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Determination of Raw Starch Hydrolytic Property of Fungal Isolates from Microbiology Laboratory

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Abstract

This study was aimed at isolating fungal strains from microbiology laboratory and determining its raw starch hydrolytic property. A totalof seven fungal strains were isolated from different places such as foot step, door mat, switch board, work bench, fan, lamp, water tap, floor, laboratory equipment surfaces and windows. The fungal isolates were incubated at room temperature inpotato infusion agar withtapioca powder as substrate and identified as Aspergillus niger, Aspergillus fumigatus, Aspergillus carbonarius, Aspergillus flavus, Mucor sp., Penicillium sp. and Rhizopus sp. Aspergillus carbonarius gave highest raw starch hydrolysing activity on tapioca starch.

Keywords: Raw **s**tarch, amylase, *Aspergillus carbonarius,* hydrolysis, laboratory.

Introduction

Starch is a polysaccharide, used widely in food industries for syrup production. Bioethanol can be obtained by the fermentation of sugar. Starch which is inexpensive is insoluble in water and resistant to enzymes and chemicals due to inter and intramolecular bonds as well as the densely packing of molecules in apolycrystalline state, In conventional enzymatic saccharification, slurry of starch is converted to a gel like consistency by heating at a temperature of about 105 °C. Heating opens the crystalline structure and enzymatic action increases, so the viscosity of the slurry increases and mixing and pumping becomes easier. The gelatinized starch is liquefied using alpha amylase (at high temperature) followed by saccharification using glucoamylase at a lower temperature (50–60 °C). This entire process requires a high-energy input, leading to increase in production cost of starch-based products. In view of energy costs, direct hydrolysis of starch below gelatinization temperature is hence desirable. This lead to the research on several raw starch digesting enzymes that does not require the gelatinisation and can hydrolyze raw starch in a single step at a moderate temperature. Amylases cannot act easily on raw starch granules than on gelatinized starch.

Amylases are a class of enzymes, capable of digesting glycosidic linkages. Enzymes like amylases, endoamylases and exoamylases are capable of hydrolysing starch. These enzymes are classified based on the attack of different types of glycosidic bond, found in the numerous glycoside hydrolase (GH) families, mainly in GH family 13[1,2]. Endoamylases cleave α, 1-4 glycosidic bonds present in the inner part of amylose or amylopectin chain. α- amylase (EC 3.2.1.1) is a well-known endoamylase, found in a wide variety of microorganisms [3]. The end products of α-amylase action are oligosaccharides having α-configuration and α-limit dextrins, constituting branched oligosaccharides of varying length. Based on the degree of hydrolysis of the substrate, α- amylases are divided into two categories, saccharifying α-amylases and liquefying α-amylases[4].

Enzymes belonging to the exoamylases group, either β-amylase (EC 3.2.1.2) that break α,1-4 glycosidic bonds or amyloglucosidase or glucoamylase (EC 3.2.1.3) that break both α,1-4 and α,1-6 glycosidic bonds and α-glucosidase (EC 3.2.1.20). Exoamylases acting on the external glucose residues of amylose or amylopectin produces only glucose (glucoamylase and α-glucosidase), or maltose and β- limit dextrin. Glucoamylase and β- amylase can convert configuration of the liberated maltose from α to β anomer. Glucoamylase and α-glucosidase differs in their substrate preference: α-glucosidase acts best on short maltooligosaccharides and liberate glucose with α- configuration while glucoamylase hydrolyzes long-chain polysaccharides best. Glucoamylases and β- amylases are found among various groups of microorganisms[3].

Starch is a glucose polymer linked by a glycosidic bond, stable at higher pH but hydrolyses at lower pH. A latent aldehyde group is present at the end of polymeric chain known as the reducing end. Starch contains two types of glucose polymers: (i) amylose and (ii) amylopectin. Amylose and starch granules are insoluble in cold water but amylopectin is soluble in water. Amylose is a linear polymer consisting of 6,000 glucose unitswhich are linked by α, 1-4 glycosidic bonds. The degree of polymerization (DP) is indicated by the number of glucose residues. Different sources of starch vary in the relative content of amylose and amylopectin[5].

Amylopectin molecules consists of short linear chains of 10–60

glucose units with α, 1-4 linkage and side chains of 15–45 glucose units with α, 1-6 linkage. The branching points in amylopectin vary with its botanical origin, average number being 5%[6]. Amylopectin molecule is one of the largest molecules in nature containing about 2,000,000 glucose units[5]. The cluster model is the most commonly accepted model for the structure of amylopectin, in which the side chains are arranged in clusters on the longer backbone chains[6,7]. The physical behaviour of granular starch is based on the internal part of amylopectin[8].

Several amylase-producing bacteria, fungi and other microorganisms have been isolated and characterized. Bacteria and fungi secrete amylase enzyme outside their cells, sothat they can carry out extra-cellular digestion. In the present study, we are trying to isolate fungal species from microbiology laboratory of SIAS and determine its hydrolytic property using soluble and raw starch as substrate.

Material and Methods

Isolation and cultivation of fungal isolates: Fungal strains were collected from microbiology laboratory of SIAS. Swabs were prepared with absorbent cotton and sterilized by autoclaving. The sabourauddextroseagar **(**SDA) and potato dextrose agar (PDA) were prepared and sterilized by autoclaving. After sterilization, the media were poured into the sterile petriplates and kept under UV to solidify. The dry swabs were taken from different areas like foot step, door mat, switch board, work bench, fan, lamp, water tap, floor, laboratory equipment surfaces and window. The swabs were inoculated to sterile SDA and PDA plates with tetracycline (50 ppm). The plates were incubated at room temperature for 28-72 hr.

After 28-72 hrs of incubation, the fungal colonies observed on the plates were sub cultured to freshly prepared SDA and PDA with tetracycline (50 ppm) plates for the purification of colonies grown. The sub cultured plates were incubated at room temperature for 28-72 hr. For the further confirmation of microorganisms, the fungal cultures were stocked on SDA and PDA slants. The fungal spores were inoculated to fresh plates by using 0.1 % tween 80 solution in sterile distilled water. The fungal plates were spreaded with 0.1 % tween 80 solution and spores were dislodged by using a sterile glass rod. The spore suspension was inoculated to SDA and PDA plates with tetracycline (50 ppm).

Identification of fungal isolates: The fungal species grown in the media were identified macroscopically and microscopically by saline wet mounting and LPCB (Lacto Phenol Cotton Blue) staining.

Determination of raw starch hydrolysing activity: The raw starch hydrolysing activities of the fungal strains were determined by using raw starch (Tapioca powder) as substrates. Tapioca was commercially purchased and washed thoroughly with distilled water, air dried, powdered, sterilized by 70% ethanol and dehydrated by acetone before use.

Raw starch hydrolysis: 100 ml of distilled water was taken in a conical flask and 40 gm of sliced potato was added. The media was boiled for 15 min. The potato pieces were removed from the conical flask and 2 gm of agar was added. The pH of media was maintained at 5.2. The media was sterilized by autoclaving for 20 min. 0.25 gm of sterile raw starch (tapioca powder) was added to the media. Different fungal cultures were spotted on the plate and incubated at room temperature for 48-72 hr. The zones of clearance indicating hydrolysis were observed.

The organism which produces maximum zonewas inoculated in potato infusion broth containing 0.5% sterile raw starch (tapioca powder) and soluble starch medium with 50 ppm tetracycline,and then incubated at room temperature for 3 days.

Results and Discussion

The fungal strains grown on SDA and PDA plates were identified macroscopically and microscopically by using staining methods like saline wet mounting and LPCB (Lacto PhenolCotton Blue) staining. Seven fungal strains were identified as *Aspergillusniger*, *Aspergillusfumigatus*, *Aspergilluscarbonarius*, *Aspergillusflavus*, *Mucor* sp., *Penicillium* sp. and *Rhizopus* sp.

The raw starch hydrolysing activities of the fungal strains were determined by using raw starch as substrates. Different fungal cultures were spotted on the plate and incubated at room temperature for 48-72 hr.A clear zone around the colony was observedafter 48-72 hr incubation.Out of seven fungal strains, *Aspergilluscarbonarius* gave best raw starch hydrolysing activity (figure-1). *Aspergilluscarbonarius* also showed better growth on potato infusion broth with tapioca starch as substrate (figure-2).

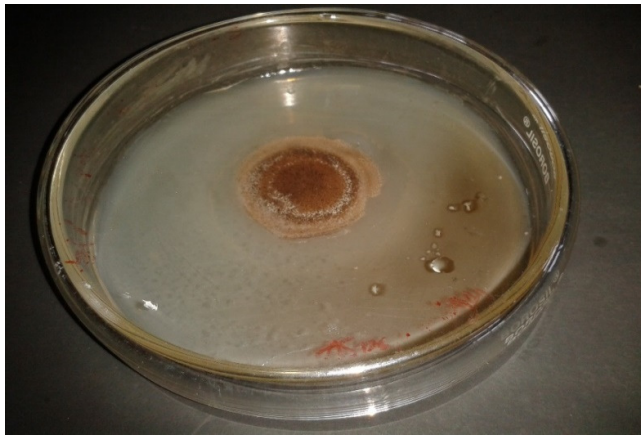

Figure-1
***Aspergilluscarbonarius* on potato infusion agar with tapioca as substrate**

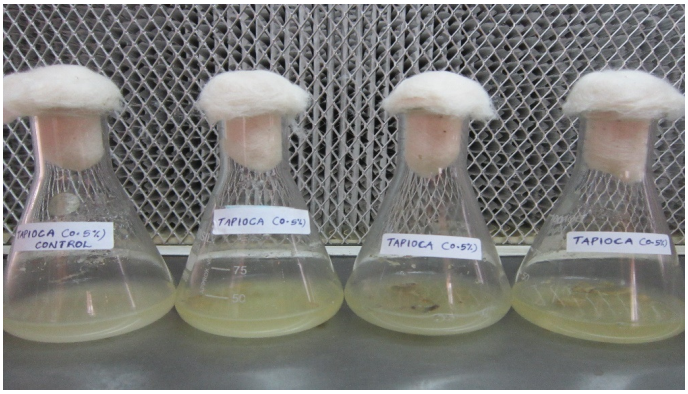

Figure-2
***Aspergilluscarbonarius*on potato infusion broth with tapioca as substrate**

Some of the mold species producing higher levels of amylase like *Aspergillusniger*, *Aspergillusoryzae*[9], *Thermomyceslanuginosus*[10], *Penicilliumexpansum*[11] and many species of *Mucor*[12-14] have been identified. Studies reported that in sawdust medium few species of *Ganoderma* mushrooms produce relatively weak amylase enzyme[15]. Amylolytic yeasts differ in the extent of starch hydrolysis and amylase secretion[16,17]. Extensive starch hydrolysis is exhibited by strains of *Filobasidiuimcapsuligenum*[18,19].

Starch is produced commercially from wheat, corn, sorghum and rice. Starch hydrolysing organisms can act only on gelatinised starch. Raw starch hydrolysing organisms can act directly on raw starch granules below the gelatinisation temperature of starch. In our study we aimed on raw starch hydrolysing property of laboratory isolates. Amylases hydrolysing raw starch vary from other amylases in their special affinity and interaction with the microcrystalline structure of the raw starch molecule[20].

We are preferring microbial sources for large scale production of enzyme because they have many advantages for the industrial production such as cost effectiveness, consistency, less time and space required for production as well as optimization. A wide variety of microorganisms including fungi, yeasts and bacteria can hydrolyse raw starch[21].

Conversion of raw starch by enzymatic treatment means that some of them could be used as raw materials by starch industry as value added products leading to reduction in wastage and improve economic gain. Starch which is inexpensive can be processed enzymatically in to variety of starch derivatives such as glucose, maltose and syrups of various dextrose levels that can be used as sweetener or can be further converted into different products.

Due to importance of our recent findingsfurther studies must focus on the partial characterisation and purification of the enzymes produced by microbiology laboratory isolates and determination of the gene sequence to understand its low pH activity and significant raw starch digesting feature.

Conclusion

Fungi are widely distributed in environment especially in soil in saprophytic mode because they produce variety of hydrolytic enzymes. The classification of fungi is based on its action; wood-rotting fungi are classified on the basis of enzymatic deterioration of wood; white-rot fungi degrade lignin and a brown-rot fungus has the capacity to degrade cellulose. Fungal hydrolytic enzymes have many industrial applications. Therefore, there is a need for screening these enzymes for improved characteristics. Keeping in view this objective, seven fungal isolates were collected from microbiology laboratory of SAFI Institute of Advanced Study (*Aspergillusniger*, *Aspergillusfumigatus*, *Aspergilluscarbonarius*, *Aspergillusflavus*, *Mucorsp*., *Penicilliumsp*. and *Rhizopussp*.). The fungal isolates grown on SDA and PDA plates were identified macroscopically and microscopically by using staining methods like saline wet mounting and LPCB (Lacto Phenol Cotton Blue) staining. These fungal strains were tested for their raw starch hydrolysing activity by using raw starch (tapioca powder) as substrate. Out of these seven fungal strains, *Aspergilluscarbonarius* exhibited raw starch hydrolysing activity.

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