J. Food Sci. Nutr.
Vol. 5, No. 4, p. 194~196 (2000)

Optimal Culture Conditions for Production of Polygalacturonase from *Bacillus* sp. DFN-75 Isolated from *Kimchi*

Min-Kyung Lee, Eun-Ae Kim, Choon-Young Kim, Gi-Nahm Kim, Seok-Hwan Kim and Inshik Park†

Department of Food Science and Nutrition, Dong-A University, Pusan 604-714, Korea

Abstract

A bacterium capable of producing polygalacturonase was isolated from *kimchi*, and identified as a strain of *Bacillus*. The effects of culture conditions and medium composition on enzyme production were investigated. Among the tested carbon sources, polygalacturonic acid or pectin was most effective for the production of the enzyme. Therefore, it seemed that the enzyme was induced when pectin or polygalacturonic acid was used as a sole source of carbon. The optimal concentration of polygalacturonic acid was 0.5%. For nitrogen sources, yeast extract was best for the production of the enzyme, at a level of 0.25%. The enzyme was maximally produced by cultivating the isolated *Bacillus* sp. at an initial pH of 8.0 and temperature of 45°C for 20 hours.

Key words: *Bacillus* sp., polygalacturonase, production

INTRODUCTION

Polygalacturonase (PG, EC 3.2.1.15) is a depolymerizing pectinase that catalyzes the hydrolysis of α-1,4-glycosidic linkage of polygalacturonan chains in pectin, thus causing structural degradation (1). The enzyme plays an important role in fruit and vegetable softening, and it is widely used in food processing industries as a processing aid, in maceration, liquefication, extraction, clarification, and filtration of fruit or vegetable juices and wine (2,3). The enzyme has been produced by various plants (4-9), and microbial sources (10-18).

Softening of *kimchi* is due to the destruction of pectic substances in the vegetable tissue by polygalacturonase. The polygalacturonase activity in *kimchi* is low in the early stage of *kimchi* ripening, and it is markedly increased after the formation of film on the surface of *kimchi* juice, indicating that the softening of *kimchi* is partly due to activities of polygalacturonase-producing microorganisms. However, there have been few reports on the production of polygalacturonase from microorganisms present in *kimchi*. This paper describes optimal culture conditions for the production of polygalacturonase by an isolated bacterium.

MATERIALS AND METHODS

Microbial strain and preparation of enzyme

The microorganism used in this research was one of our isolates from *kimchi*. It was cultivated in a medium (polygalacturonic acid 1%, yeast extract 0.25%, K_2HPO_4 0.02%, KCl 0.05%, $CaCl_2$ 0.02%, pH 8.0). The cultivation was carried out by the addition of seed culture (inoculum size: 2.0%) to culture vessel (100 mL Erlenmeyer flask) and was then incubated for 20 hrs at 45°C. After cultivation, it was centrifuged at 10,000×g for 20 min. The supernatant was used as a crude enzyme solution.

Enzyme assay

The concentration of polygalacturonic acid in the enzyme assay system was 0.45% in 50 mM sodium acetate buffer (pH 5.0). The enzyme reaction was initiated by the addition of 0.1 mL enzyme to the assay mixture, followed by incubation with shaking for 30 min at 30°C. The total volume of the assay mixture was 0.5 mL. After reaction, liberated reducing sugar (α-D-galacturonic acid) was measured by the dinitrosalicylic acid method (19). One unit of enzyme was defined as the amount of enzyme to produce one μ mole of reducing sugar *per* min under the defined conditions.

RESULTS AND DISCUSSION

The microorganisms present in *kimchi* were cultivated in a selective medium containing polygalacturonic acid as described in Materials and Methods. The isolate showed highest polygalacturonase activity among the obtained colonies. The isolated bacterium was aerobic, rod-shaped, Gram positive, and spore former. It also produced catalase. Therefore, it seems to be a *Bacillus* sp. as determined by Bergey's Manual of Systematic Bacteriology (20).

In a culture medium, it is essential to maintain the proper initial pH, culture temperature as well as medium composition in order to achieve maximum production of the enzyme. Production of polygalacturonase from the isolated *Bacillus* sp. DFN-75 was examined using various carbon sources. Production of the enzyme was observed when pectin or polygalacturonic acid was used as a sole source of carbon. When polygalacturonic acid or pectin was used as a sole source of carbon

†Corresponding author. E-mail: ispark@mail.donga.ac.kr
Phone: 82-51-200-7322, Fax: 82-51-200-7535

in the culture medium, an essential enzyme in the metabolism of this polysaccharide would be polygalacturonase. In contrast, the enzyme production was lower when the organism was grown in monosacchrides or disacchrides as shown in Table 1. This suggests that polygalacturonase would be an inducible enzyme when this bacterium is grown on polygalacturonic acid or pectin as a sole source of carbon. Polygalacturonase was also induced when *Aspergillus niger* (21), *Cryptococcus albidus* (22), or *Kluyveromyces fragilis* (23) was grown on pectic substances as sole carbon sources. However, in *Kluyveromyces. marxiannus*, pectin does not induce any increase in pectolytic activity (18). Glucose seems to be the least effective carbon source for the production of the enzyme. This result agrees with the report that glucose repressed the production of the enzyme in *Saccharomyces cerevisiae* (24) and *Kluyveromyces marxianus* (18). Table 2 demonstrates the effect of polygalacturonic acid concentration on enzyme production. The enzyme was maximally produced when 0.5% polygalacturonic acid was used in the medium.

Various nitrogen sources were employed to investigate the effect of a nitrogen source on enzyme production. As shown in Table 3, yeast extract was most suitable for enzyme production among the tested nitrogen sources. Peptone and beef extract were also good nitrogen source for enzyme production. This result seems to agree with the previous research on *Kluyveromyces lactis* that the enzyme activity increased when the media were supplied with an organic nitrogen source such as yeast extract or peptone but that when an inorganic nitrogen source was added, the enzymic activity remained unaffected (25). Table 4 exhibits the effect of yeast extract concentration on enzyme production. The optimal yeast extract concentration for enzyme production was 0.25%.

Table 1. Effect of carbon sources on the production of polygalacturonase by *Bacillus* sp. DFN-75 isolated from *kimchi*

Carbon sources	Relative activity (%)
None	50.1
Pectin	98.2
Polygalacturonic acid	100.0
Saccharose	38.4
Maltose	45.9
Lactose	26.1
Fructose	12.1
Glucose	21.8

Table 2. Effect of polygalacturonic acid concentration on the production of polygalacturonase by *Bacillus* sp. DFN-75 isolated from *kimchi*

Polygalacturonic acid concentration (%)	Relative activity (%)
0	50.1
0.5	100.0
1	89.2
1.5	88.3
2	72.0

Table 3. Effect of nitrogen sources on the production of polygalacturonase by *Bacillus* sp. DFN-75 isolated from *kimchi*

Nitrogen sources	Relative activity (%)
None	49.4
NH_4Cl	77.8
$(NH_4)_2SO_4$	74.3
Glycine	69.2
Beef extract	93.5
Yeast extract	100.0
Soytone	78.8
Peptone	94.7

Table 4. Effect of yeast extract concentration on the production of polygalacturonase by *Bacillus* sp. DFN-75 isolated from *kimchi*

Yeast extract concentration (%)	Relative activity (%)
0	49.4
0.1	62.6
0.25	100.0
0.5	92.4
0.75	89.1
1.0	87.2

The initial pH of the medium was adjusted to various pH values from 3.0 to 9.0 and cultivation was carried out for 20 hours at 45°C. As shown in Fig. 1, the maximal enzyme production was achieved when the initial pH of the medium was 8.0. But in yeast, the highest value of the enzymic activity was obtained in a medium with an initial pH of 5.0. For determining the optimal temperature for enzyme production by the isolated *Bacillus*, cultivation was carried out at various temperatures ranging from 25°C to 50°C. The optimal temperature for enzyme production was 45°C as shown in Fig. 2. Fig. 3 shows the time dependent production of polygalacturonase in addition to cell growth and pH values of the growth medium. The enzyme was maximally produced by cultivating the isolated organism at the optimal conditions described above for 20 hours.

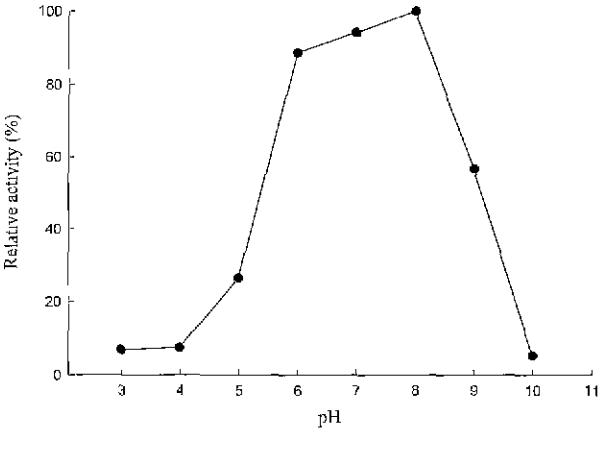

Fig. 1. Effect of initial pH on production of polygalacturonase from *Bacillus* sp. DFN-75.

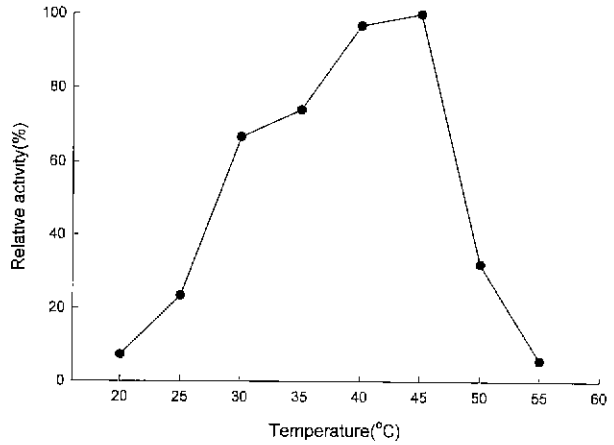

Fig. 2. Effect of temperature on production of polygalacturonase from *Bacillus* sp. DFN-75.

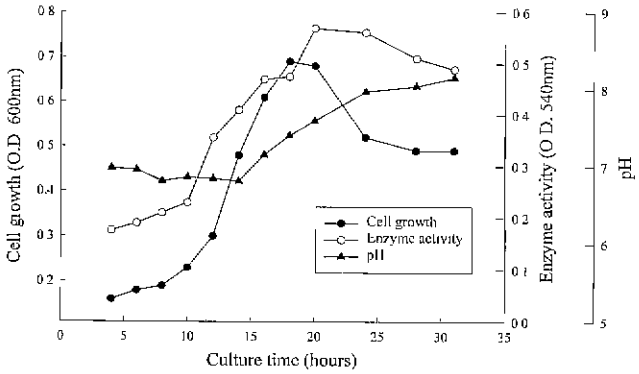

Fig. 3. Time dependent production of polygalacturonase from *Bacillus* sp. DFN-75. The inoculum size was 2%, and cultivation was carried out at initial pH of 8.0 and temperature of 45°C.

ACKNOWLEDGEMENTS

This paper was supported by the Dong-A University Research Fund, in 1998.

REFERENCES

1. Fogarty, W. and Ward, O. : Pectinases and pectic polysaccharides. *Progr. Indust. Microbiol.*, **13**, 59 (1974)
2. Cannel, E. and Moo-Young, M : Solid state fermentation systems. *Process Biochem.*, **4**, 2 (1980)
3. Dosanjh, N.S. and Hoondal, G.S. : Production of constitutive, thermostable, hyper active exo-pectinase from *Bacillus* GK-8. *Biotechnol. Lett.*, **12**, 1435 (1996)
4. Pathak, N., Mishra, S. and Sanwal, G. : Purification and characterization of polygalacturonase from banana fruit. *Phytochem.*, **54**, 147 (2000)
5. Hobson, G. : The firmness of tomato fruit in relation to polygalacturonase activity. *J. Hortic. Sci.*, **40**, 66 (1965)
6. Awad, M. and Young, R. : Post harvest variation in cellulase, polygalacturonase and pectin methyl esterase in avocado fruit in relation to respiration and ethylene production. *Plant Physiol.*, **64**, 306 (1979)
7. Bartley, I. : Exo-polygalacturonase of apple. *Phytochemistry*, **17**, 213 (1978)
8. Ahmed, A. and Labavich, J. : Cell wall metabolism in ripening pear. *Plant Physiol.*, **65**, 1009 (1980)
9. Nogata, Y., Ohta, H. and Voragen, A. : Polygalacturonase in strawberry fruit. *Phytochemistry*, **34**, 617 (1993)
10. Elegado, F.B. and Fujio, Y. : Polygalacturonase production by *Rhizopus* sp. *J. Gen. Appl. Microbiol.*, **39**, 409 (1993)
11. Kojima, Y., Sakamoto, T., Kishida, M., Sakai, T. and Kawasaki, H. : Acidic condition-inducible polygalacturonase of *Aspergillus kawachii*. *J. Molecular Catalysis B: Enzymatic*, **6**, 351 (1999)
12. Blanco, P., Sieiro, C. and Villa, T. : Production of enzymes in yeasts. *FEMS Microbiol. Lett.*, **175**, 1 (1999)
13. Devi, N. and Rao, A. : Fractionation, purification, and preliminary characterization of polygalacturonases produced by *Aspergillus carbonarus*. *Enzyme Microb. Technol.*, **18**, 59 (1996)
14. Mckay, A. : Degradation of polygalacturonic acid by *S. cerevisiae*. *Lett. Appl. Microbiol.*, **11**, 41 (1990)
15. Bailey, M.J. : Effect of temperature on polygalacturonase production by *Aspergillus niger*. *Enzyme Microb. Technol.*, **12**, 622 (1990)
16. Galiotou-Panayotou, M., Kapantai, M. and Kalantzi, O. : Growth conditions of *Aspergillus* sp. ATHUM-3482 for polygalacturonase production. *Appl. Microbiol. Biotechnol.*, **47**, 425 (1997)
17. Stratilova, E., Breierova, E. and Vadkertiova R. : Effect of cultivation and storage pH on the production of multiple forms of polygalacturonase by *Aspergillus niger*. *Biotechnol Lett.*, **18**, 41 (1996)
18. Schwan, R.F and Rose, A.H. : Polygalacturonase production by *Kluyveromyces marxianus* fermentation: effect of medium composition. *J. Appl. Bacteriol.*, **76**, 62 (1994)
19. Bernfeld, P. . Amylases, α and β. In "*Methods in Enzymology*" Colowick, S. and Kaplan, N. (eds.), Academic Press, New York, p.149 (1955)
20. Sneath, P. : *Bergey's Manual of Systematic Bacteriology*. Williams and Wilkins Co., Baltimore, Vol 2, p.1104 (1986)
21. Galiotou-Panayotou, M., Rodis, P. and Kapantai, M.: Enhanced polygalacturonase production by *Aspergillus niger* NRRL-364 grown on supplemented citrus pectin. *Lett. Microbiol.*, **17**, 145 (1993)
22. Federici, F. : Production, purification and partial characterization of an endopolygalacturonase *from Cryptococcus albidus*. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.*, **51**, 139 (1985)
23 Garcia-Garibay, M., Gomez-Ruiz, L. and Barzana, E. . Studies on the simultaneous production of single cell protein and polygalacturonase from *Kluyveromyces fragilis*. *Biotechnol. Lett.*, **9**, 411 (1987)
24. Blanco, P , Sieiro, C., Diaz, A. and Villa, T. : Production and partial characterization of an endopolygalacturonase from *Saccharomyces cerevisiae*. *Can. J. Microbiol.*, **40**, 974 (1994)
25. Murad, H. and Foda, M. : Production of yeast polygalacturonase in dairy wastes. *Bioresour. Technol.*, **41**, 247 (1992)

(Received October 6, 2000)