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Use of Pectin Rich Fruit Wastes for Polygalacturonase Production by *Aspergillus awamori* MTCC 9166 in Solid State Fermentation

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Abstract

Polygalacturonase enzyme has industrial application in extraction and clarification of fruit juices. Production of polygalacturonase (PGU) by *Aspergillus awamori* MTCC 9166 was studied in solid state fermentation using different pectin-rich fruit wastes like apple peel, banana peel, citrus (orange) peel, jackfruit rind, mango peel, and pine apple peel. Sugar free fruit peels were prepared by water treatment and dried materials were used as substrates for PGU production. Highest enzyme production was with jack fruit rind and mango peel at 65% moisture content, 28ºC, pH 5.2, 10^6 spores/gm inoculum size for jack fruit rind and 10^8 spores/gm for mango peel and 96 h incubation period. These studies indicate that locally available waste raw materials, jack fruit rind and mango peel, have good potential as substrates for PGU production. Use of such waste raw material is not only cost effective but also caters to the cause of disposing of waste at no cost, which is important for developing Indian economy. This is the first report on use of fruit wastes as substrates for production of polygalacturonases by *Aspergillus awamori* MTCC 9166 in solid state fermentation. The enzyme production by this strain is more than the reported strains.

Keywords: Solid state fermentation; Solid substrates; Polygalacturonase; Fruit wastes; Aspergillus awamori

Introduction

Pectinases are a group of enzymes that degrade pectins present in middle lamella and primary cell walls of plant tissues [1]. These have wide applications in the food industry for clarification of fruit juices, wines [2,3], coffee and tea fermentations [3] and extraction of essential oils [4] etc. Pectinases produced by different microbes are divided into depolymerizing enzymes and saponifying enzymes. Depolymerizing enzymes are polymethylgalacturonases, pectin lyases, polygalacturonases and pectate lyases and saponifying enzymes are pectinesterases [3,5]. They have significant commercial value with a share of about 25% in global sales of food enzymes [6].

The production of pectinolytic enzymes has been widely reported in bacteria and filamentous fungi [7]. Fungal polygalacturonases are very significant for clarification of fruit juices, wines and for extraction of vegetable oils [8]. Their significance in clarification of fruit juices is due to the fact that their optimal pH closer to that of many fruit juices.

Solid state fermentation (SSF) is the process of cultivation of microbes on solid material in the near absence of free water. Polygalacturonase (PGU) production is reported to be significantly higher in solid state fermentation than in submerged fermentation (SMF) [9].

In the present study pectin rich solid wastes were used as substrates for production of PGU by *Aspergillus awamori* MTCC 9166. Various fruit wastes used were apple peel, banana peel, orange peel, jackfruit rind, mango peel, and pine apple peel. Different fermentation conditions like moisture content, temperature, pH, inoculums size and incubation period were optimized using the best substrates, jackfruit rind and mango peel, for production of PGU in SSF.

Methodology

Microorganism

Aspergillus awamori MTCC 9166 strain was isolated from vegetable dumpyard soil and maintained on PDA slants in refrigerator [10].

Pretreatment of solid substrates

Peels of various fruits like apple, banana, citrus (orange), jackfruit rind, mango and pine apple were subjected to water treatment till sugar free. These were dried and cut into small pieces in the size range of 0.5- 0.75cm. Their pectin content was determined by carbazole method [10,11].

Media and their moisture

Different fruit wastes as solid support and pectin substrate sources were taken and various volumes of Czapek nutrient solution was added to optimize moisture content.

Inoculum optimization

Fungal spores were scrapped from PDA slants to water suspension and added in various concentrations to media flasks to optimize inoculum size. One ml of spore suspension with a spore count in the range of 105-109spores/gm was inoculated to each 10gm of substrate.

Cultural conditions

Experiments were carried out in 100 ml flasks with 10gm solid substrate. Fermentation parameters like pH, temperature and incubation period were tested for optimization. The ranges were pH 4.5 - 6, temperature 25 - 40°C and incubation period 2 - 10 days.

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Enzyme extraction and assay

Enzyme was extracted using acetate buffer at pH 5.2 and assayed by measuring the D-galacturonic acid realeased from polygalacturonic acid as substrate by Miller's method [12]. One unit of enzyme activity is defined as the amount of enzyme required to produce 1µ mole of galacturonic acid per minute at room temperature.

Results

Pectin rich fruit wastes were studied for selection of solid substrates for polygalacturonase (PGU) production (Figure 1). Figure 1 indicates shows polygalacturonase production using different pectin rich substrates at various moisture levels. It also indicates that 65% moisture was best for all substrates studied. Two substrates, jack fruit rind and mango peel, showed comparatively higher enzyme production. Therefore these were used as solid substrates for further study on optimization of fermentation conditions for PGU production.

Inoculum size for polygalacturonase production was optimized and the results are presented in Figure 2. The optimized inoculum size was 106 spores/gm for jack fruit rind and 108 spores/gm for mango peel. The pH range was tested from 4.5 to 6 and optimum pH was 5.25 (Figure 3). The effect of temperature was tested in the range of 25° - 40°C and the optimum temperature was 28° C (Figure 4).

The fermentation cycle by solid state fermentation (SSF) for polygalacturonase was studied for a period of 240 hours or 10 days (Figure 5). Peak production was at 96 hrs or 4th day for both jack fruit rind and mango peel as substrates. The overall polygalacturonase production by *Aspergillus awamori* MTCC 9166 was good by using jack fruit rind (412 U /gm) than mango peel (217 U /gm) as solid substrate.

Discussion

Solid state fermentation (SSF) is advantageous compared to submerged fermentation as microbe-substrate interactions are efficient in this providing scope for reaching high product concentration, and also there is less liquid effluent that makes product recovery more efficient [13]. SSF was selected as there are reports of highest PGU production by this method [14-16]. Moisture content plays a significant role in SSF as it gives a good compromise between water availability and substrate swelling. It is also significant as both oxygen availability and its diffusion depend on this [14,17]. Therefore it was determined for different raw pectin substrates selected for study and all showed good response at 65% moisture level as shown in figure1. Comparitive study of 65% moisture level for PGU production in different pectin substrates used both as solid support and pectin rich inducers indicated (Figure 2) that jack fruit rind and mango peel are good substrates as they are also rich in pectin [11,10]. Inoculum size is important as it influences efficient substrate microbe interaction and 106 spores/gm was optimum for jack fruit rind and 10^8 spores/gm for mango peel (Figure 3). Optimum fer-

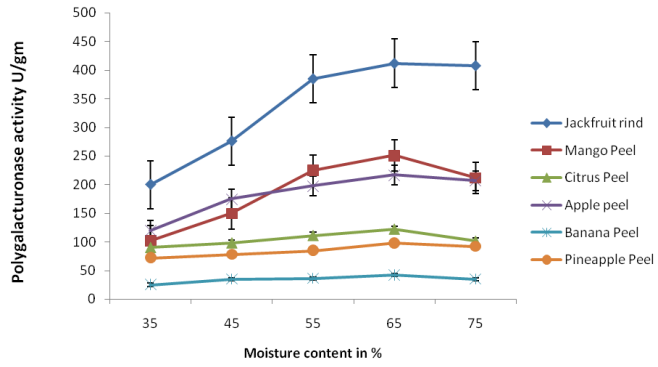

Figure 1: Effect of moisture content on polygalacturonase production by *Aspergillus awamori* MTCC 9166 in solid state fermentation using different pectin rich substrates.

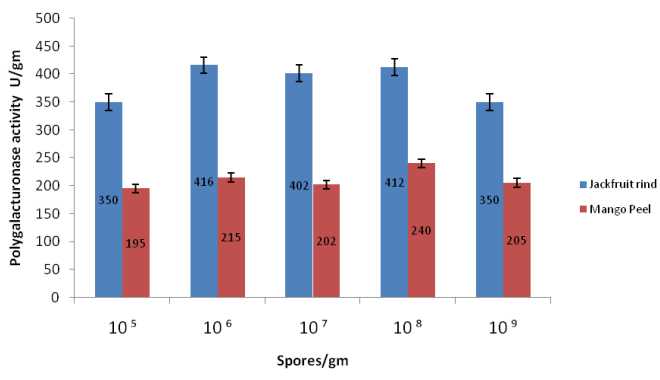

Figure 2 : Effect of inoculum size on polygalacturonase production by *Aspergillus awamori* MTCC 9166 in solid state fermentation. The p value is 0.0000 .

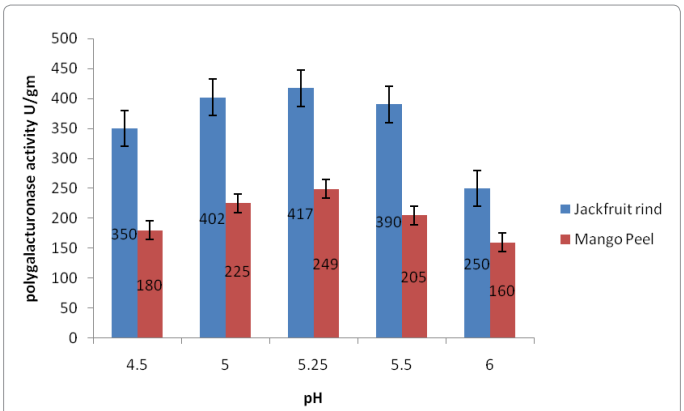

Figure 3 : Effect of pH on polygalacturonase production by *Aspergillus awamori* MTCC 9166 in solid state fermentation. The p value is 0.001641.

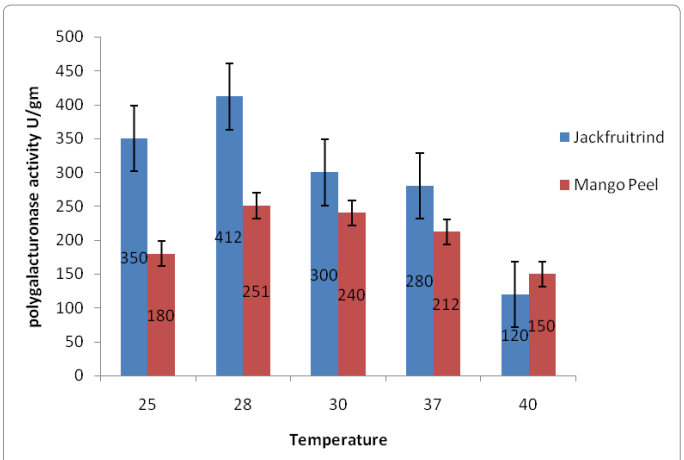

Figure 4 : Effect of temperature on polygalacturonase production by *Aspergillus awamori* MTCC 9166 in solid state fermentation. The p value is 0.139135.

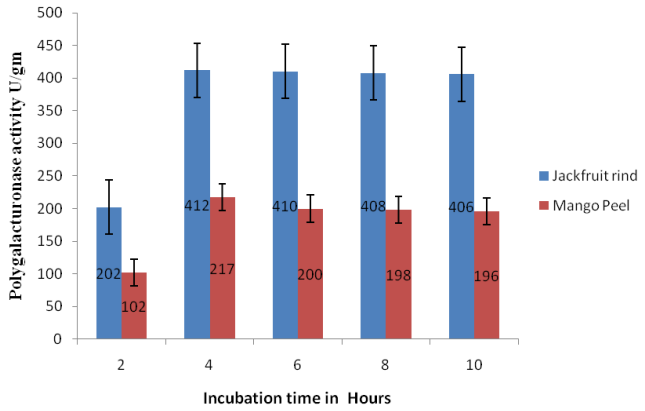

Figure 5 : Effect of incubation time for polygalacturonase production by *Aspergillus awamori* MTCC 9166 in solid state fermentation. The p value is 0.00392845.

mentation conditions bring out the highest product from any producer strain [18]. When these were tested as indicated in Figures 4 and 5, the response was good for an acidic pH 5.25 and a mesophilic temperature 28° C as it is a fungal organism and the result is similar to reported observations [19-21]. Producer strains need to have shorter fermentation cycles as this would be cost effective. In the present study the strain showed peak production at 4th day or 96 hours for both jack fruit rind and mango peel which is less than the reported for the same species. The organism studied could be an efficient producer strain as it responded well to a cheaper locally available solid substrates like jack fruit rind which is a waste that if not disposed could cause waste disposal problems. Response of this strain to submerged fermentation with jack fruit rind was also significant as reported earlier [10]. The shorter fermentation cycle also makes the strain cost effective for commercial exploitation. For a developing and comparatively poorer economy like that of India, use of such waste raw material is not only cost effective but also caters to the cause of disposing off waste at no cost.

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