

Original Research Paper

Screening of Yeast Strains for Pectinolytic Activity: Effects of Different Carbon and Nitrogen Sources in Submerged Fermentations

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Article history

Received: 01-12-2014

Revised: 15-03-2014

Accepted: 18-06-2015

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Abstract: Twenty yeast strains were screened for pectinolytic activity, among the strains, only nine were positive for pectinase production. The best strain was *Kluyveromyces marxianus* NRRL-Y-1109 which gave high quantities of pectinase activity, by submerged fermentation. Different parameters such as incubation time, pH (3.5-7), temperature (20-45°C), nitrogen and carbon source, were optimized. The optimal incubation time, temperature and pH for pectinase production were found to be 48 h, 30°C and 6, respectively. Studies were conducted on the production of pectinase in submerged fermentation using agro-industrial residues such as wheat bran, grape waste, brewer's malt, beet molasses and corncob at a concentration of 1.5% (wt./vol.). Under optimized fermentation conditions, maximal enzyme were produced when citrus pectin (4.8 U/mL) was used as the carbon source, but high enzyme production was also obtained on wheat bran (2.2 U/mL) and grape waste (1.8 U/mL) in shaking conditions (120 rpm) for 48 h. Peptone and yeast extract used together as nitrogen source gave best enzyme production. The effects of temperature (30-70°C), pH (3.5-8) and salt concentration (1, 2, 5 and 10%) on the pectinase activity were determined. The optimum activity was obtained when temperature 45°C and pH at 5.5. The enzyme was stable at 45°C for more than 50 min, suggesting that it is relatively thermostable and being of interests for food processing.

Keywords: Agro-Industrial, Food Processing, *Kluyveromyces marxianus*, Pectinase Activity, Polygalacturonase, Yeasts

Introduction

The production of enzymes has evolved rapidly and nowadays, enzymes are the most important products obtained for human needs through microbial sources. Enzymes have application in a variety of areas including food biotechnology, environment, animal feed, pharmaceutical, textile, paper and others technical and chemical industries. Due to the large industrial application and significant cost, there is a necessity to develop processes able to minimize the production costs. In this sense, the production of enzymes by a variety of agro-industrial wastes may be used as support material, carbon and nitrogen sources that would allow obtaining more economical fermentation processes avoiding the use of expensive chemical components in the media formulation (Mussatto *et al.*, 2012).

Pectinases comprise several enzymes that promote the natural degradation of pectins, which are high molecular

weight acid polysaccharides, present in plant cell walls, primarily made up of α -(1 \rightarrow 4) linked D-galacturonic acid residues with a small number of rhamnose residues in the main chain and arabinose, galactose and xylose in the side chain (Blanco *et al.*, 1999; Kashyap *et al.*, 2001; Pedrolli *et al.*, 2009). The presence of pectic substances causes problems in the elaboration of fruit juices, particularly during the clarification and filtration steps (Gummadi and Panda, 2003; Serrat *et al.*, 2011). Microbial pectinolytic enzymes are known to play a commercially important role in the current biotechnological area widely used in industry, with applications such as the extraction and clarification of fruit juices, wastewater treatment, tea and coffee fermentations, bleaching of paper, the production of fermentable sugars from plant biomass, oil and pigment extraction and processing of textile fibers (Favela-Torres *et al.*, 2006; Sharma *et al.*, 2012). They are also

used in formulations of in poultry feed additives, contributing to better nutrient assimilation. In the industrial production of fruit juices, it is necessary to eliminate the pectin released during fruit processing in order to reduce the time of filtration and enhance the production at the end of the process (da Silva *et al.*, 2005; Serrat *et al.*, 2011; Mehraj Pasha *et al.*, 2013).

Pectinase production used in industry has been reported from microorganisms including bacteria (Swain and Ray, 2010; Roosdiana *et al.*, 2013), actinomycetes (Kuhad *et al.*, 2004; Jacob and Prema, 2006), filamentous fungi in particular of the *Aspergillus niger* (Solis-Pereira *et al.*, 1993), *Penicillium pinophilum* (Ahmed and Mostafa, 2013), *Penicillium viridicatum* (Silva *et al.*, 2002), *Mucor circinelloides* (Thakur *et al.*, 2010) and some yeast (da Silva *et al.*, 2005; Oliveira *et al.*, 2006; Serrat *et al.*, 2011; Taskin, 2013). *Aspergillus niger* is known worldwide for production of secondary metabolites and extracellular enzymes of commercial value, including industrial production of pectinases. Yeasts have advantages compared to filamentous fungi with regard to the production of pectinases, because they are unicellular, the growth is relatively simple and the growth medium does not require an inducer. Yeasts have a great potential for the production of microbial enzymes for the food industry and they offer an alternative source of these enzymes (da Silva *et al.*, 2005; Martos *et al.*, 2013). Although some yeasts produced one or two types of pectinolytic enzymes, the production of pectinase is not so widespread in them. Very few yeasts show this ability namely those belonging to the genera *Saccharomyces*, *Kluyveromyces*, *Cryptococcus*, *Rhodotorola* and *Candida* (Schwan *et al.*, 1997; Miura *et al.*, 2001; Moyo *et al.*, 2003).

At present filamentous fungi, produce almost all the pectinolytic enzymes used for industrial applications. There are a few reports of pectinase production by yeast strains. Therefore, aim of the present study, was to select yeast strains, which are able to secrete PGase and characterize some of the properties of the enzymes produced by submerged fermentation (SmF) processes. In addition, enzyme productions on various agro-industrial residues, nutritional and environmental conditions required for the production of extracellular pectinase activity have been described.

Materials and Methods

Yeast Strains and Culture Conditions

The yeast strains (Table 1) obtained from the Biology Department of Ege University, was kept on Yeast extract-Peptone-Glucose (YPG) slants at 4°C and subcultured at 15-21 days intervals. Propagation medium for inocula was formulated as follows: Glucose 10, yeast extract 5, peptone 5, K₂HPO₄ 1, (NH₄)₂SO₄ 0.25, MgSO₄·7H₂O 0.25 g/L, trace element solution (Shirling and Göttlieb, 1966) 1 mL/L, pH 6.

Selection for Pectinolytic Activity (Plate Assays)

For plate assay, yeasts were spot inoculated in the solid medium including citrus pectin (1%) and incubated for 72 h at 28°C. After, iodine-potassium iodide solution or 6 M HCl was added to detect clearance halos around the colonies. Strains presenting large clearing zones were used for enzyme production assays on liquid medium. The strain, *Kluyveromyces marxianus* NRRL-Y-1109 with the best PGase production in the qualitative assay, was used for further experiments.

Selection of Suitable Culture Conditions for the Optimum Production of PGase

Various different cultural parameters such as pH, temperature, carbon and nitrogen sources and time incubation in hours were studied to monitor their effect on PGase production of the *K. marxianus* was optimized.

To determine, influence of initial pH value of culture medium on growth and enzyme production; the strain was cultivated in the medium with different initial pH values (3.5-7). The pH was adjusted using 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. The initial pH of culture medium achieved maximum enzyme production was used for subsequent study.

The optimum temperature for cell growth and enzyme yield was assayed by incubating the production medium at different temperatures varying from 20-45°C, maintaining all other conditions at optimum levels at original concentration.

For determinations of the influence of different carbon (glucose, glycerol, starch, fructose, galactose, xylan, carboxymethyl cellulose and sucrose) and nitrogen sources (yeast extract, peptone, tryptone, asparagine, alanine, potassium nitrate, soybean meal and urea) were added to the pre-optimized medium before sterilization at a concentration of 1% (wt./vol.) and 0.1% (wt./vol.), respectively. The carbon and nitrogen source supporting the maximum production of enzyme were selected for the further studies. The temperature and pH were set to 30°C and at 6, respectively.

During fermentation, up to 72 h at a regular interval of 12 h, cell free supernatant of fermentation broth was harvested by centrifugation at 11000 rpm for 10 min at 4°C and assayed for enzyme activity.

Determination of the Influence of Agro-Industrial Residues for PGase Activity

Wheat straw, beet molasses, wheat bran, grape waste, brewer's malt and corncob were ground to particles of 0.2-0.5 cm. For delignification, 100 g of substrate in 500 mL Erlenmeyer flasks were soaked in 1% NaOH and autoclaved at 121°C for 20 min. After the alkali treatment, the materials were washed with tap water until neutral and oven-dried. Treated substrates were passed through 0.5 mm screens (Pham *et al.*, 1998).

Table 1. List of the yeast strains and their pectinase activity in solid media

Yeast strains	Pectinase activity (mm) ^a
<i>Kluyveromyces marxianus</i> NRRL-Y-1109	16.3±1.53 ^a
<i>K. marxianus</i> TEM-4	13.3±0.58
<i>K. lactis</i> NRRL-Y-8279	0
<i>Pichia pastoris</i> NRRL-Y-7556	13.0±1.0
<i>P. ananola</i> NRRL-Y-366	0
<i>Candida rugosa</i> NRRL-Y-95	12.3±0.58
<i>C. zeylanoides</i> NRRL-Y-1774	5.3±0.58
<i>C. albicans</i> ATCC 10231	0
<i>C. sake</i> NRRL-Y-1622	0
<i>Saccharomyces cerevisiae</i> NRRL-Y-12632	11.7±0.58
<i>Galactomyces geotrichum</i> TEM-1	5.3±0.58
<i>Debaryomyces hansenii</i> TEM-24	4.3±0.58
<i>D. hansenii</i> TEM-16	0
<i>D. hansenii</i> TEM-25	0
<i>Torulasporea delbrückii</i> TEM-5	4.7±1.15
<i>T. delbrückii</i> TEM-6	0
<i>Yarrowia lipolytica</i> T-10	0
<i>Y. lipolytica</i> T-46	0
<i>Y. lipolytica</i> T-47	0
<i>Y. lipolytica</i> T-53	0

^aThe pectinase activity of the strains (mm): ≥15-Very good producers; 10-15-good producers; 5-10-weak producers; +, 4 > poor producers. ^a Data presented as the mean value of three determinations ± standard deviation, 0- negative, clear zones not in

Agro-industrial wastes as a carbon sources replaced with citrus pectin, by adding them separately at a final concentration of 1.5% (wt./vol.) in the optimized medium.

PGase Production and Fermentation

For PGase production, initially in a 100 mL Erlenmeyer flask containing 25 mL of propagation medium the strain was inoculated with a single loopful of a 48 h yeast culture from YPG plates for development of inoculums. The culture was then incubated at 30°C in an orbital shaker at 150 rpm for 12 h, after which the growing culture (1%, v/v) was transferred into a 250 mL Erlenmeyer flask containing 50 mL of propagation medium and incubated for an additional 10 h under the same conditions. This culture was used as the inoculum in subsequent experiments. SmF was carried using 250 mL Erlenmeyer flasks with 40 mL of optimized production medium in a rotary shaker (120 rpm) at 30°C. After 48 h the biomass was separated by centrifugation at 11000 rpm for 10 min at 4°C and the supernatant was used to evaluate PGase activity.

Assay of PGase Activity in Supernatants

Assay of PGase activity was determined by measuring the release of reducing groups using the modified dinitrosalicylic acid (DNS) method described by Miller (1959). Briefly, the reaction mixture containing 250 µL of 1% citric pectin in 250 µL citrate- phosphate, pH 6.0 buffer and 100 µL of cell free culture supernatant, was incubated at 30 °C for 5 min under static conditions. The reaction was stopped using the 3, 5-dinitrosalicylic acid reagent followed by keeping at 100°C for 5 min for development of color. After heating for 5 min in boiling water, the reaction

mixture was centrifuged (8000 rpm for 5 min) to separate out the insoluble pectinolytic materials formed during reaction. A control mixture deprived of pectin and another mixture deprived of cell free supernatant were assayed in parallel tests. The absorbance read at 540 nm using Varian Carry50 UV-visible Spectrophotometer. One Unit (U) of PGase activity was defined as the amount of enzyme required to liberate one µmol of galacturonic acid per minute under the assay conditions.

Effect of Temperature, pH and Salt Concentration on PGase Activity

The optimum temperature of PGase activity was determined by incubating the enzyme at different temperatures ranging from 30 to 70°C at pH 5.5 and the pH profile (3.5-8) at 45°C was evaluated in the presence of citrus pectin solubilized in appropriate buffers. Residual enzyme activity was measured by the standard assay. The effect of the presence of different concentrations of NaCl (1, 2, 5 and 10%) on PGase activity was also measured under optimized conditions. Thermal stability of the enzyme extract was determined by first incubating the enzyme at 50°C for intervals of 5, 25, 45, 50 and 60 min at pH 5.5 and the residual enzyme activity measured.

Results

Pectinolytic Activity of the Yeast Strains

Twenty yeast strains were evaluated for their potential to pectinase activity in solid medium containing citrus pectin. Based on the size of the clear zones, among the

strains, only nine were positive for pectinase activity (45%). Yeast strains were classified as very good producers of pectin depolymerizing enzymes when presented clear halos around colonies of at least 15 mm (*Kluyveromyces marxianus* NRRL-Y-1109), good producers when the halos were of at least 10 mm (*Pichia pastoris*, *Candida rugosa*, *Saccharomyces cerevisiae* NRRL-Y-12632 and *Kluyveromyces marxianus* TEM-4), weak producers when halos were at least 5 mm (4 strains), poor producers when no pectinolytic activity and no clear lysis zones were observed (11 strains) (Table 1). The yeast *K. marxianus* NRRL-Y-1109 was found excrete high level of extracellular pectinolytic activity on pectin agar and was selected for further studies.

Optimization of the Suitable Culture Conditions for the PGase Production

Preliminary fermentation studies were conducted in medium containing pectin as the primary carbon source to production of pectinase of yeast strain at pH 6 and temperature at 28°C under shaking conditions (150 rpm). Maximum pectinase values of *K. marxianus* were ranging from 0.4 to 3.6 U/mL, obtained at 72 h incubation (Data not shown). Various different cultural parameters were tested such as pH (3.5-7), temperature (20-45°C), commercial carbon and nitrogen sources and time incubation in hours (12-72) were studied to monitor their effect on optimum PGase production of *K. marxianus*. The optimum pectinase activity was achieved at pH 6, 30°C and shaking conditions at 120 rpm after 48 h of incubation (Table 2). PGase production of *K. marxianus* in liquid medium has been shown to be influenced by the type and source of carbon and nitrogen. The highest (4.2 U/mL) of PGase was produced on citrus pectin (1% wt./vol.) as a pectinase inducer, was the best source followed by glucose (2 U/mL), galactose (1.8 U/mL) and starch (1.6 U/mL), (Table 3). On the other hand, amongst various nitrogen sources tested for PGase activity, best nitrogen sources

were found yeast extract and peptone (0.1%, 1:1) (4.8 U/mL) supplemented to production medium under optimized physiological conditions (Table 3). Yeast extract (3.8 U/mL) and soybean meal (3.6 U/mL) alone gave comparable activity. When the *K. marxianus* was grown on urea, as a sole nitrogen source, pectinase activity was detected in the supernatant very low (0.4 U/mL). As seen from Table 3 nitrogen free production medium decreased the PGase activity (1.6 U/mL).

Influence of the Agro-Industrial Residues for PGase Activity

The influence of different agro-industrial residues on extracellular PGase production was investigated. In these experiments, substrat dosage has been chosen according to the literature reported value and further optimization will have to be carried out. Enzyme activities were measured after 48 h of incubation at 30°C at pH 6. The highest of PGase was produced on citrus pectin (4.8 U/mL) following treated wheat bran (2.2 U/mL) and treated grape waste (1.8 U/mL) whereas much lower levels of activities were produced with wheat straw and corncob (0.6 U/mL) (Table 4).

Effect of Temperature, pH and Salt Concentration on PGase Activity

The optimum reaction pH, temperature and NaCl concentrations were investigated with crude culture supernatant. The optimum pH and temperature for the enzymatic activity were 5.5 and 40-45°C, respectively. When the effect of NaCl concentrations was studied, optimum PGase activity observed at 1% NaCl and was not stable up to 5 and 10% NaCl. The extracellular pectinase of *K. marxianus* was relatively thermostable and the long-term temperature stability of crude PGase at pH 5.5 was stable at temperatures up to 45°C even after 50 min as it retained over 100% for activity (Table 5).

Table 2. Effect of different pH and different temperature on production of PGase from *K. marxianus*

		PGase activity (U/mL) in different fermentation time (h)					
		12	24	36	48	60	72
pH	3.5	0 ^a	0.0	0.0	0.0	0.0	0.0
	4.0	0	0.0	0.4	0.4	0.0	0.0
	5.0	0	0.6	1.6	2.2	1.8	1.6
	5.5	0	0.6	2.4	3.8	3.4	2.8
	6.0	0	0.6	3.8	4.4	4.4	3.6
	7.0	0	0.0	0.4	1.1	0.6	0.4
Temperature (°C)	20	0	0.0	0.8	1.4	1.2	1.0
	25	0	0.4	2.2	2.8	2.4	2.0
	30	0	0.8	3.6	4.6	4.2	3.6
	35	0	0.4	0.8	1.2	1.0	0.6
	40	0	0.0	0.0	0.0	0.0	0.0
	45	0	0.0	0.0	0.0	0.0	0.0

^aIn production medium, carbon and nitrogen sources were citrus pectin (1%) and yeast extract (0.1%), respectively. Mean values, n = 3, enzyme activity was expressed as the U/mL±0.1

Table 3. Effect of different commercial carbon and nitrogen sources on production of PGase by *K. marxianus*

		PGase activity (U/mL) in different fermentation time (h)					
		12	24	36	48	60	72
Carbon sources (1%)	Glucose	0 ^a	0.6	1.8	2.0	1.4	0.8
	Starch	0	0.0	1.4	1.6	1.2	0.6
	Glycerol	0	0.0	0.0	0.0	0.0	0.0
	Fructose	0	0.0	0.0	0.4	0.0	0.0
	Sucrose	0	0.0	0.6	0.8	0.6	0.0
	Xylan	0	0.0	0.0	0.0	0.0	0.0
	CMC	0	0.0	0.0	1.2	0.0	0.0
	Galactose	0	0.4	0.8	1.8	1.6	1.2
	Control (citrus pectin)	0	1.2	1.8	4.2	3.7	2.6
Nitrogen sources (0.1%)	Yeast extract	0	0.8	3.0	3.8	3.4	3.0
	Peptone	0	0.4	2.2	2.6	2.4	2.0
	Tryptone	0	0.0	1.6	1.8	1.4	1.2
	Asparagine	0	0.0	0.8	1.8	1.4	1.4
	Alanine	0	0.0	0.4	1.2	0.8	0.0
	Potassium nitrate	0	0.0	1.8	2.4	2.0	1.6
	Soybean meal	0	0.6	2.8	3.6	3.4	3.2
	Urea	0	0.0	0.4	0.4	0.0	0.0
	Yeast Extract + Peptone (1:1)	0	0.8	3.4	4.8	4.0	3.6
Control (nitrogen free)	0	0.0	0.8	1.6	1.4	1.2	

^aIn production medium, pH and temperature were 6 and 30°C, respectively. Mean values, n = 3, enzyme activity was expressed as the U/mL ± 0.1

Table 4. Effect of different agro-industrial residues on production of PGase by *K. marxianus*

		PGase activity (U/mL) in different fermentation time (h)					
		12	24	36	48	60	72
Agro-industrial residues (1.5%)							
Wheat straw		0 ^a	0	0.8	0.6	0.4	0.0
Grape waste		0	0.6	1.2	1.8	1.4	1.0
Brewer's malt		0	0.4	0.8	1.0	0.6	0.6
Beet molasses		0	0.4	0.6	1.4	0.8	0.6
Wheat bran		0	0.8	1.8	2.2	2.0	1.6
Corn cob		0	0.0	0.4	0.6	0.6	0.0
Control (citrus pectin)		0	1.2	1.6	4.4	3.2	2.6

^aIn production medium; pH, temperature and nitrogen source were 6, 30°C and yeast extract (0.1%), respectively. Mean values, n = 3, enzyme activity was expressed as the U/mL ± 0.1

Table 5. Influence of the pH, temperature and salt concentration on the activity of pectinase and its thermal stability profile

pH	3.5	4.00	5.00	5.50	6.0	7.0	8.0
Residual enzyme activity (%)	0	27.00	82.00	100.00	91.0	59.0	0.0
Temperature (°C)	30	35.00	40.00	45.00	50.0	60.0	70.0
Residual enzyme activity	77	86.00	100.00	100.00	95.0	36.0	0.0
NaCl concentration (%)	0	1.00	2.00	5.00	10.0		
Residual enzyme activity	100	105.00	86.00	54.50	27.0		
Temperature stability	0	5.00	25.00	45.00	50.0	60.0	
Residual enzyme activity	100	100.00	100.00	100.00	100.0	59.0	

^aMean values, n = 3

Discussion

It has been reported that strains belonging to the yeast species, *K. marxianus* have been isolated from a great variety of habitats, which results in a high metabolic diversity and a substantial degree of intraspecific polymorphism. This yeast is most important in different biotechnological applications: Production of enzymes (β -galactosidase, β -glucosidase, inulinase and polygalacturonases, among others), of single-cell protein, of

aroma compounds and of ethanol (including high-temperature and simultaneous saccharification-fermentation processes); reduction of lactose content in food products; production of bioingredients from cheese-whey; bioremediation; as an anticholesterolemic agent; and as a host for heterologous protein production have been investigated (Serrat *et al.*, 2004; Fonseca *et al.*, 2008).

Twenty yeast strains subjected to the preliminary screening in solid medium, only nine of them were positive for pectinase activity. *K. marxianus* selected

further screening to verify its consistent enzymatic activity. Schwan *et al.* (1997) isolated 12 yeast strains from cocoa fermentations, among them only four showed extracellular pectinase activity. They also reported *K. marxianus* was the most pectinolytic activity.

Results of the primary fermentation studies suggested that the maximum pectinase values of *K. marxianus* were ranging from 0.4 to 3.6 U/mL in medium containing pectin as the sole carbon source at pH 6 and temperature at 28°C under shaking conditions (150 rpm) obtained at 72 h incubation. On the other hand, in the optimized culture conditions: Optimum pectinase activity was achieved at pH 6, 30°C and shaking conditions at 120 rpm after 48 h of incubation (Table 2). The pectinase secreted by *K. wickerhamii* has optimum conditions were as follows: pH 5.0, Temperature, 32°C and an incubation period of 91 h (Moyo *et al.*, 2003). Earlier, the optimal conditions for PGase activity (9.8 U/mL) reported from *K. marxianus* were pH, 4.6 and temperature, 31°C when beet juice was the used as a substrate (Serrat *et al.*, 2011). In our study, the highest (4.2 U/mL) of PGase was produced on citrus pectin, was the best carbon source followed by glucose (2 U/mL), galactose (1.8 U/mL) and starch (1.6 U/mL). The wild-type yeast *Wickerhamomyces anomalus* (*Pichia anomala*) was able to produce PGase in liquid medium containing glucose and citrus pectin (51 U/mL) (Martos and Zubreski, 2013). PGase from *Aspergillus niger* in SmF, was strongly decreased when glucose or sucrose (3%) was added to culture medium containing pectin (Solis-Pereira *et al.*, 1993). PGase activity from culture broths which including the different nitrogen sources in a basal medium were determined for testing maximum enzyme production, best nitrogen sources were found yeast extract and peptone (0.1%, 1:1) (4.8 U/mL) under optimized physiological conditions (Table 3). Swain and Ray (2010) indicated the optimum temperature, pH and incubation period for exo-PGase production (82-83.2 U/mL) from *Bacillus subtilis* were 50°C, 7 and 36 h, respectively. However, in their study, culture medium with peptone (1%) was found best nitrogen source for enzyme production. It has been reported that maximum an extracellular PGase activity of *Mucor circinelloides*, was obtained in 48 h at 30°C and pH 4 with pectin methyl ester (1% wt./vol.) as carbon source and a combination of casein hydrolysate (0.1% wt./vol.) and yeast extract (0.1% wt./vol.) as nitrogen source (Thakur *et al.*, 2010).

It has been reported that wheat bran was proved the best substrate for *Streptomyces lydicus* (Jacob and Prema, 2006), *Penicillium viridicatum* (Silva *et al.*, 2002), *Moniliella* sp. and *Penicillium* sp. (Martin *et al.*, 2004) for the maximal activity value of PGase in submerged fermentation. Similarly, in this study the

highest of PGase was achieved with treated wheat bran (2.2 U/mL) and treated grape waste (1.8 U/mL) as an agro-industrial substrate. It should be noted that the differences and lower PGase production by yeast strains grown on agro-industrial end products might be attributed to less accessible sites in the substrate for enzyme attack due to the presence of pectin.

Effect of pH, temperature and NaCl concentration on PGase activity of *K. marxianus* were 5.5, 40-45°C and 1%, respectively. The optimum pH and temperature are also consistent with values that have been found for *K. marxianus* pectinase production of 5 and 40°C respectively (Schwan *et al.*, 1997). Our results are in agreement with those reported by other authors (Masoud and Jespersen, 2006; Martos *et al.*, 2013) who indicated the most yeasts secreted typical of pectinolytic enzymes.

Based on the results of these experiments, the yeast strain *K. marxianus* produced PGase possessing different activity of pH, temperature, NaCl concentration and thermal stability. The enzyme solution of yeast in this study has specific properties that may be applied directly to fruit juice industries without the pH modification. Nevertheless, because of the temperature stability of the enzyme offer advantages at processing temperature of 40-50°C, this is sufficient for industrial process, although enzyme preparation could be improved.

Acknowledgement

This study was partly supported by the Scientific and Technological Research Council of Celal Bayar University (Project No: 2015-093).

Author's Contributions

Mustafa Oskay: Designed the research plan and organized the study, participated in all experiments, coordinated the data analysis and has drafted the article.

Hüsnüye Tansel Yalçın: Isolated and identified some of the yeast strains, performed the first fermentation studies, participated in data analysis and contribute in writing of the manuscript.

Ethics

Both authors declare that there is no conflict of interests regarding the publication of this paper.

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