ACTA ABP BIOCHIMICA POLONICA

Vol. 62, No 4/2015
651–657
http://dx.doi.org/10.18388/abp.2015_1081

Regular paper

Enhanced production of polygalacturonase in solid-state fermentation: selection of the process conditions, isolation and partial characterization of the enzyme*

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Polygalacturonase (PG) production by *Penicillium chrysogenum* during solid-state fermentation was accompanied by decomposition of orange peels. A leaching procedure was developed through the selection of solvent, time and intensity of stirring. A maximum PG activity was observed after 48 h peel inoculation. Further cultivation decreased the enzyme activity significantly, up to 60% of the maximum PG activity. During fermentation, a rapid acidification of the solid medium which inhibited the pectinolytic enzyme, was observed. Buffering agents with different pH values and different ionic strengths were examined to identify the most suitable medium to avoid this problem. Buffer addition counteracted acidification and enhanced active protein production, which was observed for all of the applied pH values (6.5–8.0) of the buffering agent. The most satisfactory results were obtained when using the highest pH at 8.0. The protein content and PG activity increased from 3.5 mg/g and 1.09 U/g to 7.7 mg/g and 7.11 U/g during cultivation, with uncontrolled and pH-controlled medium, respectively. Measurements at wide pH and temperature ranges indicated an optimum for PG activity at pH 5.0 and 43°C; however, high thermal stability corresponded to lower temperatures, and a temperature of 37°C is thus recommended. Under these conditions, the operational stability was determined to be $t_{1/2}$=570 h.

Key words: polygalacturonase; solid-state fermentation; constant pH maintaining; moisture content; phosphorus source; *Penicillium chrysogenum*

Received: 11 June, 2015; revised: 27 August, 2015; accepted: 14 October, 2015; available on-line: 26 October, 2015

INTRODUCTION

Polygalacturonases (PGs) are naturally occurring enzymes that are produced by various organisms, such as plants (Bird *et al*., 1988, Hadfield *et al*., 1998), bacteria (Jayani *et al*., 2010, Tariq & Latf, 2012) and fungi (Martin *et al*.2004, Anuradha *et al*., 2014). These proteins belong to a large group of pectinases, which synergistically mediate the complete decomposition of pectin substances that are abundantly present in plant tissues, primarily in fruit. Polygalacturonases are pectin-degrading proteins that catalyze hydrolysis of α-1,4-glycosidic linkages in pectate or other galacturonans at the end (EC 3.2.1.67) or randomly in the middle (EC 3.2.1.15) of polymeric chains.

PGs function at different optimal pH values, which are typically acidic or rarely basic, and determine the potential application of these enzymes (Alkorta *et al*., 1997, Gummadi & Panda, 2003). In particular, PGs are used in the beverage industry for fruit and vegetable processing, but they have other biotechnological applications as well (Jayani *et al*., 2005). The importance of these enzymes is reflected in their annual sales which were once accounted at $75 million (Kashyap *et al*., 2001).

PGs are typically derived from fungal (mould) isolates and are produced through fermentation. Basically, depending on the substrate solubility in water, two types of fermentation processes are used. Submerged fermentation (SmF) utilises water-soluble substrate and can be accomplished through either bacteria or fungi, whereas fermentation on a water non-soluble nutrient material (SSF – Solid State Fermentation) is typically accomplished by fungi. The latter system reproduces natural microbiological processes, such as composting and ensiling, and imitates the natural growth of moulds (Nigam & Pandey, 2009). This process occurs on moistened surfaces, where microorganisms gradually colonize the substrate area until the organic substances and water are eventually depleted. Therefore, solid substrate fermentation is a batch process in nature. To some extent, SSF presents more advantages over the SmF technique, primarily through the utilization of low-cost, abundant plant biomass, a higher volumetric productivity, the low demands of aseptic conditions and many other crucial aspects, which have been previously described in detail (Szewczyk, 1993, Hölker *et al*., 2004, Singhania *et al*., 2009).

Nonetheless, there are some limitations concerning SSF. The main disadvantages are the heterogeneous fermentation conditions that result from various substrate compositions or mycelium damage through mechanical mixing and the low amenability of the process to preserving the temperature, moisture content and pH. The control of the last parameter is extremely difficult in solid state fermentation (Mitchell *et al*., 2000) because this regulation is critical for certain SSF processes.

The first aim of the present study was to enhance polygalacturonase production in the solid-state fermentation system through the moisture content and pH control of the *Penicillium chrysogenum* (*notatum*) growth conditions. The supply of phosphorus source was also considered as a crucial factor for increased enzyme production. Furthermore, the selection of the extraction parameters for efficient protein isolation from decaying orange peels was performed. The second aim of the present study was to determine the optimal thermal and pH conditions

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*The results were presented at the 6th International Weigl Conference on Microbiology, Gdańsk, Poland (8–10 July, 2015).

and operational stability for polygalacturonase activity in crude enzyme extract.

MATERIALS AND METHODS

Microorganism and inoculum preparation. A wild type of *Penicillium chrysogenum* (*notatum*) isolated from decaying orange peel was used for this study. Identification of the mould on selective media and microscopic examination (morphology and microscopic features) were done at the Division of Phytopathology and Mycology at the Wroclaw University of Environmental and Life Sciences, Poland. The strain was maintained on potato dextrose agar at 4°C and periodically transferred.

For experiments, the spores were transferred on Petri dishes with Czapek-dox agar containing 0.1% citrus pectin (Sigma) and kept at ambient temperature up to visible growth and sporulation. The inoculum was prepared by harvesting the spores from 12 to 14 day culture with 20 ml of 0.1% Tween80® solution (1×10^8 spores/ml).

Substrate and culture conditions. Fresh, ripe oranges (*Citrus sinensis* (L.) Osbeck) were purchased from a local market. The fruits were carefully washed and peeled. The peels were cut into small cubes with an average edge of 5 mm. A total of 15 g of substrate was transferred to 250 ml conical flasks that were closed with cotton plugs and autoclaved at 121°C, 1.5 atm for 20 min. After cooling, the peels were supplemented with a sterile solution of inorganic salts, according to Silva *et al.* (2007). For that purpose, the mixture of $MgSO_4$ and $(NH_4)_2SO_4$ with final concentration of 10 mg/ml of each salt in water was prepared. The volume of 5 ml was added to solid substrate and left for soaking for two hours at 24°C. Subsequently, the prepared orange peels were inoculated with 1.0 ml of the *P. chrysogenum* spore solution. The peels were cultivated for 5 days at 24°C, and the moisture content, corresponding to 0.997 of water activity (a_w), was determined. Polygalacturonase production was examined in 24-hour intervals based on the protein content and polygalacturonase activity. In pH-controlled media, McIlvaine buffer supplementation was applied with varying pH values, ranging from 6.5 and 8.0, in 2.5 or 5.0 ml. Buffering agents were incorporated into fermentation media on the day of the highest PG production (2nd day) under uncontrolled pH conditions. Thereafter, the controlled pH experiments were conducted for another three days and the protein content and PG activity were assayed. All batch cultivations were performed in duplicate.

Product isolation. To obtain detectable amounts of the active protein, the enzyme was isolated through fermented matter leaching, followed by extract filtration and low-molecular compound diafiltration.

A total of 90 ml of distilled water or McIlvaine buffer (pH 5.0), corresponding to 15 grams of orange peels, was used as solvents. Further experiments were performed at 50–230 rpm for 4–120 min. The extracts were separated through a sterile cotton filter and centrifuged (horizontal centrifuge Hettich Universal 320R Centrifuge) at 4°C, 9000 rpm for 30 min. The supernatant was dialysed through Membra-Cel MD44-14×100 CLR with a 14-kDa cut-off against McIlvaine buffer at pH 5.0 for 12 hours. The buffer was changed at least twice. The purified extract was centrifuged at 4°C, at 9000 rpm for 30 min and analysed for the total protein concentration and polygalacturonase activity. The measurements were performed in duplicate.

Polygalacturonase characterization. The PG activity was measured in McIlvaine buffer for 15 and 30 min. Thermal and pH enzyme optima were determined for temperatures ranging from 4 to 57°C at pH 5.0 and for pH values ranging from 2.5–8.0 at 37°C, respectively. Similar ranges of temperatures and pH were applied to quantify the PG stability, and the activity was measured after preincubation for 60 min at 37°C. Furthermore, the inactivation test was conducted at the indicated pH and temperature. The experiments were performed in triplicate.

Analytical procedures. Protein content was determined using Lowry's method (Lowry *et al.*, 1951) and a standard curve was performed for albumin serum bovine, $Abs(750) = 2.273 \cdot C$ (mg/ml). The protein amount was expressed as mg of protein obtained from 1g of orange peels ($mg/g_{orange\ peels}$).

The standard assay for the polygalacturonase activity was tested *vs.* 8 mg/ml D-polygalacturonic acid (Sigma-Aldrich) as a substrate, in McIlvaine buffer at 37°C and pH 5.0 for 15 and 30 min. Reducing sugars released during the reaction were detected by the 3,5-dinitrosalicylic acid procedure (Miller, 1959) and established according to a standard curve for D-galacturonic acid, $Abs(550)=0.634 \cdot C$ (mg/ml)–0.0654. One unit (U) of polygalacturonase activity was defined as the amount of reducing group equivalent (mg) released in 1 min under the reaction conditions. The PG activity was expressed as a unit of total activity of the enzyme per 1 g of fresh substrate, $U/g_{orange\ peels}$. All analytical methods were performed in triplicate.

The pH measurements of the cultivated medium were monitored with 24 hours intervals for 5 days. Fermenting biomass was stirred with 90 ml of deionized water for 30 minutes with intensity of 180 rpm. An extract was separated from medium by filtration and centrifuged for 20 min, 9000 rpm at 4°C, followed by pH measurements of the filtrate using a Crison Basic 20 pH meter and a Crison 52 09 pH electrode at room temperature.

RESULTS AND DISCUSSION

In biotechnology, fermentation on solid substrate (SSF) is one of the microbial cultivation techniques for the production of various enzymes and secondary metabolites. The advantages of the process are plant material utilization with simultaneously high production of the desired product or group of products.

In the present study we used orange peels as a cultivation medium. Large quantities of citrus peels remain from the production of juices, purees and jams. According to Marin *et al.* (2007), the peels of ripe orange fruits primarily are comprised in 37.08% of cellulose, 23.02% pectin, 11.04% hemicellulose, 9.75% free sugars and 9.06% protein, based on dry weight. Therefore, this type of solid substrate might serve as a good nutrient medium in which pectin substances act as inducers of pectinolytic enzyme production. The results of a previous study (Zaslona *et al.*, 2015) showed that the fresh orange peels include relatively high amounts of water bound to plant matrices. The average moisture content of the material is 66.0% (*w/w*), corresponding to water activity at 0.973. Furthermore, we observed that using peels with 72.0% moisture (a_w=0.997) supplemented with inorganic salts, i.e., $(NH_4)_2SO_4$ and $MgSO_4$, resulted in the improved production of polygalacturonase compared with enzymes obtained solely from the fresh peels. Nevertheless, PG production decreased after the 2nd day of cultivation.

Table 1. Protein concentration and PG activities of the pH uncontrolled and controlled cultivation media.
[a]pH S.D. ±0.1–0.2; S.D., standard deviation

Annotation	Cultivation conditions	Protein content $mg/g_{orange\ peels}$	S.D.	Enzymatic activity $U/g_{orange\ peels}$	S.D.	pH[a] on 2nd day	pH[(l)] on 5th day
K1	Uncontrolled pH and moisture	3.53	0.04	1.09	0.01	3.2	3.0
K2	Uncontrolled pH, 5 ml water	4.16	0.03	3.37	0.03	3.2	3.3
A	pH 6.5; 5 ml buffer	4.48	0.18	3.40	0.14	3.5	3.4
B	pH 7.5; 5 ml buffer	4.47	0.19	4.32	0.05	4.5	4.4
C	pH 8.0; 5 ml buffer	7.67	0.10	7.11	0.02	5.6	4.7
D	pH 7.5; 2.5 ml buffer	3.89	0.14	3.51	0.04	3.6	3.9
E	pH 8.0; 2.5 ml buffer	4.48	0.09	3.50	0.02	4.1	4.0

Previous studies have shown remarkable changes in the pH of the substrates as result of fungal fermentation (Raimbault, 1998). These changes reflect the production of organic acids due to incomplete substrate oxidation and the consumption of ammonium ions, which cause pH to decline. Raimbault and Alazard (1980) reported that the pH of fermenting cassava meal could be stabilized after adding ammonium salts and urea to the solid substrate at the beginning of the process. Similarly, Saucedo-Castaneda *et al.* (1992) investigated the use of urea as a sole nitrogen source in a medium. The urea addition increased the initial pH, thereby facilitating the maximum starch substrate utilization by *Schwanniomyces castellii*. Nagel *et al.* (1999) also conducted studies on pH control, investigating the effect of initial medium supplementation on the maximum specific growth rate of *Rhizopus oligosporus*. These authors used different nitrogen sources and buffering components and observed that the use of different buffering agents did not affect microbial growth. In some fungal fermentation processes, an increase in the pH of the medium was detected during A*cremonium chrysogenum* cephalosporin C production (Cuadra *et al.*, 2007). A buffered medium with lower initial fermentation pH values maintained this parameter at suitable level for cephalosporin production.

In the present study, pH control and "a real time control" were conducted, which represents a different approach from the methods described above, where the reaction medium was adjusted at the beginning of the SSF process. The results of pH change on the 2nd day of fermentation, after adding concentrated McIlvaine buffer (0.2:0.4 M citrate-phosphate components ratio), expressed as the total protein production and PG activity, are presented in Table 1.

The reference process, K1, corresponds to cultivation at an uncontrolled pH and a moisture content for which the highest PG activity of 1.09 $U/g_{orange\ peels}$ was detected on the 2nd day of fermentation. On the 5th day of fermentation, the enzyme activity decreased to 60% of the maximum value (data not shown). The pH measurements of the extracts showed that the initial pH of the fresh orange peels varied from 4.0 to 4.4, and during the next two days of the cultivation, this value declined to a pH of approximately 3.2. This intermediate acidification was maintained until the end of the process.

As presented in Table 1 (A–E), two variables changed during SSF: pH (6.5–8.0) and the buffer volume incorporated in the fermented biomass (2.5 or 5.0 ml). To distinguish the effect of increasing moisture content in cultivation medium from buffering agent action, additional control experiment, K2, was performed. In the K2 experiment, 5 ml of sterile water was added on the 2nd day of cultivation, whereas the results were determined on the 5th day of cultivation. The enzyme production was not reduced at the end of cultivation for K2 and A-E experiments, as observed for the reference process (K1). In general, increased moisture of the orange peels had a positive effect on mould growth and product formation. Although the amount of total protein did not differ significantly, 4.16 and 3.53 mg/g for respective K2 and K1, the protein extract exhibited 3 times higher PG activity, 3.37 U/g, in comparison to 1.09 U/g (Table 1). Further comparison the results for K2, A-C experiments, characterised with the same amount of applied water volume, showed the influence of increasing reaction medium on polygalacturonase production. The highest PG activity was detected in the C process (pH 8.0, 5 ml). 5 ml of this buffer included approximately 5.30 mg of citric acid (0.2 M) and 276 mg of disodium hydrogen phosphate (V) (0.4 M) which corresponds to 0.07 and 3.7 g/l per g of orange peels, respectively. Such proportion and composition could serve not only as a buffering agent but also as a phosphorus source. Both roles could be noticed when analysing the pH of the fermenting peels in A, B and C experiments. For A and B, the reaction media varied by one unit but remained almost unchanged between 2nd and 5th day (Table 1). Furthermore, the amount of proteins was similar, while the PG activity increased by approximately one U/g in the higher reaction medium. Thus, these phenomena indicated the buffering role of the mixture as well as proved that protein was more stable at pH>4. The pH of the reaction medium in the C experiment dropped during the cultivation unexpectedly. The pH decreased significantly from 5.6 to 4.7 with simultaneously the highest PG production. This effect suggested that an excess of phosphate salts was utilised as a phosphorous source for intense microbial growth and polygalacturonase production. The extensive studies on phosphorus metabolism in moulds were performed and described by Mann (1944). He found that an increase in the content of phosphate in the culture medium with *Aspergillus niger* enhanced both the utilization of carbohydrate and the formation of organic acids. Mycelia grown in the presence of high phosphate concentrations had a much higher nitrogen metabolism in comparison with those that grew on phosphate free media and were capable of forming a considerable reserve of various valuable nitrogenous substances. Similar results were obtained by Liang *et al.* (2012). The authors investigated, the influence of phosphorus concentration (KH_2PO_4, 0–4 g/l) on *Phanerochaete chrysosporium* (white-rot fungi) growth and manganese peroxidise production in liquid medium. They observed enhanced fungal growth and enzyme production in the presence

of a relatively high concentration (2 g/l) of phosphate in the medium in comparison to phosphorus free media. Above results clearly indicate that the applied procedure contributed to three synergistic effects. The first effect was associated with maintaining the reaction in the fermentation system, confirmed through the pH measurements at the end of the process. In A–B and D–E processes, the pH of the medium was similar during fungal growth compared with the approximately one unit decline observed in the C process. This high difference between C and the remaining processes might explain the significant production of active protein as being a result of an excess of phosphorus presented in McIlvaine buffer. The addition of 5 ml of buffer at pH 8.0 resulted in pH values of 5.6 and 4.7 on the 2nd and 5th days, respectively. These values correspond to the pH range of polygalacturonase stability in McIlvaine buffer (see section "PG characterization"). The nutrient substances available for mycelium development over the substrate increased with increasing amounts of active enzyme in the moistened solid substrate additionally enriched with phosphorus source, thereby inducing *Penicillium chrysogenum* growth and polygalacturonase production. The third effect is associated with the medium moisture. Some amount of water was utilized during metabolic processes, such as fungal growth, polygalacturonase production, etc. and this water was replenished through buffer addition. Thus, increased water content could improve the physicochemical properties of solids, such as the solubility of the nutrient components, the distribution of mycelium/spores and the nutrient substances formed as a result of enzymatic activity in the fermenting medium. The increased moisture could also protect the mycelium from drying. This aspect is well observed for K1 and K2 processes because the pH value did not change the medium acidity but visibly improved PG production. However, enhanced active protein production was clearly observed for all buffers applied, and the most significant pH and moisture effects on PG production were observed for the C process.

Isolation of PG

In SSF, polygalacturonase is secreted into the orange peels from *P. chrysogenum* mycelium. Therefore, the most effective method for isolating the desired product is leaching (Rostagno & Prado, 2013). In the present study, we optimized the isolation process using C type cultivation (Table 1).

Previous studies have shown that different types of media have been successfully applied for the isolation of different enzymes. Among these media, the most commonly applied solvents were water, buffers or surfactants (Diaz *et al.*, 2007, Abbasi *et al.*, 2011, Galbas *et al.*, 2013). In the present study two extractants, water and McIlvaine buffer at pH 5.0, were examined (90 ml against 15 g of orange peels). The influence of the solvent on the protein isolation was observed based on PG activity. The total protein content in the extracts was comparable in both cases: 2.01 and 1.98 mg/g_{peels} for water and buffer, respectively. In water, the PG activity was almost half of that in the buffer: 2.76 *vs.* 4.90 U/g. This result likely reflects the stabilization of the enzyme structure at an appropriate ionic strength. Ahmed and Mostafa (2012) reported similar results for polygalacturonase isolated from a mixture of orange bagasse and molokihia stalk through SSF using *Penicillium pinophlilum*.

The effect of leaching time and the intensity of stirring on PG isolation was also verified. The level of protein isolation, including proteins with polygalacturonase activity, increased with increasing rotation speed (Fig. 1).

A significant difference in the PG activity in extracts obtained after 4, 10 and 30 min was observed. After 60 minute, the protein content increased, but only slight changes in the PG activity were observed. Additional experiments conducted at 230 rpm, higher buffer volumes of 180 ml or extraction times of 120 min resulted in comparable product content and polygalacturonase activity as obtained at 60 min, 230 rpm and 90 ml (data not shown). Therefore, the following parameters were used for leaching: 60 min, 230 rpm, and 6 ml of medium per 1 gram of fresh substrate.

The dialysis of the crude extract resulted in the purification of monosaccharides, low molecular proteins and peptides (cut-off 14 kDa). The monosaccharide concentration (estimated using the DNS test) in the extracts depended on the day of cultivation. The highest content was observed at the beginning of the process: 33.7 mg per 1 g of fresh orange peels. At the end of the process, this value declined to 2.3 mg/g (Zasłona *et al.*, 2015). The high concentration of the monosaccharides in the extracts could affect the reliable analysis of the activity measurements through the inhibition of the enzyme and

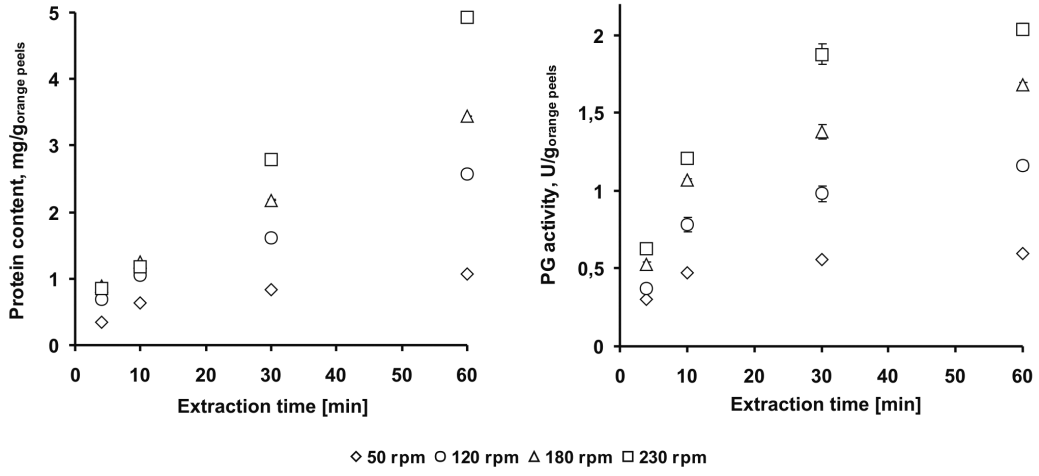

Figure 1. Effect of extraction time and intensity of rotation (series at 50, 120, 180 and 230 rpm) on extraction yield expressed in total protein content (left diagram) and polygalacturonase (PG) activity (right diagram).
Processes were performed at room temperature in McIlvaine buffer at pH 5.0.

the inaccuracy in product detection during the enzymatic reaction. Therefore, this purification step was necessary. The average purification from sugars was 83 to 89%.

PG characterization

Many fungal polygalacturonases are acidic enzymes (Jayani *et al.*, 2005). Nevertheless, the temperature and pH optima activity of the PG obtained through SSF differ among *Penicillium* sp. The optimal temperature and pH of polygalacturonase activity produced by *P. solitum* during fermentation in apple fruit was between 20–37°C and pH 4.0–5.0 (Jurick *et al.*, 2009). These authors also reported that the PG activity of *P. expansum* is consistent with these values (Jurick *et al.*, 2009). Chellegatti *et al.* (2002) reported that polygalacturonase produced in submerged fermentation through *Penicillium frequentas* presents optimal activity at pH 3.9, which is comparable to that of the enzymes produced in SSF. However, some *Penicillium* species produce protein with biochemical properties higher than the typical values. For example, *P. citrinium* cultivated on sugar beet pulp presents PG activity at pH 6.0 and 40°C (El-Batal *et al.*, 2013). Similarly, the exo-PG obtained from *P. viridicatum* RFC3 cultivated on wheat bran and orange bagasse exhibits optimal activity at pH 6.0, with a significantly higher temperature optima at 60°C (Silva *et al.*, 2007). Such divergent pH and temperature optima observed for PG from various *Penicillium* sp. suggest that these parameters should be determined respectively for each protein. A characterization of the polygalacturonase from *P. chrysogenum* has not been previously described in detail, and the data presented below are different from those obtained in the present study. Polygalacturonase synthesized in submerged fermentation in defined medium containing citrus pectins (Banu *et al.*, 2010) had exhibited optimal activity at 35°C and pH 6.5. Amin *et al.* (2013) calculated the optimal PG production on wheat bran after 2.18 days at 33°C using ammonium chloride as the nitrogen source. This prediction was verified in experiments where the maximum PG production was achieved on the 3rd day of cultivation on wheat bran at 30°C and pH 1.318 using ammonium chloride as the nitrogen source. In the present study, the polygalacturonase from *P. chrysogenum* showed considerable activity at pH values ranging from 4.0–6.0 (Fig. 2).

The highest enzyme activity was maintained over 95% of the maximal value at a pH range of 4.5–5.5 with visible maximum at pH 5.0. The enzyme was stable at a broader range, preserving 57 and 45% of the initial activity at pH 3.0 and 7.0, respectively. Relatively high PG stability with over 85% of the initial activity was maintained at pH values between 4.0 and 6.0, with activity similar to the maximum activity detected at pH 5.0.

The measurements of thermal activity and stability are shown in Fig. 3.

The activity of the polygalacturonase extract slowly but steadily increased when the temperature was increased from 4 to 43°C. The activity rapidly declined above optimal value, preserving 30% of the relative activity at 50°C. At 57°C, the extract exhibited no detectable PG activity. The enzyme presented at least 60% of the maximal activity at temperatures between 27 and 47°C. Although the highest enzyme activity was observed at 43°C, 76% of the initial activity was maintained after incubation for 60 min at 43°C. The protein was stable at a temperature range of 4–37°C. Therefore, further experiments were performed

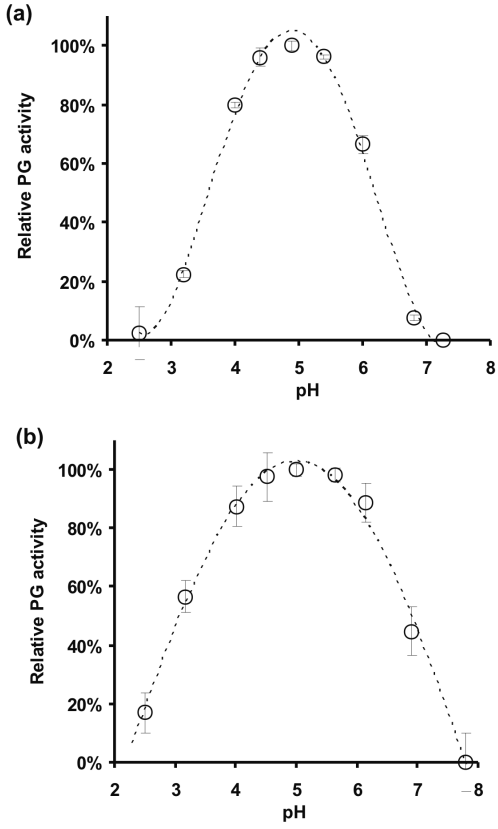

Figure 2. The pH dependent activity (a) and stability (b) of polygalacturonase from *Penicillium chrysogenum*.
The activity measurements were performed against polygalacturonic acid in McIlvaine buffer at 37°C.

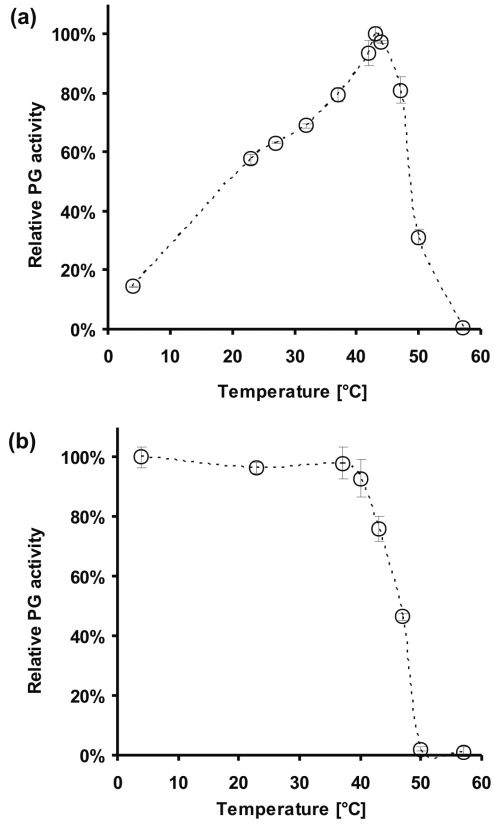

Figure 3. The effect of temperature on activity (a) and stability (b) of polygalacturonase from *Penicillium chrysogenum*.
The activity measurements were performed against polygalacturonic acid in McIlvaine buffer at pH 5.0.

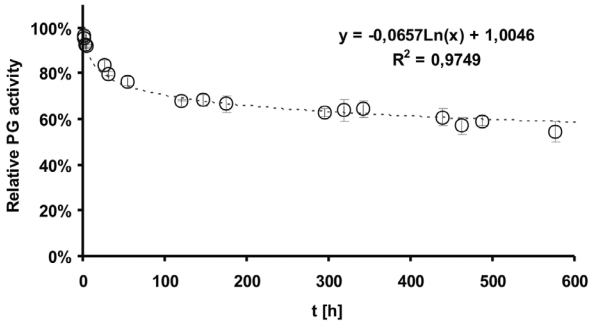

Figure 4. Inactivation test of Polygalacturonase from *Penicillium chrysogenum* performed at 37°C and pH 5.0.

at 37°C, which corresponded to 80% of the maximum U/g value. The enzyme stability decreased with increasing temperature. At 47°C, the extract presented only 46% of the relative activity and at 50°C, the enzyme was inactive.

The results of the operational stability measurements performed at pH 5.0 and 37°C are presented in Fig. 4.

During the course of 32 hours, the highest decrement of 20% was detected, showing a slow decline in PG activity. The half time ($t_{1/2}$) of the polygalacturonase activity was estimated as approximately 570 hours. Thus, the examined polygalacturonase presented high stability under these conditions.

CONCLUSIONS

Orange peels were used as a solid medium rich in pectin and other nutrient substrates necessary for *P. chrysogenum* growth. The agro industrial wastes were fully decomposed with simultaneous polygalacturonase production. A successful enhancement of PG production during SSF was achieved. The pH of the medium changed during fermentation process by approximately one unit, from pH 4 to 3, which inhibited the production and activity of the enzyme. We proposed that introduction of buffering agents during cultivation would counteract this effect. Maintaining the reaction medium pH, providing an additional phosphorus source and relatively constant moisture content significantly improved active protein production compared with the pH-uncontrolled processes. The best results were obtained after applying 5 ml of McIlvain buffer at pH 8.0. Indeed, McIlvain buffer was a suitable extraction solvent. The optimal isolation parameters were 230 rpm and 60 min for the high recovery of PG.

Enzyme preparation exhibited a temperature and pH optima of PG activity at 43°C and 5.0, respectively, however an improved thermal stability was observed at 37°C. Under these conditions, the half time was determined as 570 hours.

Acknowledgements

This project was supported by Wroclaw Centre of Biotechnology, The Leading National Research Centre (KNOW) program for years 2014-2018.

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