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Original Research Paper

Effect of physical factors on pellet morphology of *Aspergillus awamori* MTCC 9166 and polygalacturonase production

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ABSTRACT

Polygalacturonase enzyme has its industrial application in extraction and clarification of fruit juices. Growth as pellet is an important character for any industrial fungal strain as product recovery becomes easy and simple. The simultaneous effect of fermentation conditions like pH, temperature, agitation and inoculum size were studied on pellet morphology and polygalacturonase production by *Aspergillus awamori* MTCC 9166 in submerged fermentation using crude pectin. The studies were done by unidimensional approach in which conventionally one parameter was selected and studied at a time. Optimum fermentation conditions for maximum polygalacturonase production were at pH 5.5, temperature 28 °C, agitation speed 200 rpm and inoculum size 1×10^6 spores/ml. The highest enzyme production at these conditions was 17.8 U/ml. The study revealed a significant fact that the same optimum fermentation conditions promoted both pellet formation and maximum enzyme production.

1. Introduction

Pectin is a hetero polysacharide polymer found in the middle lamella of cell wall of plants. Pectin is degraded by the action of several enzymes included under the generic term pectinases. Pectinases produced by different microorganisms are classified into depolymerizing enzymes and saponifying enzymes. Depolymerizing enzymes are those that catalyze the hydrolytic cleavage of the α-(1–4)-glycosidic bonds in the D-galacturonic acid moieties of the pectic substances. They are polymethylgalacturonases, pectin lyases, polygalacturonases and pectate lyases. Saponifying enzymes are esterases that catalyze the de-esterification of pectins by the removal of methoxy esters and are called pectinesterases (Whitaker, 1990).

About 75% of the estimated sale of industrial enzyme is contributed by pectinases (Sathyanarayana and Panda, 2003). These have wide applications in food industry for clarification of fruit juices, wines (Alkorta et al., 1998; Whitaker, 1984) coffee and tea fermentations (Jayani et al., 2005) and extraction of essential oils etc. The production of pectinolytic enzymes has been widely reported in bacteria and filamentous fungi (Naidu and Panda, 1998). Fungal polygalacturonases are very significant for clarification of fruit juices, wines and for extraction of vegetable oils (Castilho et al., 2000). Their significance in clarification of fruit juices is due to the fact that their optimal pH closer to that of many fruit juices. Pellet morphology in fungi results in formation of spherical agglomerates of hyphae that not only increase the efficiency of nutrient transfer but also make recovery of product easy and simple (Cui et al., 1997; Ryoo, 1999; Zhaou et al., 2000).

In the present study the effect of fermentation conditions on both pellet morphology and production of polygalacturonase (PG) by *Aspergillus awamori* MTCC 9166 was studied. There was variation in size and number of pellets with variation in fermentation conditions like pH, temperature, agitation speed and inoculum size. Optimum fermentation conditions for polygalacturonase production were also identified in the study.

2. Materials and methods

2.1. Microorganism

A. awamori MTCC 9166 was isolated from vegetable dumpyard soil and maintained on PDA slants in refrigerator (Anuradha et al., 2010).

2.2. Inoculum preparation

Fungal spores were scrapped from PDA slants to water suspension and added at a concentration 10^6 spores/ml to fermentation broth.

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2.3. Fermentation conditions

Experiments were carried out in 250 ml flasks with 50 ml Czapek's broth containing (g/l) – K_2HPO_4 0.2, KCl 0.5, $NaNO_3$ 0.2, $FeSO_4$ 0.03, and 1% crude pectin as sole carbon source. Fermentation parameters like pH, temperature agitation and inoculum size were tested for optimization. The ranges were pH 2–7, temperature 20–40 °C, agitation speed 140–220 rpm and inoculum size 10^4–10^8 spores/ml.

2.4. Study of pellets

The size of different types of pellets formed under different physical conditions was determined. Experiments for pellet studies were carried out in 250 ml flasks with 100 ml Czapek's broth. The number of pellets and their total dry weight were simultaneously determined.

2.5. Recovery and enzyme assay

Polygalacturonase (PG) enzyme was extracted using acetate buffer at pH of 5.2 and assayed by measuring the D-galacturonic acid realeased from polygalacturonic acid as substrate by Miller's method (Miller, 1959). One unit of enzyme activity is defined as the amount of enzyme required to produce 1 μ mole of galacturonic acid per minute at 37 °C.

3. Results

The simultaneous effect of fermentation conditions like pH, temperature and agitation on pellet morphology and polygalacturonase production was studied. Pellet formation is a character of some fungi and fermentation conditions influence both the size and number of pellets formed. The size of *A. awamori* MTCC 9166 pellets formed ranged 0.5–1.8 mm and the number varied from 70–145/100 ml for pH range 4–6. The optimum pH was 5.5 as a maximum of 145 pellets/100 ml of 0.9 mm size weighing 1.05 g/100 ml were formed (Table 1). Effect of temperature on pellet morphology was also significant as pellets ranging in size 0.5–1.8 mm, with dry weight 0.9 g/100 ml and number variation of 90–145 were formed. The optimum temperature was 28 °C as a maximum of 145 pellets/100 ml of 0.9 mm size weighing 0.9 g/100 ml were formed (Table 2). The ranges of pellet size, number and dry weight formed in response to variation of agitation speed was almost similar to that of temperature (Table 3). The optimum agitation speed was 200 rpm as a maximum of 145 pellets/100 ml of 0.9 mm size weighing 1 gm/100 ml were formed (Table 3).

The studies for optimization of fermentation conditions like pH, incubation temperature, agitation and inoculum size were done by uni-dimensional approach in which conventionally one parameter was selected and studied at a time. As pH of the medium is one of the important factors effecting enzyme production its effect on PG production when studied at various pH ranging 2–7, it was observed that the PG production was maximum at pH 5.5 (17.6 units). Enzyme yields were low at pH 2–3.5 pH and also beyond pH 6 (Fig. 1). Effect of temperature was studied at various

Table 1
Effect of pH on pellet morphology and polygalacturonase production by *Aspergillus awamori* MTCC 9166.

Parameter	pH				
	4	4.5	5	5.5	6
Pellet number/100 ml medium	90	95	100	145	70
Pellet morphology (size in mm)	Very small 0.5	Very small 0.5	small 0.7	medium 0.9	large 1.8
Biomass dry weight (g/l) at 72 h	5	5	6	10.5	7.5
Maximum enzyme activity/ml	11.5	12.5	14	17.6	11.5

Table 2
Effect of temperature on pellet morphology and polygalacturonase production by *Aspergillus awamori* MTCC9166.

Parameter	Temperature (°C)				
	20	25	28	30	37
Pellet number/100 ml medium	90	100	145	120	100
Pellet morphology (size in mm)	Very small 0.5	small 0.7	medium 0.9	large 1.8	large 1.8
Biomass dry weight (g/l) at 72 h	6	8	9	8	7
Maximum enzyme activity/ml	14.5	16	17.8	16.3	15

Table 3
Effect of agitation on pellet morphology and polygalacturonase production by *Aspergillus awamori* MTCC 9166.

Parameter	Agitation speed (rpm)				
	140	160	180	200	220
Pellet number/ 100 ml medium	100	125	130	145	80
Pellet morphology (size in mm)	Very small 0.5	small 0.7	small 0.7	medium 0.9	large 1.8
Biomass dry weight (g/l) at 72 h	6	8	9	10	6.5
Maximum enzyme activity/ml	12.5	15	16	17.8	11.5

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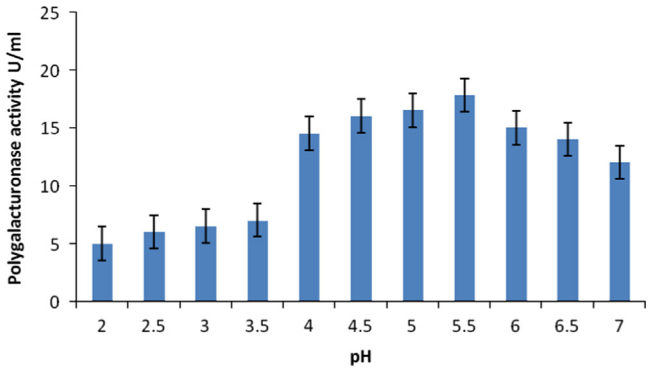

Fig. 1. Effect of pH on polygalacturonase production *by Aspergillus awamori* MTCC 9166. The *p*-value is 0.000213 and it is less than α.

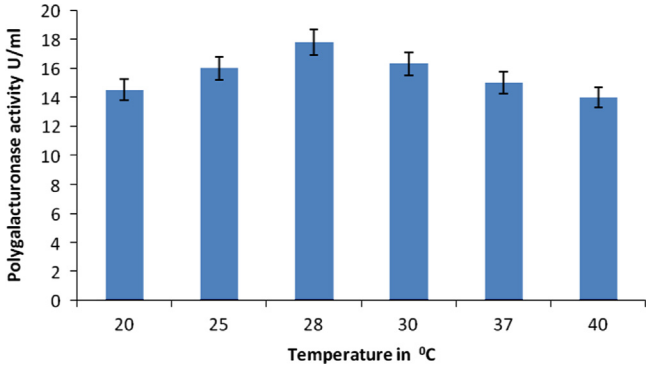

Fig. 2. Effect of temperature on polygalacturonase production by *Aspergillus awamori* MTCC 9166. The *p*-value is 0.005579 and it is less than α.

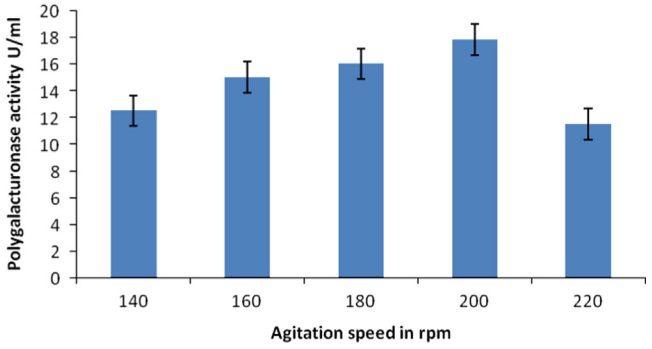

Fig. 3. Effect of agitation speed on polygalacturonase production by *Aspergillus awamori* MTCC 9166. The *p*-value is 0.000309 and it is less than α.

temperatures ranging from 20 to 40 °C. *A. awamori* MTCC 9166 was found to produce maximum PG (17.8 U/ml) at 28 °C (Fig. 2). Agitation speed in the range of 140–220 rpm was maintained and its effect studied for PG production by *A. awamori* MTCC 9166. Increased agitation was found to improve enzyme production with 17.8 U/ml, at 200 rpm and beyond that speed there was decrease in enzyme production (Fig. 3). When the inoculum size ranging in a spore count (10^4–10^8 spores/ml) was studied for its effect on PG production, it was observed that inoculum size of 10^6 spores/ml showed maximum PG production (Fig. 4).

4. Discussion

The *A. awamori* MTCC 9166 under present study exhibited pellet morphology which is an important character as it makes

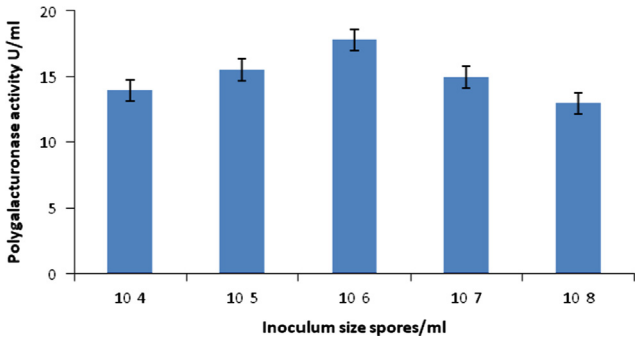

Fig. 4. Effect of Inoculum size on polygalacturonase production by *Aspergillus awamori* MTCC 9166. The *p*-value is 0.0053 and it is less than α.

both fermentation and downstream processing easy. Many studies on growth morphology in terms of different products have concluded that pellet morphology is more favorable for industrial strains as it not only improves culture rheology but also promotes better microbe nutrient interaction for efficient product yield (Lopez et al., 2005; Zhaou et al., 2000). Fermentation conditions are known to affect the growth morphology and enzyme production by *Aspergillus sojae* and *Rhizopus oryzae* (Oncü et al., 2007; Tari, et al., 2011). The physical factors influenced the size, number and dry weight of pellets and also the primary metabolite production like PG enzyme. In the present study it was found that a pH 5.5, temperature 28 °C and agitation 200 rpm were the optimum as they increased both the pellet formation (size and number) and PG enzyme production (17.8 U/ml) by *A. awamori* MTCC 9166. There was better enzyme production with medium sized pellets (0.9 mm) as these could promote more microbe-nutrient interaction and better oxygen transfer which is necessary for an aerobic organism like *Aspergillus*. Studies on optimization of inoculum size for both pellet formation and PG production revealed that 10^6 spores/ml was optimum and this is important to form both biomass (pellets) and enzyme as a primary metabolite. Similar results were obtained in earlier studies where the authors described the effects of the inoculum concentration for better production of enzymes (Kiro, 2010; Shah and Madamwar, 2005; Qinnghe et al., 2004). This study on pellet morphology provides significant information for PG production as there are no such reports on *A. awamori*. Therefore the present findings serve as a base line study on pellet morphology and PG production or any other enzymes by fungal organisms.

5. Conclusion

A pellet forming fungal isolate *A. awamori* MTCC 9166 showed good polygalacturonase production. Optimum fermentation conditions for pellet formation and maximum polygalacturonase production were pH of 5.5, temperature 28 °C, agitation speed of 200 rpm and inoculum size of 1×10^6 spores/ml. The present study is significant for fungal polygalacturonase producer with pellet morphology, which is an important character in industrial product recovery.

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