room temperature for 120 hours.
17) Filter the medium
18) From that medium take 100 ml and add 75g Ammonium sulphate, let the precipitation form.
19) After precipitation centrifuge it at 5000rpm for 15 mins
20) **Enzyme Activity on Azocasein**
21) Take 1ml of glycine buffer (ph 4.5).
22) Add 0.1ml of enzyme and 20mg casein in the respective buffer. It is for test.
23) For blank 0.8ml of TCA keep it for 50°C for 15 min (hot water bath).
24) Add it with the test and centrifuge it 5000rpm for 20 min.
25) The activity was estimated spectrophotometrically by reading the absorbance at 400nm

Result

β-galactosidase

The production of β-galactosidase by using fungi was examined in the presence of various agro-industrial wastes. Extracellular (Surface Fermentation) β-galactosidase production was induced by orange peel and by using *Aspergillus niger*. And also, Intracellular (Submerge Fermentation) β-galactosidase production was also induce by the orange peel and using the same organism. In both fermentation the highest value was examined as compare to other carbon sources. When both the values were compared, the submerge fermentation had the highest value then the surface fermentation. Enzyme activity was determined from the amount of released o-nitrophenol, one unit of enzyme activity was defined as the amount of the enzyme capable of releasing 1µmol of o-nitrophenol per minute under the assay conditions used. The reading for both submerges and surface fermentation is taken with the help of spectrophotometer at 410 nm.

The value of submerge fermentation was observed: 2.36µ/mol.

The value of surface fermentation was observed: 2.32µ/mol.

Fructofuranosidase

The production of β-fructofuranosidase by using fungi was examined in the presence of various agro-industrial wastes. Extracellular (Surface Fermentation) β-fructofuranosidase production was induced by orange peel and by using *Aspergillus niger*. And also, Intracellular (Submerge Fermentation) β-fructofuranosidase production was also induce by the orange peel and using the same organism. In both fermentation the highest value was examined as compare to other carbon sources. When both the values were compared, the submerge fermentation had the highest value then the surface fermentation. One unit of enzyme activity was defined as the amount of enzyme activity capable of releasing 1µ/mol of reducing sugar per minute under the experimental condition used. The reading for both submerge and surface fermentation is taken with the help of spectrophotometer at 410 nm.

1. The value of submerge fermentation was observed: 0.63µ/mol.
2. The value of surface fermentation was observed:0.5µ/mol

Fig-1 submerged fermentation

Fig-2 surface fermentation

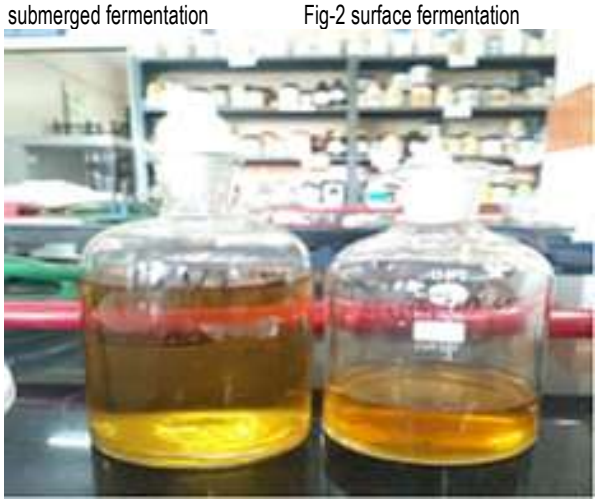

Fig-3 After filtration

Keratinase

After incubation of 120 hours the media is purified. Media contain *Aspergillus niger* and keratinous substrate *i.e.*, human hair and chicken hair which get degraded and its activity is determined by the spectrophotometrically at 440 nm after every 72 hours.

Days	24HRS	72HRS	144HRS
Blank	1.2	1.34	1.3
Test	2.35	1.96	1.6
Test-Blank	1.15	0.62	0.3

Fig-4 centrifugation

Fig-5 Human Hairs and Chick Feather

Fig-6 After Filtration

Fig-7 After Filtration

Conclusion

In this study, screening for strong fungal producers of β-galactosidase and β-fructofuranosidase was performed using carbon source obtained from agro-industrial substrate. The highest β-galactosidase production was found as an intracellular enzyme produced by *Aspergillus niger* using orange peel and even in fructofuranosidase the highest production was found as an intracellular enzyme produced by *Aspergillus niger* using orange peel. It was therefore, concluded that these fungi can be exploited to generate significant amounts of β-galactosidase and β-fructofuranosidase using alternative agro-industrial substrate that abundantly. available, mainly in agriculture production region. Feather meal was selected as substrate for protease and keratinase production by *Aspergillus niger*. This strain produces aspartic protease with general proteolytic activity and a serine protease with keratinolytic activity. The optima conditions were different for the production of each enzymes and the models were validated and can predict the enzymatic activity. *Aspergillus niger* has potential to be use in biotechnological process involving keratin hydrolysis, mainly because of the GRAS status of these product. Thus, more studies are necessary are better exploit this keratinolytic potential.

Future Prospect:

This method can apply on large scale industry. The production of β-galactosidase and fructofuranosidase as well as keratinase will be get more improve by having a proper scale production. It can be use in cosmetology, pharmacology, in food industries and many more.

Applications of research:

1. Production of enzymes
2. Better utilization of agriculture waste product
3. Enzymes can be utilized in cosmetology pharmacology and various other industrial productions.

Research Category: Exploitation of industrial waste and enzyme productions

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Author statement: All authors read reviewed, agreed and approved the final manuscript. Note-All authors agreed that written informed consent was obtained from all participants prior to publish/enrolment

Study area/sample collection: *A. niger* culture obtained from Department of Microbiology, Sophia College, Mumbai,

Cultivar/Variety Name: Orange

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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