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Effect of metal salts on production of pectin methyl esterase by solid state fermentation using *Penicillium notatum* NCIM No.923 utilizing agricultural wastes

SOUMI GAYEN AND UMA GHOSH*
Department of Food Technology & Bio Chemical Engineering. Jadavpur University, Kolkata, West Bengal

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In this study an attempt was made to increase the production of pectin methyl esterase (PME) by incorporating some metal salts in the wheat bran (WB) and citrus waste (CW) by solid state fermentation (SSF) using *Penicillium notatum* NCIM No.923. Optimum conditions for production of PME showed that addition of 0.02 % (w/v) $NaNO_3$ in the solid substrates may increase the relative activity up to 10.72% at 30°C for 120 h of incubation at pH 5.4

Key words: Wheat bran, orange peel, solid state fermentation, *Penicillium notatum*

INTRODUCTION

The potential of solid state fermentation (SSF) systems has been recognized in recent years as reported by Pandey (1992) and Lonsane *et al.*(1992) Although, this method has many advantages over submerged culture, it has a few limitations as studied by Hesseltine (1972) and Shankaranand *et al.*(1992). SSF could be a better biodegradation alternative.

SSF is defined as the growth of microorganisms on a solid matrix with a low content of free water. According to Favela-Torres *et al.* (1997) there are several advantages of SSF over SmF in regard to hydrocarbon biodegradation and they include: (i) high initial concentrations of carbon and energy source (up to 50% w/w). (ii) lower degree of catabolic repression as stated by Solis-Pereira *et al.*(1993), (iii) microbial metabolism not limited by oxygen transport as reported by Durand *et al.* (1988), (iv) low water content, (v) soluble substrates not required for microbial growth, and (vi) better fungal growth because the conditions are similar to their natural habitat as reported by Mitchell and Lonsane, (1992). Pilnik and Voragen (1970) have stated that most fruit juices, the pectin fraction is often identified as causing the most hindrance to juice filtration performance. Consequently, pectinase enzymes are typically used to modify colloidal interaction behavior as reported by Rombouts and Pilnik(1979). The quality of such products can be improved by treating fruit juice with cell wall degrading enzyme (such as pectinase) which reduces the viscosity and can increase the yield of the juice as stated by Sreenath *et al.* (1995). According to Pilnik and Voragen (1970) pectin, a structural cell wall polysaccharide found in all higher plants, is primarily composed of linear polymers of D-galacturonic acid joined by α-D-1,4 glycosidic linkages.

MATERIALS AND METHODS

Microorganisms

Czapek Dox agar slants were inoculated with *Penicillium notatum* NCIM No.923 and incubated at 30°C for 7 days before storing at 4 °C

*ughoshftbe@yahoo.co.in

Materials

Dried wheat bran and orange peel were used in the whole experiment.

Fermentation

Solid state fermentation was carried out by taking 10 g of solid substrate (1:1,w/w) in 500 ml Erlenmeyer flasks and distilled water was added to the substrate to obtain final moisture content 50%. Inoculum of 10^7/ml spores were added in the sterile medium and inoculated at 30°C for 5 days under stationary condition.

Crude enzyme extraction

After 5 day when fermentation was over, the substrate was mixed with distilled water in the ratio of 1:10 (w/v) and mixed thoroughly then centrifuged at 8000-10,000 rpm for 30 min. The filtrate was used as crude enzyme.

Enzyme assay

For enzyme assay of pectin methyl esterase, pectin was used as a substrate. The amount of acid produced was neutralized by NaOH solution by titrimetry (Assis *et al.*,2002). One unit of PME activity is defined as the amount that released 1 µmole of carboxyl groups per minute.

Optimization of enzyme production
Effect of metal salts

Media was supplemented with 0.1(%,w/v) solution of different metal salts such as $NaNO_3$, $CuSO_4$, $MgSO_4$, Hg_2Cl_2, $HgCl_2$, and ZnS to study their effect on production of pectin methyl esterase. Further studies were made with salt of $NaNO_3$ (as its activity was found the best among all) at a concentration viz. 0.01 to 1%,w/v.

RESULTS AND DISCUSSION

From the Figures (Figs.1-4) highest activity $NaNO_3$ was considering the 100%. $MgSO_4$ showed activity 91.68%. Different metal salts such as $NaNO_3$, $MgSO_4$, $CuSO_4$, ZnS, Hg_2Cl_2, $HgCl_2$ etc. were added in the medium Cu^{++}, Zn^{++}, Hg^{++}, and Hg^{+} had no positive effect on PME production as they had lower

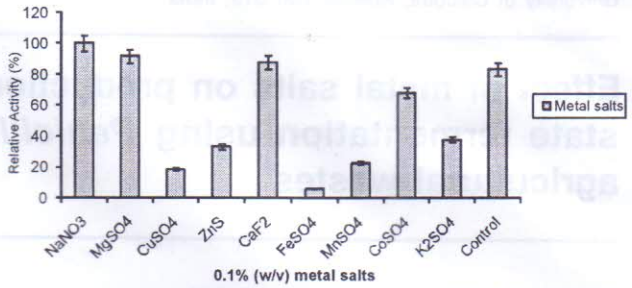

Fig. 1 : Effect of metal salts for the production of pectin methyl esterase.

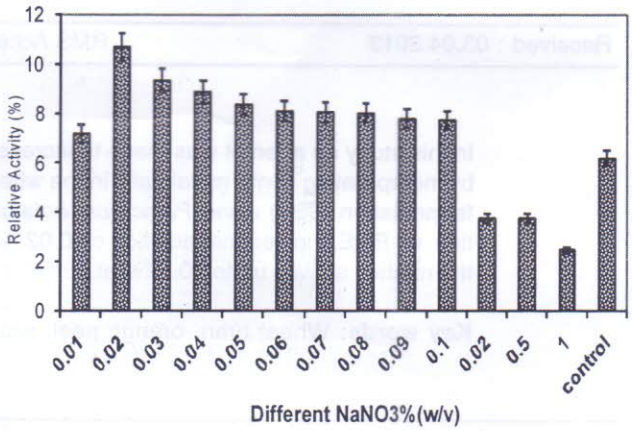

Fig. 2 : Effect of various concentration of $NaNO_3$ for the production of pectin methyl esterase in the medium.

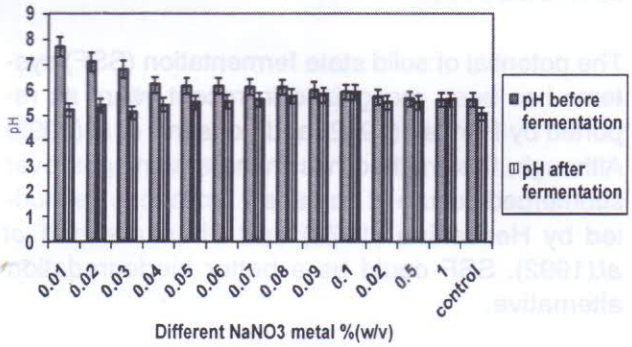

Fig. 3 : Measurement of pH with variation of concentration of $NaNO_3$ for the production of pectin methyl esterase in the medium.

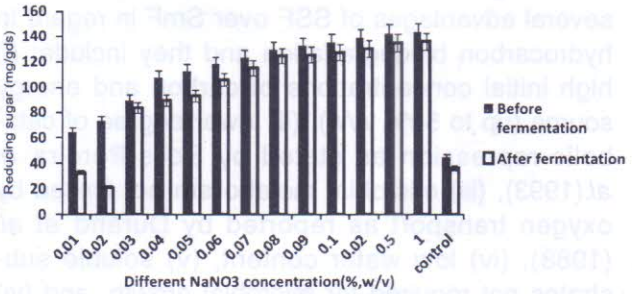

Fig. 4 : Measurement of reducing sugar with variation of concentration of $NaNO_3$ for the production of pectin methyl esterase in the medium.

activities than control. $MgCl_2$ and $ZnCl_2$ inhibited pectinase enzyme activity to the level of 21.2 and 14.8% as reported by Banu *et al.,* (2010). Similarly $HgCl_2$, $CoCl_2$ and $CuSO_4$ have been reported to inhibit activity of pectinase of *P. chrysogenum* up to 60%. Alana *et al.* (1990) reported that Ca^{2+}, Mg^{2+}, Zn^{2+} and Mn^{2+} did not affect pectin lyase activity of *P. italicum* at 5 mM, while Co^{2+} reduced it by 14%. However Cu^{2+} and Fe^{2+} at the same concentration produced complete inhibition.

Further studies were made with $NaNO_3$ salt at a various concentration from 0.01 to 1 % (w/v) and their pH was measured. It was found that addition of 0.02% $NaNO_3$ in the medium increase the PME activity maximum at a pH of 5.3 with the reducing sugar content of 32.7 mg/gds. Alexandre Maller *et al.,* (2011) found that PG activity was predominantly acidic, presenting two plateaus (pH range of 3–4.5 and 5–6.5, suggesting more than one enzymatic form). For *A. niger* (Mohamed *et al.*, 2006) and *Fusarium moniliforme* (Friedrich *et al.*,1994) the maximum PG activity occurred at pH 5.0, and for *T. reesei* PGs at pH 4.5 and 4.2 (Mohamed *et al.*, 2006). The enzyme from *A. niveus* was stable in a pH range of 3.0–5.0, for 24 h at 4–6 ?C. The PG from *T. harzianum* (Mohamed *et al.*, 2006) was stable at pH 5.0, and PG from *A. fumigatus* (Hoondal *et al.*,2002) was stable in a pH range of 3.0–9.0.

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