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Batch and fed batch production of pectin lyase and pectate lyase by novel strain *Debaryomyces nepalensis* in bioreactor

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Abstract

The effect of various parameters such as pH, agitation and aeration was studied for maximum production of pectin lyase (PL) and pectate lyase (PGL) by a novel yeast strain *Debaryomyces nepalensis* in bioreactor. The optimal levels of pH, aeration and agitation rate was found to be 7.0, 300 rpm and 1 vvm, respectively. Under these conditions, *D. nepalensis* produced 14,200 U/L of PL and 12,000 U/L of PGL corresponding to a productivity of 600 U/L h and 500 U/L h of PL and PGL, respectively. Fed-batch production was studied by feeding inducer (lemon peel), carbon source (galactose) individually and in combination at 12 h of growth for enhanced production of PL and PGL. Combined feeding of inducer and carbon source at 12 h was found to be the best strategy for enhanced production of PL and PGL. Under these conditions, production of PL and PGL increased to 23,300 U/L and 22,400 U/L, respectively which corresponded to a productivity of 728 U/L h of PL and 700 U/L h of PGL, respectively. The production was increased by 1.6- and 1.8-fold and productivity by 1.2- and 1.4-fold for PL and PGL, respectively when compared to batch culture.
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Keywords: Pectin lyase; Pectate lyase; *Debaryomyces nepalensis*; Fed-batch culture; Bioreactor

1. Introduction

Pectic transeliminases or pectic lyases are one among the pectinases that degrade the pectic substances by transelimination mechanism yielding unsaturated oligogalacturonates. Pectate lyase (PGL), acting on polygalacturonic acid, and pectin lyase (PL), acting on pectin are the two important transeliminases. It has been reported that PL is mostly produced by fungi whereas PGL is produced by pathogenic fungi (Gummadi and Kumar, 2005). Yeasts like *Saccharomyces cerevisiae* and *Candida* sp. have been reported to produce pectinolytic enzymes (pectin lyase, polygalacturonases, and pectinesterases) (Gainvors et al., 1994). Pectic transelimanses were enzymes of interest in the last decade owing to their varied industrial applications. PL has extensive applications in extraction, clarification and cloud stabilization of fruit juices (apple lemon, orange, mango, etc.) and maceration of plant tissues (Kilara, 1982; Naidu and Panda, 1998; Alkorta et al., 1998; Blanco et al., 1997; Rebeck 1990). These are also used in the isolation of protoplasts (Takebe et al., 1968) and saccharification of biomass (Beldman et al., 1984). PGL has potential applications in cotton scouring (Hoondal et al., 2002), degumming of plant fibers (Bruhlmann et al., 1994) improve the quality of fiber (Beg et al., 2000), decreasing the cationic demand of pectic solutions in paper processing (Reid and Ricard, 2000), purification of plant viruses (Salazar and Jayasinghe, 1999), treatment of effluents from food processing industries (Tanabe et al., 1987) and enhancing the fermentation step for tea and coffee processing (Godfrey, 1985). Keeping in view of the increasing industrial applications of PL and PGL it becomes crucial for production of both these enzymes from a single non-pathogenic microorganism. Till now only a couple of reports were available where both the

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pectic transeliminases being produced by *Candida boidinii* and *Paenibacillus* sp. (Nakagawa et al., 2000, Soriano et al., 2005). However, detailed studies on the production of these two enzymes in are bioreactors are not reported.

In this context, we have previously isolated a yeast strain *Debaryomyces nepalensis* from rotten apple capable of utilizing pectin as the sole source of carbon and produce both the transeliminases (Gummadi and Kumar, 2006a). It has been found that production of PL and PGL by *D. nepalensis* are higher to those reported in literature, 9 U/L of PL by *Aspergillus niger* NCIM 548 (Panda and Naidu, 2000), 60 U/L of PL by *Penicillium griseoroseum* CCT 6421 (McCarthy et al., 1985), 400 U/L of PGL by *Bacillus licheniformis* (Singh et al., 1999), 960 U/L of PGL by *Pseudomonas marginalis* (Hayashi et al., 1997) and 673 U/ml of PL and PGL by *Candida boidinii* (Nakagawa et al., 2000). However, the production of PL and PGL is slightly higher for *Paenibacillus* sp. (Soriano et al., 2005). *D. nepalensis* has been exploited for increasing the production of PL and PGL in our laboratory. Optimization studies on media components for maximum production of PL and PGL and the optimal conditions for maximum activities were determined under shake flask conditions (Gummadi and Kumar, 2006a,b). Microbiological parameters were optimized in order to develop an effective seed culture (Gummadi and Kumar, 2006c). Bioreactors have an edge over shake flasks, as there is better control on process parameters like aeration, agitation and pH maintenance. In this study, the possibility to enhance PL and PGL production by *D. nepalensis* was evaluated in a laboratory bioreactor. Various parameters like pH, aeration and agitation were studied in batch fermentation. Fed-batch studies were performed to enhance production by feeding carbon source and inducer.

2. Methods

2.1. Chemicals

Pectin and polygalacturonic acid were purchased from Sigma. All other chemicals used in the present study were of analytical grade procured in India.

2.2. Strain

Debaryomyces nepalensis was used for all experiments and is deposited in National collection of yeast cultures (NCYC), Norwich, UK with accession number D3893 (Gummadi and Kumar, 2006a). The isolate was maintained on YEPD agar plate at 4 °C and sub-cultured every two weeks.

2.3. Media

The seed medium consists of (g/l) yeast extract, 10; peptone, 20; dextrose, 20. The production medium had the following composition (g/l): galactose 9.6, yeast extract 19, lemon peel 24, Na_2HPO_4 6.0, K_2HPO_4 3.0, NaCl 5.0, $MgSO_4$ 0.1 and $FeCl_3$ 1.0 (Gummadi and Kumar, 2006a).

2.4. Cultivation

Conditions of seed culture like plate age, inoculum age and percentage of inoculum were optimized previously in our laboratory (Gummadi and Kumar, 2006c). A loopful of the strain from YEPD agar plates, which was previously incubated for 32 h in an incubator at 30 °C, was transferred to 100 ml sterile YEPD medium. The pH of the medium was adjusted to 7.0 and the culture was incubated on a rotary shaker at 180 rpm and 30 °C. The seed culture was allowed to grow for 12 h and then 5% of the seed culture was transferred to production medium in bioreactor.

Batch and fed-batch studies for production of PL and PGL were carried out in a 2.4 L bioreactor (Bioengineering, Switzerland). The bioreactor is equipped with a DO analyzer and an automatic pH controller controlled in PID (proportional integral derivative) mode. Optimal initial pH for maximum enzyme production was tested by growing the organism in production media with different initial pH ranging between 6.0 and 9.0. The organism was grown at a temperature of 30 °C, agitation of 300 rpm and an aeration of 1 vvm. At regular intervals of time, samples were withdrawn and analyzed for cell growth, carbon source consumption and measurement of PL and PGL activities. In order to determine the optimum agitation for PL and PGL production experiments were performed at 150, 300, and 450 rpm. Different levels of aeration (0.5, 1.0, and 2.0 vvm) were used to find out the optimum aeration for enzyme production.

Fed-batch experiments were performed by the addition of feed solutions, consisting of galactose (100 g/l) and lemon peel (100 g/l) to the production medium at specified time of growth. In the first experiment, 50 ml of 100 g/l lemon peel was added to the growing culture at 12 h of fermentation, when the growth was about to reach stationary phase. In the next experiment, galactose (100 g/l) was fed to the culture around 12 h of growth when galactose concentration was less than 5 g/l to bring back the galactose concentration to 10 g/l. The same procedure was repeated at 24 and 34 h of fermentation when the galactose was found to be less than 5 g/l. In order to check the combined effect of feeding galactose and lemon peel, stock solution of galactose (100 g/l) and lemon peel (100 g/l) was added together ~12 h of fermentation.

2.5. Enzyme assays

Culture supernatant obtained from the bioreactor was used as the source for enzyme activity. PL and PGL activities were assayed by measuring the formation of unsaturated oligogalacturonates at 235 nm. The reaction mixture contained 0.19% (w/v) pectin in 100 mM citrate phosphate buffer (pH 6.4) and suitably diluted enzyme (10 times diluted enzyme with appropriate buffer). To test PGL activity,

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