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Isolation, purification and some properties of pectate lyase synthesized by *Xanthomonas campestris campestris*

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ABSTRACT: Pectase lyase (PL) was isolated from *Xanthomonas campestris campestris* and partially purified by ammonium sulphate precipitation. Some properties of the enzyme were determined. The PL activity was stimulated at 20mM, 3mM, 1mM, 1mM and 0.01mM of Ca^{++}, Ba^{++}, $K^{+,}$ Na^{+}, Mg^{++} and NH_4^{+} respectively. Below and above 0.1mM of each of ethylenediaminetetraacetic acid (EDTA), iodoacetic acid (IAA) and 2,4-dinitrophenol (DNP), the PL activity was inhibited. Optimum PL activity was obtained at 40°C and incubation period of 1 hour. The enzyme was inactivated at 50mins of boiling.

Key Words: *Xanthomonas compstris compestris*, Pectate Lyase, Characterization.

Introduction

Xanthomonas campestris campestris, a phytopathogenic bacterium causes leaf spot in banyan trees (1). Pectolytic strains of *X. compestris compestris* causes soft-rot in fruits and vegetables (2,3) and black-rot in crucifers (4). Pathogenicity of *X. compstris compestris* is related to its ability to synthesize pectic-substance-degrading enzymes such as pectate lyase. Pectate lyase (EC. 4.2.2.2) is an extracellular non-hydrolytic pectic enzyme which cleaves pectic substance at α-1,4-glucoside linkage between adjacent uronic acid units by trans-elimination with the formation of galacturonic acid residue having an unsaturated bond between carbons 4 and 5 (4, 5). Pectic substance-degrading enzymes are associated with the phenomenon of tissue maceration and are considered to function in aiding pathogens to spread within their hosts (6).

Production and activities of enzymes are affected by diverse physiological factors (7,8). Pectate lyase is an inducible enzyme. Polygalacturonic acid and pectin induced the synthesis of pectate lyase in a culture medium of *X. compestri compsestri* and its mutants (9). Pectate lyase from some fungi and bacteria have been characterized (10, 11, 12, 13, 14, 15). No report on thecharacteristics of pectate lyase produced by *X. compestris compestris* has been documented. To control the synthesis and activity of PL formed by *X. compenstris compenstris*, the enzyme has been isolated, partially purified and some of its properties studied.

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Materials and Methods

Bacterial Strain and growth Conditions

Strain 2350 of *X. compestris compestris* was obtained from the National Institute of Applied Sciences, Lyon, France and routinely subcultured on nutrient afar. Cells were grown at 30°C for 24 hours in synthetic minimal medium M63 (16) supplemented with 0.2%(v/v) glycerol and 0.4% (w/v) polygalacturonic acid (PGA) grade III (Sigma) (17).

Extraction and Partial Purification of Pectate Lyase

After 24hour of incubation, cultured cells were broken open by the method of Moran and Starr (18) and pelleted by centrifugation at 3500g for 15 mins at 4°C. Protein contained in the supernatant was precipitated with $(NH_4)_2SO_4$ (Analytical grade) to 80% saturation (18). The precipitate was redissolved in 0.2M Tris-HCl buffet, (pH 7.2) and dialysed at 4°C against changes of the same Tris-HCl buffer for 24 hours. Protein content was determined spectrophotometrically by absorbance at 280nm.

Assay for Pectate Lyase (PL) Activity

PL activity was determined by the modified thiobarbituric acid reagent method of Weissbach and Hurwitz (19). The reaction mixture contained 1.0ml of the enzyme, 3.5ml of 1% (w/v) PGA (Sigma Grade III) at pH 8.0 and 1mM of $CaCl_2$. The reaction was carried out at 35°C for 1h and PL activity was measured at 540nm. One unit of activity was defined as the amount of PL requiredto produce an increase in absorbance of 0.01 per minute (9). This assay condition was used to determine PL activity throughout this study except otherwise indicated.

Characterization of Pectate Lyase

Assay for Effect of Some Cations and Chemical Compounds

Effects of Ca^{++}, Ba^{++}, K^+, Na^+, Mg^+, NH_4^+, DNP, EDTA and IAA on PL activity was determined at concentrations of 0 to 5mM except for Ca^+ which was increased to 50mM due to increase in PL activity above 5mM. Each of the chemicals was substituted for $CaCl_2$ in the enzyme assaymixture.

Test for Effect of Temperature

PGA solutions were adjusted to different temperatures ranging from 0 to 50°C and used in the enzyme assay mixture.

Test for Effect of Boiling

1ml of the partially purified PL was boiled at 100°C for various lengths of time ranging from 0 to 60mins. The boiled enzyme was rapidly cooled and PL activity was assayed for.

Test for Effect of Incubation Time

The enzyme mixture was incubated at 35°C for different periods of time ranging from 30mins to 5hours. PL activity was measured as described above.

Results

The enzyme was purified 7-fold with 96% recovery of the initial enzyme activity (Table 1). The enzyme degraded PGA in the absence of the cations and other chemicals used in this experiment. Addition of the cations increased the PL activity. Optimum PL activity was achieved at 20mM, 3mM, 1mM, 1mM. 1mM and 0.01mM of Ca^{++}. Ba^{++}, Mg^{++}, K^{+}, Na^{+}, and NH_4^{+} respectively. Aty above 0.1mM of each of DNP, EDTA and IAA, PL activity was inhibited (Fig. 1). Optimum pH and temperature for the PL activity were 9.0 and 40°C (Fig. 2a, b). PL showed highest activity (39.8 units/ml) at incubation period of 1 hour (Fig. 2c). Boiling decreased activity of the PL, but the enzyme retained some of its activity after boilingfor 40mins (Fig. 2d). The enzyme was completely inactivated at 50mins of boiling.

Table 1: Partially Purified Pectate Lyase Isolated from *Xanthomonas compestris compestris*.

Fraction	Total volume (ml)	Total Activity (Units)	Enzyme (Units/ml)	Yield (%)	Relative purification (Fold)
Culture supernatant	240	9240	38.5	100	1
Ammonium sulphate	150	8850	59.0	96	7.09

Discussion

Increased activity of the PL in the presence of the cations is related to activation of the enzyme. Metals activate enzymes by various mechanisms. The cations may function by forming enzyme-metal-substrate complex in which the cation may tie down or hold the PGA in the required position for the PL to act (20). Cations may also displace ineffective metal ion from combining with the active centre of the PL or with the functional group of the substrate (21).

DNP prevents oxidative phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) resulting in uncoupling of the energy-yielding reaction from energy-consuming reaction (8). The interference of DNP with aerobic phosphorylation processes suggests the importance of energy yielding breakdown of PGA to galacturonic acid by PL.

EDTA, a metal chelator acts as an inhibitor by forming a complex with the inorganic group which results in lowering substrate-enzyme affinity (8). Iodoacetic acid is a sulpfhydryl reagent hindering enzyme activity by combining with thiol group ("-SH") of the protein to form inactive alkylated derivative (22). It is therefore likely that PL which actrivity was inhibited by IAA contains thiol group with which the compound could form inactive complex. Also, IAA could hinder PL activity by a mechanism of auto-digestion. This is similar to the inhibition of protease activity caused by IAA which resulted from auto-digestion of the enzyme (23).

High PL activities obtained at observed optimum pH and temperature were due to increased frequency of molecular action of the reactants in the reaction mixture. The PL will be more stable and have greater affinity to form the PL-PGA complex at these pH and temperature values than others (8, 21, 24).

Effect of boiling and incubation time on PL activity are also associated with the frequency of molecules (8). Sensitive of PL to boiling can be explained by the phenomenon of thermal denaturation (21, 24, 25). PL as other proteins can be denatured at high temperatures. PL from *X. campestris campestris* can be regarded as heat-stable in comparison with some enzymes which when heated at 70°C lost all their activities in 10 minutes (26, 27).

It is evident from this investigation that the effect of incubation on PL action is influenced by the prevailing temperature. It is therefore suggested that incubation period of 1hour at 40° with the addition of

20mM Ca^{++} should be considered for the assay condition to obtain the optimum PL activity isolated from *X. campestris campestris.*

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