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Influence of phytic acid and its metal complexes on the activity of pectin degrading polygalacturonase

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ABSTRACT

Polygalacturonase is one of the important requirements of different microorganism to cause pathogenicity and spoilage of fruits and vegetables that involved in degradation of pectin during plant tissue infections. In current study, 20 mM phytic acid inhibited 70% activity of polygalacturonase. The effect of different concentration of metal ions such as Cu^{+2}, Al^{+3} and V^{+4} were studied separately and it was found that the 20 mM of these metal ions inhibited 37.2%, 79%, and 53% activity of polygalacturonase, respectively. Finally, the complexes of phytic acid and these metals ions were prepared and 1:1 ratio of phytic acid and metal ions complexes showed maximum inhibitory activity of enzyme as compared to complexes having 1:2 and 1:3 ratio except phytate–copper complexes which showed no inhibitory effect on the activity of polygalacturonase.

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1. Introduction

The plant cell wall composed of several complex polysaccharides includes cellulose, hemicelluloses, pectin and xylan and play important role in the prevention of plant tissues and its distinguished components which are essential for their survival (Carpita & Gibeaut, 1993). Pectin is a complex and high molecular weight heteropolysaccharide mainly composed of α-1,4 linked D-galacturonic acid residues backbone that may be methylated and substituted with L-rhamnose, arabinose, galactose, and xylose. The microorganisms (whether a pathogen or saprophytic association) first face pectin in the middle lamella of cell wall to initiate colonization on plant tissues (Prade, Zhan, Ayoubi and Mort, 1999). The production of pectinases by microorganism is prime important for the degradation of pectin for colonization of plant tissues (Collmer & Keen, 1986; Alghisi & Favaron, 1995). Pectinase is a group of enzymes including polygalacturonases, pectin lyases and pectin esterases that capable of degrading pectin molecule using different mechanism of action and naturally produced by different organisms such as bacteria, fungi, yeast, insects, nematodes, protozoan and plants (Hoondal, Tiwari, Tewari, Dahiya, & Beg, 2002). Pectinases are essential for pathogenic microorganisms which do not have any specific penetration structure as well as for necrotrophic pathogen during the final phases of their invasion process (De, Castoria, Bellincampi, & Cervone, 1997). Among these enzymes, polygalacturonases are the initial and important cell wall degrading enzyme secreted by many pathogenic microorganisms when they attack on plant tissues to cause diseases (Idnurm & Howlett, 2001). The polygalacturonases are extremely significant pathogenicity factors for different fungi such as *Aspergillus flavus*, *Alterniria citri* and *Claviceps purpurea*, and bacteria such as *Agrobacterium tumefaciens* and *Ralstonia solanacearum* (Matteo, Bonivento, Tsernoglou, Federici, & Cervone, 2006). Phytic acid is a natural carbohydrate compound from plant with six phosphate groups attached to each carbon and also known as *my*-inositol hexaphosphate (Shamsuddin, 2002). Phytic acid can be obtained by most of cereal grains, legumes, nuts, oil seeds, tubers, pollen, spores, and organic soils (Febles, Arias, Hardisson, Rodriguez-Alvarez, & Sierra, 2002). The strong antioxidant and iron (metal) chelating properties of phytic acid make this compound a distinctive and resourceful for using as a food preservative. Phytic acid is usually used as preservative and added to fruits and vegetables to prevent spoilage of these products (Zhang, Yanga, Lin, Ren, & Hou, 2012). In U.S. sodium phytate was recognized as GRAS substance and has been applied as a preservative for baked goods since 1997 (Hix, Klopfenstein, & Walker, 1997). However, there were no reports available to study the effect of phytic acid and its metals complexes on the activity of polygalacturonase which is one of starting cell wall degrading enzyme used by many pathogenic microorganism to cause diseases and spoilage to different fruits and vegetables plants.

Current study deals with the influence of phytic acid and different metal ions such as Cu^{+2}, Al^{+3} and V^{+4} on the polygalacturonase

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activity. Finally, different complexes of phytic acid with these metal ions were also prepared and studied their influence on polygalacturonase activity.

2. Materials and methods

2.1. Production of polygalacturonase

Bacillus licheniformis KIBGE IB-21 was used for the production of polygalacturonase using submerged fermentation in the production medium containing pectin (1.0%), yeast extract (0.3%), potassium nitrate (0.2%), di-potassium hydrogen phosphate (0.2%) and potassium di-hydrogen phosphate (0.2%) and pH 7.0 at 37 °C for 48 h (Rehman, Qader & Aman, 2012). After 48 h cells were separated by centrifugation at 10,000 rpm for 15 min at 4 °C and the crude enzyme was precipitated using 50% saturation of ammonium sulfate. The precipitates were obtained after centrifugation at 10,000 rpm for 15 min at 4 °C, then dissolved in glycine–NaOH buffer (pH 10.0) and dialyzed against same buffer. This partially purified polygalacturonase enzyme was used for further study.

2.2. Enzyme assay

Polygalacturonase activity was determined by 3,5-dinitrosalicylic acid method using D-(+)-galacturonic acid monohydrate as a standard and pectin as a substrate (Miller, 1959). The unit of polygalacturonase was defined as the amount of polygalacturonase required to release one μmole of galacturonic acid per minute under standard assay conditions.

2.3. Effect of phytic acid on the activity of enzyme

The effect of phytic acid on the activity of polygalacturonase was analyzed by pre-incubation of different concentration of phytic acid (1.0, 5.0, 10 and 20 mM) with enzyme (1:1) for 30 min at 37 °C and enzyme assay was performed to observe the effect of phytic acid on polygalacturonase.

2.4. Effect of some metallic cations on the activity of enzyme

The effect of different concentrations of metal ions (Cu^{+2}, Al^{+3}, and V^{+4}) on the activity of polygalacturonase was determined by pre-incubating these metal ions with enzyme solution (1:1) for 30 min at 37 °C. The aliquots were taken to perform enzyme activity under standard assay conditions.

2.5. Effect of metal complexes of phytic acid on the activity of enzyme

The metal–phytate complexes were prepared in different ratio of phytate to metal (Cu^{+2}, Al^{+3} and V^{+4}) (1:1), (1:2), (1:3), (1:4), (1:5) and (1:6).The concentration of phytate ion was kept constant (0.2 mM) in all the complexes while concentration of metal ions were in the range of (0.2–1.2 mM). The enzyme was pre incubated with these complexes for 30 min at 37 °C and than aliquots were used for enzyme activity.

3. Results and discussion

3.1. Effect of phytic acid on the activity of polygalacturonase

Different concentrations of phytic acid showed significant effect on the activity of polygalacturonase. It has been observed that the increased in the concentration of phytic acid increased the inhibition rate of enzyme activity and 20 mM of phytic acid was found

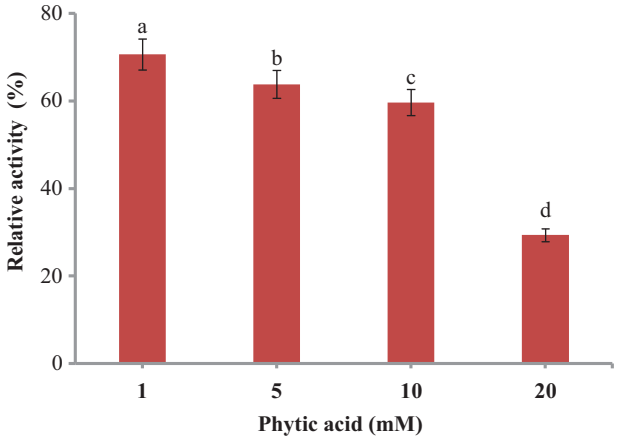

Fig. 1. Effect of phytic acid (1.0–20.0 mM) on the activity of polygalacturonase from *B. licheniformis* KIBGE IB-21. Symbols (means ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

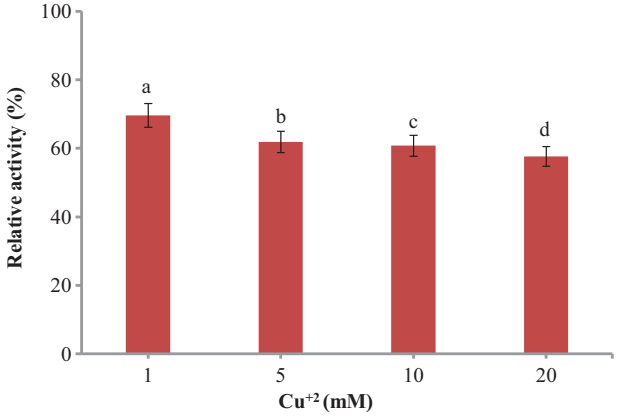

Fig. 2. Effect of Cu^{+2} (1.0–20.0 mM) on the activity of polygalacturonase from *B. licheniformis* KIBGE IB-21. Symbols (means ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

to be enough for the inhibition of more than 70% activity of polygalacturonase (Fig. 1). Basically, highly negative charged phosphate in phytic acid strongly binds to amino groups (NH_4) of proteins, carbohydrate and metallic cations and affects their biological activities to perform their physiological functions. So it has been suggested that the binding of negatively charged phosphates in phytic acid to N-terminal amino acids of polygalacturonase might be one of the reason to caused some conformational changes to the structure of enzyme which ultimately affect its activity.

3.2. Effect of metallic cations on the activity of polygalacturonase

The effect of different metallic cations such as Cu^{+2}, Al^{+3} and V^{+4} having different concentrations ranging from 1–20 mM were investigated on the activity of polygalacturonase and it has been observed that all metal ions imposed some inhibitory effect on the activity of polygalacturonase. It was observed that as the concentration of Cu^{+2} increased the activity of polygalacturonase decreased and maximum inhibition (42.36%) was seen at concentration of 20 mM of Cu^{+2} (Fig. 2). However, the effect of V^{+4} on the activity of polygalacturonase was higher to some extent as compared to Cu^{+2} and more than 50% loss of activity was obtained by using 20 mM of Vanadium (IV) (Fig. 3). The effect of Al^{+3} on the activity of polygalacturonase showed that as the concentration of aluminum increased from 1.0 mM to10 mM, relative activity of polygalacturonase activity decreased from 53.57 to 19.9%, respectively (Fig. 4).

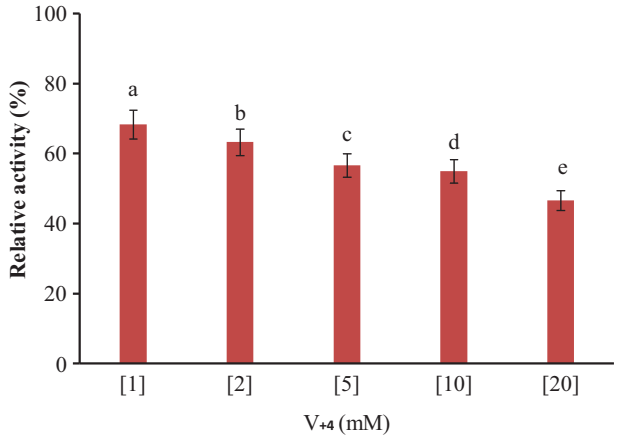

Fig. 3. Effect of V^{+4} (1.0–20.0 mM) on polygalacturonase activity from *B. licheniformis* KIBGE IB-21. Symbols (means ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

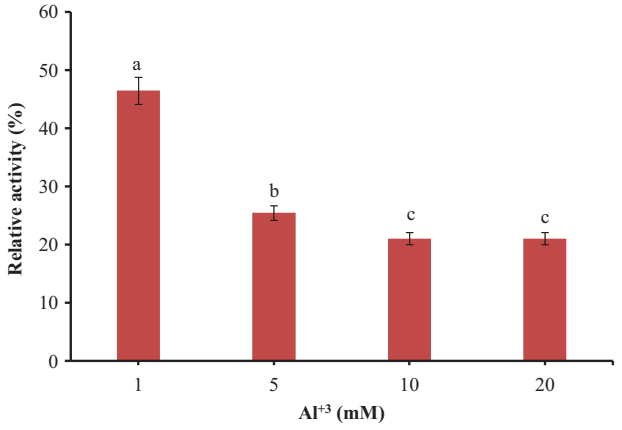

Fig. 4. Effect of Al^{+3} (1.0–20.0 mM) on the rate of pectin degradation by polygalacturonase from *B. licheniformis* KIBGE IB-21. Symbols (means ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

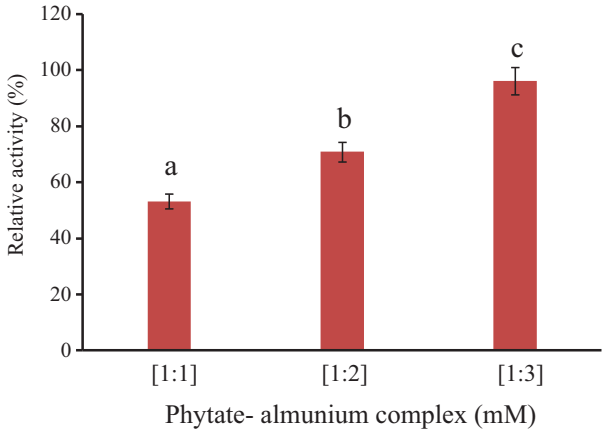

Fig. 5. Effect of phytate–aluminum complexes on the activity of polygalacturonase from *B. licheniformis* KIBGE IB-21. Symbols (means ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

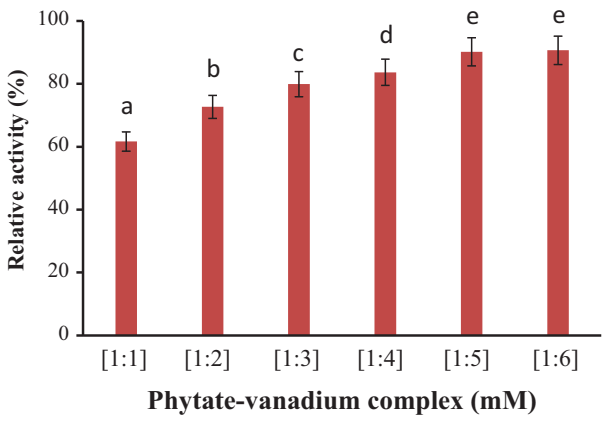

Fig. 6. Effect of phytate–vanadium complexes on the activity of polygalacturonase from *B. licheniformis* KIBGE IB-21. Symbols (means ± S.E., $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

It was also noted that no activity loss was observed beyond 10 mM Al^{+3} and also even after using 20 mM of Al^{+3}.

3.3. Effect phytic acid and metals cations complexes on activity of polygalacturonase

After observing the effect of phytic acid and metals cations on the activity of polygalacturonase, the effect of complexes of phytic acid with Al^{+3}, Cu^{+2} and V^{+4} were analyzed on rate of hydrolyzing of pectin by polygalacturonase. The complexes of phytic acid with these metallic cations were prepared in constant ratios ranging from 1:1 to 1:6 keeping the phytic acid concentration. The low concentrations of metal ions (0.2–1.6 mM) were prepared and the reason is that the high concentration of metal ions reduces the solubility of complexes. It has been previously reported that the maximum six divalent metallic cations are capable to bind per molecule of phytic acid (Martin & Evans, 1989). The complexes were treated with partially purified enzyme and it was found that each metal–phytate complexes affect the activity of polygalacturonase in different manner. The aluminum–phytate complexes in ratio of 1:1, 1:2 and 1:3 approximately showed 46.8, 29.13 and 3.82% loss of polygalacturonase activity, respectively, while 1:4, 1:5 and 1:6 showed no effect on the activity of polygalacturonase (Fig. 5). No activity loss of polygalacturonase was observed with treatment with phytate–copper complexes, therefore no data is mentioned here. While in the case phytate–vanadium complexes the relative activity of polygalacturonase increases as the ratio of phytate–vanadium complexes increases from 1:1 to 1:4 (Fig. 6). However, no significant loss of relative activity was observed in complexes having 1:5 and 1:6 ratio of phytate–vanadium complexes.

4. Conclusion

Polygalacturonase is a pectinolytic enzyme and play important role in microorganism's pathogenicity and spoilage of fruits and vegetables. In present study, the effects of a highly negatively charge and simple ringed carbohydrate compound with six phosphate groups in each carbon and its metallic cations complexes on the activity of polygalacturonase were analyzed. The phytic acid significantly inhibited the activity of polygalacturonase and loss of more than 70% of its initial activity was observed after treatment with 20 mM of phytic at 37 °C for 30 min. Among metals cations, 10 mM of aluminum was found to be superior as compared to vanadium and copper and inhibited 79% activity of polygalacturonase. The phytate–metallic complexes inhibited the polygalacturonase activity to some extent but lower than the phytic acid and metals cations alone. Therefore, it is suggested that the treatment of phytic acid and Al^{+3} to fruits and vegetables plants might be able to reduce the spoilage of fruits and vegetables caused by infectious microorganisms of these plants by inhibiting the polygalacturonase.

References

Alghisi, P., & Favaron, F. (1995). Pectin-degrading enzymes and plant–parasite interactions. *European Journal of Plant Pathology*, *101*, 365–375.

Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, *3*, 1–30.

Collmer, A., & Keen, N. T. (1986). The role of pectic enzymes in plant pathogenesis. *Annual Reviews of Phytopathology*, *24*, 383–409.

De, L. G., Castoria, R., Bellincampi, D., & Cervone, F. (1997). Fungal invasion enzymes and their inhibition. In G. C. Carroll, & P. Tudzynski (Eds.), *The mycota V, part B: plant relationship* (pp. 61–83). Berlin: Springer.

Febles, C. I., Arias, A., Hardisson, A., Rodriguez-Alvarez, C., & Sierra, A. (2002). Phytic acid level in wheat flours. *Journal of Cereal Science*, *36*, 19–23.

Hix, D. K., Klopfenstein, C. F., & Walker, C. E. (1997). Physical and chemical attributes and consumer acceptance of sugar-snap cookies containing naturally occurring antioxidants. *Cereal Chemistry*, *74*, 281–283.

Hoondal, G. S., Tiwari, R. P., Tewari, R., Dahiya, N., & Beg, Q. K. (2002). Microbial alkaline pectinases and their industrial applications: a review. *Applied Microbiology and Biotechnology*, *59*, 409–418.

Idnurm, A., & Howlett, B. J. (2001). Pathogenicity genes of phyto-pathogenic fungi. *Molecular Plant Pathology*, *2*, 241–255.

Martin, C. J., & Evans, W. J. (1989). Phvtic acid-enhanced metal ion exchange reactions: the effect on carboxypeptidase A1. *Journal of Inorganic Biochemistry*, *35*, 267–288.

Matteo, A. D., Bonivento, D., Tsernoglou, D., Federici, L., & Cervone, F. (2006). Polygalacturonase-inhibiting protein (PGIP) in plant defence: a structural view. *Phytochemistry*, *67*, 528–533.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*, 426–428.

Prade, R. A., Zhan, D., Ayoubi, P., & Mort, A. J. (1999). Pectin pectinases and plant–microbe interaction. *Biotechnology Genetic Engineering Reviews*, *16*, 361–391.

Rehman, H. U., Qader, S. A. U., & Aman, A. (2012). Polygalacturonase: production of pectin depolymersing enzyme from *Bacillus licheniformis* KIBGE IB-21. *Carbohydrate Polymer*, *90*, 387–391.

Shamsuddin, A. M. (2002). Anti-cancer function of phytic acid. *International Journal of Food Science and Technology*, *37*, 769–782.

Zhang, H., Yanga, Q., Lin, H., Ren, Z. L., & Hou, J. (2012). Phytic acid enhances biocontrol efficacy of Rhodotorula mucilaginosa against postharvest gray mold spoilage and natural spoilage of strawberries. *Food Science and Technology*, http://dx.doi.org/10.1016/j.lwt.2012.01.0