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Process optimization for the production of polygalacturonase from citrus wastes by *Saccharomyces cerevisae*

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Abstract

The production of polygalacturonase (PG ase) by an yeast strain, *Saccharomyces cerevisae* in submerged fermentation (SmF) of various citrus wastes was optimized. The effects of the fermentation parameters namely initial pH, temperature and cultivation time on enzyme production were studied using both pure pectin and citrus wastes as the sole carbon source at a time method. It was found that although dried citrus pulps did not show sufficient inducing activity, citrus peels were proved to be competent carbon source for extra cellular polygalacturonase production. Although pure pectin at a concentration of 0.5% (w/v) was found to be most effective for enzyme production, higher concentration of lemon peel was proved to be better than pure substrate. The optimum pH for enzyme production was found to depend on the type of carbon source used as for orange peel, pure pectin and lemon peel supplemented cultures, the preferred pH s were 4, 6 and 7 respectively. Highest production was achieved at a temperature of 27°C. The enzyme production kinetics indicated that the strain took a longer time (5 days) to hydrolyse waste pectins for enzyme production than the pure substrate (3 days).

Key words: Citrus wastes, polygalacturonase, *Saccharomyces cerevisae*, submerged fermentation and yeast.

Introduction

The current annual world production of citrus fruits is greater than 80 million tonnes Kuivanen *et al.* (2014), which leads to the generation of a huge amount of citrus processing wastes. As only a small part of these wastes is used in the nutrition of livestock or in solid fuel production Li *et al.* (2006), a large amount is left unutilised or underutilized. Utilization and valorization of citrus peels has been a subject of various investigations Karsheva *et al.* (2013), as citrus wastes can be used for the production of various value added materials including enzymes. The pectin content in citrus wastes is around 25% on a dry mass basis, corresponding to about 5% on a wet mass basis Pourbafrani *et al.* (2010). Pectolytic enzymes are involved in the degradation of pectin, a complex colloidal acidic polysaccharides that show a backbone of galacturonic acid residues with α-1,4-glycosidic linkages Yadav *et al.* (2009). Pectinases have extensive applications not only in the extraction, preparation and clarification of fruit juices and wine, but also wastewater treatment, paper manufacturing, oil extraction, coffee and tea fermentation, processing and degumming of many plant fibers Hoondal *et al.* (2002). Among the pectinases, poly-galagturonase (EC 3.2.1.15) hydrolyses the 1,4 α-D galacturonic acid linkages of pectin Sur *et al.* (2014).

Pectinase have a share of 25% in the global sales of food enzymes Jayani *et al.* (2005) and 10% of total enzyme production Mukesh Kumar *et al.* (2012), but higher cost of the production is the major constraint in commercialization of new sources of enzymes Siddiqui *et al.* (2012). Different wastes must be used as the sole nutrient source in place of expensive pure substrate for the growth of the microbe to curtail the cost of production. Hence, citrus wastes could be used as the sole carbon source for the production of polygalacturonase.

Although pectinolytic enzymes are found in some bacteria and yeasts, they are mainly found in moulds Fernández-González *et al*. (2004), Jayani *et al*. (2005) and Oliveira *et al*. (2006). A few yeast strains are reported to produce polygalacturonase McKay (1990), Fernández-González *et al*. (2004), Favela-Torres *et al*. (2005), Arévalo-Villena *et al*. (2011), but till today most commercial pectinase preparations used in the food industry are derived from *Aspergillus niger* producing some other less desirable by-products. Arévalo-Villena *et al*. (2011). On the other hand, according to Arévalo-Villena *et al*. 2011, for a genuine product can only be obtained from yeasts, enzymes derived from *Saccharomyces cerevisiae* would provide a useful alternative to mould derived pectinases. Hence extensive research is warranted on the production of pectinase from highly productive yeast strain utilising a low cost substrate.

The aim of this research was to optimize the culture medium using various citrus wastes as substrate for the growth and synthesis of polygalacturonase by a locally isolated strain of *Saccharomyces cerevisiae*.

Materials and methods

Cultivation of microorganism

A strain of yeast was isolated locally Chatterjee *et al*. (2011), identified as *Saccharomyces cerevisiae* was used as the present working strain. The yeast strain was cultivated in 100 mL Erlenmeyer flasks each containing 10 mL Basal Medium (BM) composed of (g L-1): peptone 0.9; $(NH_4)_2HPO_4$ 0.4; KCl 0.1; $MgSO_4.7H_2O$ 0.1 and pectin 5. (pH: 6) for 48-120 hours. To study the effect of citrus wastes as sole carbon source for polygalacturonase production, the citrus wastes namely peels and pulps of orange, lemon and sweet lime were collected from domestic and market effluents, dried, pulverized and sieved before using in fermentation media in place of pure pectin.

Enzyme extraction and assay

The grown culture was centrifuged at 6,000 rpm for 5 min at 4°C and the supernatant was used as the crude enzyme. To measure the activity of polygalacturonase, the assay mixture (1 mL) containing an equal volume of crude enzyme and 1% (w/v) pectin dissolved in 0.1(M) phosphate buffer (pH-7) was incubated at 37°C for 15 min. The reducing sugar released was measured by the dinitrosalicylic acid method (Bernfeld, 1955). Blanks were prepared with inactivated enzymes. One unit of enzymatic activity (U) was defined as one µmol of galacturonic acid released per minute (Dey *et al*., 2011).

Optimization of production parameters

The concentrations of pure and waste pectin used as sole carbon source were varied from 0.25% - 2% (w/v) to optimize the substrate concentration of submerged culture of *Saccharomyces cerevisae*. The optimum pH was determined by adjusting the initial pH of the fermentation media at a range from pH 4.0-8.0.

Most favourable production temperatures were studied by growing the strain at different temperatures (17- 37°C). The time course of growth and enzyme production by the yeast strain under optimized culture conditions were studied by checking the enzyme production kinetics for 48 to 168 hours at 27°C.

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All chemicals used were of analytical grade and each experiment was performed thrice and the average value was taken.

Result and discussions

Carbon source is an essential constituent of the fermentation medium, which affects the cellular metabolism of the microorganism Kumar *et al*. (2014). Hence the inducing efficacy of pure pectin and other citrus wastes for the production of poly-galacturonase was investigated. Among the carbon sources used, pure pectin gave the best activity for the production of polygalacturonase (PGase) followed by other citrus pectins present in the peels of orange, lemon and sweet lime (Table - 1) suggesting that the organism utilized pure pectin more efficiently as compared to citrus pectin. However, similar efficacy of utilizing citrus wastes for Pg ase production was reported by Maller *et al*. (2011), Dey *et al*. (2011), Amande *et al*. (2012). On the other hand, dried pulp of orange and sweet lime failed to induce polygala-cturonase synthesis by the present strain. Hence, the subsequent experiments involved only dried peels of the citrus fruits as a replacement for pure pectin.

Substrate concentration plays a vital role in enzyme production. Although the submerged culture of the present strain showed highest production in a cultivation medium supplemented with pure pectin at a concentration of 0.5% (w/v) (Fig.- 1), but about 1.125 times increase in yield was obtained if the growth medium was supplemented with dried pulverised lemon peel at a concentration of 1.5% (w/v). Requirement of a higher concentration of waste substrate than the pure pectin might be due to the lesser availability of digestible substrate to the strain. The enzyme synthesis gradually decreased with further increase in substrate concentration, which might be for enzyme limitation Dey *et al*. (2011).

Maximum enzyme activity was obtained when the initial pH of the production media supplemented with pure pectin and with sweet lime peel was adjusted to 6.0 (Fig.- 2), and there was a sharp decrease in enzyme activity at pH of 8.0. Similar pH preference was reported from *Aspergillus foetidus* Pasha *et al.* (2013). But the enzyme yield from orange peel was found to be maximum at acidic medium (pH 4), which gradually declined with further increase in pH, an identical pattern of pH pattern was found in *Aspergillus niveus* Maller *et al*. (2011). On the other hand, lemon peel containing medium showed highest PG ase production at pH 7.0, similar to the optimum pH reported from the various bacterial strains Dey *et al*. (2011), Soares *et al*. (1999) and *Trichoderma pseudokoningii* (Sur *et al*., 2014).

Most favourable production temperatures for PGase production by *Saccharomyces cerevisae* was found to be 27°C (Fig.- 3) similar to another strain of *Saccharomyces cerevisae* Arévalo-Villena *et al.* (2011). Since the present strain showed diminished growth at higher temperature, enzyme production dropped above a temperature of 32°C. Generally an optimal temperature of 30°C was found for PGase production by *Aspergillus* sp. Galiotou-Panayotou *et al.* (1997) *Mucor circinelloides* Thakur *et al.* (2010) and *Peacilomyces clavisporus* Souza *et al*. (2003) and a higher temp of 37°C was required for *Bacillus* sp. AD 1 Dey *et al.* (2011).

The kinetics of enzyme production (Fig.- 4) indicated that the strain showed highest PG ase production at 72 hours only when grown on pure pectin supplemented culture medium, whereas it took more time for achieving the highest production from citrus wastes. This longer time duration might be due to the complex nature of pectin residues in the waste substrates, which made their hydrolysis by the strain

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Table - 1. Effect of carbon source on Polygalacturonase production by *Saccharomyces cerevisae*

Carbon sources used (1% w/v)	Relative efficiency of Polygalacturonase production (%)
Pure pectin	100
Orange peel	70.2
Orange pulp	15
Lemon peel	60
Lemon pulp	11
Sweet lime peel	51
Sweet lime pulp	3

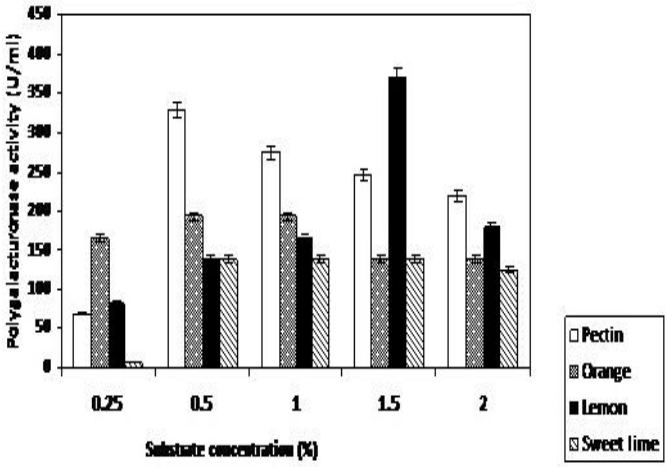

Fig 1. Effect of substrate concentration on Polygalacturonase production by *Saccharomyces cerevisae*

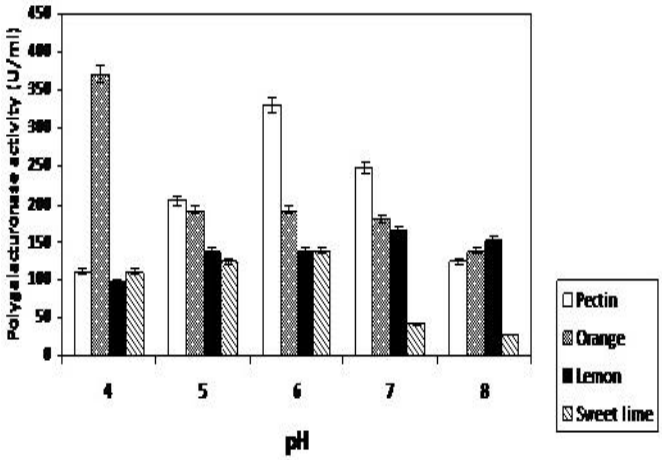

Fig. - 2. Effect of pH on Polygalacturonase production by *Saccharomyces cerevisae*

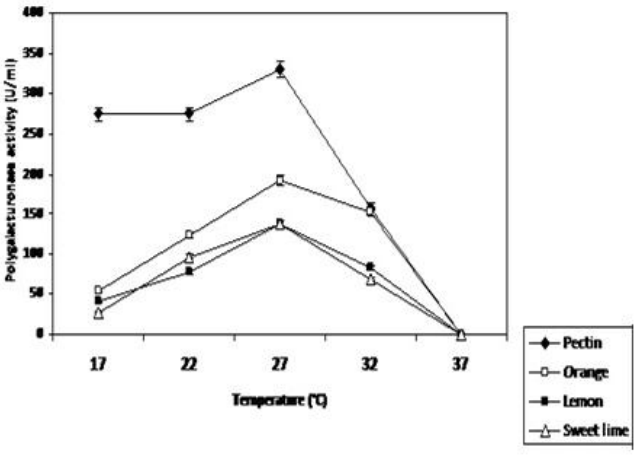

Fig.- 3. Effect of cultivation temperature on Polygalacturonase production by *Saccharomyces cerevisae.*

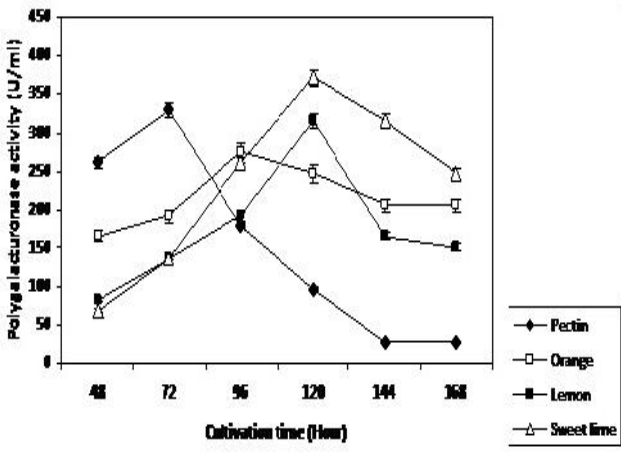

Fig.- 4. Kinetics of polygalacturonase production by *Saccharomyces cerevisae.*

more difficult and time taking. In *Aspergillus niveus* Maller *et al.* (2011) maximum PG production occurred after five days of incubation, whereas Amande *et al*. (2012) found the maximum yield of PG by different genera of *Aspergillus*, *Fusarium* and *Mucor* after day 6 of cultivation on medium with in mango peels, plantain and banana peels.

Conclusion

Yeasts are an alternative source for the large-scale production of commercial enzymes, and they have advantages compared with filamentous fungi with regard to the production of pectinases, because their growth is relatively simple, and the growth culture medium does not require an inducer Silva *et al*. (2005). The present working yeast strain of *Saccharomyces cerevisae*, due to its ability to produce a large amount of polygalacturonase under submerged cultivation of citrus wastes could represent a promising source of enzymes for biotechnological purposes and needs to be thoroughly characterized before it could be regarded as Safe (GRAS) and employed in the food industry.

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