Volume 280, number 2, 335–340 FEBS 09524 March 1991

ADONIS 001457939100231G

# Purification and some properties of the pectin lyase from *Penicillium italicum*

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Received 7 November 1990; revised version received 18 January 1991

For the first time, a pectin lyase (poly(methoxygalacturonide)lyase; EC 4.2.2.10) from a member of the genus *Penicillium* was isolated, purified to homogeneity and characterized. The monomeric enzyme from *Penicillium italicum* CECT 2294 culture filtrates showed a molecular mass of 34 kDa after SDS-electrophoresis in polyacrylamide gradient gels, and the isoelectric point was 8.6 as determined by isoelectric focusing. The optimum pH (9.0), the high pH and temperature stabilities, the ability to degrade pectins from different sources and with a wide range of degrees of esterification (from 37% to 86%) as well as the importance of this type of biocatalysts in the food industry make this enzyme an interesting subject of study.

Food industry; Pectin; Pectin lyase; Pectolytic enzyme; Purification; *Penicillium italicum*

## 1. INTRODUCTION

Pectin lyase is the only enzyme currently known to be able to cleave, without the prior action of other enzymes, the α-1,4 glycosidic bond of highly esterified pectins such as fruit pectin [1]. The importance of this enzyme in both phytopathological processes and in recycling carbon compounds in the biosphere demand the isolation of homogeneous preparations to find out its properties and mechanism of action. In addition, the industrial applications of pectin lyase [2,3] (usually available as a member of complex enzyme mixtures) have developed a current need for pure enzyme preparations to allow more specific and controllable effects. Nevertheless, both in fungi and bacteria, the information about this enzyme is very scarce [4].

As far as the post-harvest spoilage of citrus products is concerned, pectin lyase also has a great significance. Among the microorganisms responsible for this process, *Penicillium* spp. (mainly *P. italicum* and *Penicillium digitatum*) are the most important examples in terms of both quantity and quality [5]. Although several studies about pectin lyases from *Penicillium* spp. have been made [4], neither the isolation nor the characterization of the homogeneous enzyme after SDS-electrophoresis and IEF have been described. This paper reports a purification procedure capable of yielding a pure preparation of pectin lyase from a *P. italicum* strain and describes some relevant physicochemical and catalytic properties of this enzyme.

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*Abbreviations:* SDS, sodium dodecyl sulphate; DE, degree of esterification; IEF, isoelectric focusing; FPLC, fast protein liquid chromatography

## 2. MATERIALS AND METHODS

### 2.1. *Materials*

Mono P, Phenyl-Superose and Superose 12 columns, PhastGel 8-25, SDS buffer strips, Polybuffer 96, ampholytes (8–10.5), and molecular weight and isoelectric point markers were obtained from Pharmacia (Uppsala, Sweden). DEAE-cellulose (DE-52) was from Whatman (Maidstone, UK). CM-cellulose, Phenyl-Sepharose CL-4B, acrylamide, *N,N'*-methylene-bisacrylamide, SDS, and citrus pectin were purchased from Sigma (St. Louis, MO). Apple pectin was a kind gift from OBI Pektin (Bischofszell, Switzerland). Gel filtration molecular weight markers were obtained from Boehringer (Mannheim, Germany). The potato dextrose agar medium was from bioMèrieux (Charbonnières les bains, France). The protein assay dye-reagent was from Bio-Rad (Munchen, Germany). All other chemicals were reagents of analytical grade supplied by Merck (Darmstad, Germany).

### 2.2. *Growth of* P. italicum

*P. italicum* CECT 2294 (also available as ATCC 66636) was obtained from Colección Española de Cultivos Tipo, Valencia, Spain, and maintained in potato dextrose agar medium. Cultures were grown in 5-liter Erlenmeyer flasks, containing 250 g of wheat bran and 250 ml of water at 28°C in a constant-temperature room. The flasks were inoculated with a suspension of germinated spores ($10^6$/ml in 20% (v/v) glycerol) grown for 4 days in potato dextrose agar. Filtrates were prepared after 72 h of growth by adding distilled water to the bran (100 ml of water to 10 g of dry bran) and allowing the suspension to remain with gentle shaking. After 1 h, the liquid was decanted, filtered through a nylon filter, and finally centrifuged at 10 000 × *g* and 4°C for 30 min. After this time, the supernatant was dialysed at 4°C against distilled water and considered as the starting crude extract.

2.3. *Purification of pectin lyase*
All steps were carried out at room temperature.

2.3.1. DEAE-cellulose chromatography
The pH value of the dialysed crude extract (about 6.15) was adjusted with NaOH to pH 8.0 and passed though a DE-52 column (9.0 × 12.5 cm) equilibrated with buffer A (0.05 M Tris-HCl, pH 8.0) at an approximate flow rate of 25 ml/min. Pectin lyase activity was not retained in the column and was recovered as the DEAE-cellulose eluate.

2.3.2. CM-cellulose chromatography
The above eluate was acidified with HCl until pH 6.0 and passed through a CM-cellulose column (9.0 × 11.5 cm) equilibrated with 0.01 M citrate-phosphate, pH 6.0. After properly washing the column with the same buffer, pectin lyase activity was eluted with 0.25 M citrate-phosphate, pH 7.0.

2.3.3. Phenyl-Sepharose CL-4B chromatography
The active fractions were pooled and solid ammonium sulphate was added up to 0.75 M. The resulting solution was adsorbed to a Phenyl-Sepharose column (1.7 × 10.0 cm) equilibrated with 0.75 M ammonium sulphate in 0.25 M citrate-phosphate, pH 7.0 at a flow rate of 2 ml/min. After washing the column with the equilibration buffer, pectin lyase activity was eluted with buffer B (0.025 M 1,3-diaminopropane-HCl, pH 10.5).

2.3.4. First Phenyl-Superose chromatography
The active fractions were pooled, adjusted to 1 M with solid ammonium sulphate and loaded (flow rate of 0.5 ml/min) onto an FPLC Phenyl-Superose (HR 5/5) column equilibrated with 1 M ammonium sulphate in buffer B using FPLC Pharmacia equipment (Uppsala, Sweden). After washing with 15 ml of equilibration buffer, pectin lyase was eluted applying a linear gradient of 22.5 ml from 1 M to 0 M of ammonium sulphate in buffer B. The active fractions were pooled and dialysed overnight at 4°C against buffer B.

2.3.5. Mono P chromatography
The dialysed preparation was applied to an FPLC-chromatofocusing Mono P (HR 5/20) column equilibrated with buffer B. A flow rate of 1.0 ml/min and a pH gradient from 9.0 to 6.0 were used. After equilibration of the column, and once the preparation was injected onto the column, the pH gradient was formed by applying 30 ml of 1:10 diluted Polybuffer 96-HCl (pH 6.0).

2.3.6. Second Phenyl-Superose chromatography
The active fractions were collected and dialysed overnight against buffer A. Solid ammonium sulphate was added to the dialysate up to 1 M and the resulting solution was adsorbed to an FPLC Phenyl-Superose (HR 5/5) column equilibrated with 1 M ammonium sulphate in buffer A at a flow rate of 0.5 ml/min. Pectin lyase was eluted with a linear gradient of 22.5 ml from 1 M to 0 M of ammonium sulphate in buffer A. The purified pectin lyase was dialysed against buffer A and stored at −20°C until used.

2.4. *Amino acid determination*
The amino acid composition was determined by the Pico-Tag method (Waters Associates, Milford, MA) by monitoring at 254 nm. The hydrolysis method used damages the Trp residues and does not work with Cys.

2.5. *Enzyme assays*
Pectin lyase activity was determined spectrophotometrically (Shimadzu UV-260 spectrophotometer) by monitoring the increase in $A_{235}$ as previously described [6]. The reaction mixture (1.25 ml) during the purification studies contained: 0.25 M citrate-phosphate, pH 6.0, 0.5% w/v citrus pectin (DE 70%) and the appropriate amount of enzyme. The assays with the pure enzyme were carried out with the same final volume but with 0.25 M 1,3-diaminopropane-HCl, pH 9.0, 1% w/v of citrus pectin (DE 86%) and 0.1 μg of enzyme. Preincubations were carried out at 40°C for 10 min, and the reaction was started by adding the enzyme. Both assays were checked following the Selwyn's test [7] and no enzyme inactivation was detected under experimental conditions. Control tubes contained distilled water in substitution for the enzyme. Pectin lyase catalytic studies with apple pectin as substrate were carried out in the same conditions, but with a substrate concentration of 0.4% w/v. One unit of activity is the amount of enzyme which produces an increase of one unit of $A_{235}$ per minute. The increase of the $A_{235}$ originated by the formation of the unsaturated methyl-oligosaccharides can be transformed in μmoles of product by using the extinction coefficient of 5500 $M^{-1} \cdot cm^{-1}$ [1].

2.6. *Absorption spectrum*
The absorption spectrum was recorded at room temperature against a buffer blank in a Shimadzu UV-260 spectrophotometer with cuvettes of 1 cm pathlength.

2.7. *Purification and hypermethoxylation of pectin*
Pectin was purified and highly esterified as described by Van Houdenhoven [8].

2.8. *Polyacrylamide gel electrophoresis*
Polyacrylamide gel electrophoresis was carried out in a Pharmacia PhastSystem equipment (Uppsala, Sweden). SDS-electrophoresis was performed in 8-25% w/v acrylamide gradient gels (PhastGel 8-25) with PhastGel SDS buffer strips. Native-electrophoresis was carried out in homogeneous (25 % w/v) gels with native buffer strips, lab-made as described in the PhastSystem Separation Technique File No. 300 (Pharmacia). The electrophoretic conditions were those described in the PhastSystem Separation Technique Files No. 110 and 300 (Pharmacia), and the SDS-and native-electrophoresis were stopped when the Bromophenol blue marker had reached the anode or the fuchsin the cathode. Proteins were stained using silver staining (PhastSystem Development Technique File No. 210, Pharmacia). Pectin lyase was located in the gels by analysing the activity [4] after cutting the gels and soaking the resulting pieces in buffer A.

2.9. *Isoelectric point determination*
Isoelectric point was determined by IEF in a Pharmacia PhastSystem equipment as described in the PhastSystem Separation Technique File No. 100. Polyacrylamide (5% w/v) gels used contained an ampholyte concentration of 22 μmol · (ml · pH unit)$^{-1}$, capable of generating a pH gradient from 10.5 (cathode) to 8.0 (anode). pI markers were cytochrome *c* (pI 10.25), trypsinogen (pI 9.30), lentil lectin-basic (pI 8.65), lentil lectin-middle (pI 8.45), and lentil lectin-acidic (pI 8.15).

2.10. *Analytical methods*
Protein content was determined by the method of Bradford [9] with bovine serum albumin as the standard.

## 3. RESULTS

Table I summarizes a typical purification from 5 litres of culture filtrate. Pectin lyase was purified more than 1500-fold, with a final yield of 41%. The purified enzyme had a specific activity of 3934 units/mg protein at 40°C.

After the last step, the enzyme preparation gave a single protein band on silver stained native-electrophoresis (Fig. 1, Native) with a positive reaction for increasing absorbance at 235 nm activity. The purity of the pectin lyase preparation was also confirmed by the single protein band detected in the silver-stained

Table I

Purification of pectin lyase from *P. italicum*

| Purification step | Volume (ml) | Total activity (U) | Total protein (mg) | Specific activity (U/mg protein) | Purification (-fold) | Yield (%) |
|---|---|---|---|---|---|---|
| Crude Extract | 4880 | 1747 | 664 | 2.6 | 1 | 100 |
| DEAE-cellulose | 4890 | 1590 | 23.2 | 68.5 | 26 | 91 |
| CM-cellulose | 486 | 1361 | 13.6 | 100 | 38 | 78 |
| Phenyl-Sepharose | 10 | 1350 | 1.6 | 844 | 325 | 77 |
| Phenyl-Superose I | 3 | 1039 | 0.57 | 1823 | 701 | 59 |
| Mono P | 2 | 742 | 0.44 | 1686 | 648 | 42 |
| Phenyl-Superose II | 2 | 720 | 0.18 | 3934 | 1513 | 41 |

The enzyme was assayed at pH 6.0 (0.5 M citrate-phosphate) and 40°C, in a final volume of 1.25 ml. Citrus pectin (DE 70%) at 5 mg/ml was used as substrate.

SDS-PAGE and isoelectric focusing (Fig. 1, SDS and IEF).

The molecular mass of pectin lyase was estimated by two methods: SDS-electrophoresis in acrylamide gradient gels and gel filtration. The molecular mass obtained by the first method was 34 kDa (Fig. 1, SDS). However, when the same preparation was chromatographed through a gel filtration Superose 12 column, a much lower value (22 kDa) was calculated.

The same gel filtration column was utilised to determine the Stokes radius of the pectin lyase (2.44 nm). The frictional coefficient (1.32) calculated for this enzyme from the data obtained with the Superose 12 column is not very different from that calculated by assuming a molecular mass of 34 kDa (1.14). These values are similar to those reported for two isoenzymes purified from *Aspergillus niger* (Stokes radius values of 2.44 and 2.28 nm, and frictional coefficient values of 1.10 and 1.06, respectively) [8].

The isoelectric point of the enzyme determined by isoelectric focusing was 8.6 (Fig. 1, IEF).

Table II shows pectin lyase amino acid composition. Lys, Asp, and Ala seem to be the most abundant residues among the amino acids determined. On the contrary, Phe, Pro, His and Arg show a minor presence. As far as the majority and minority residues are concerned, these results are similar to those reported for the pectin lyase from *A. niger* [8].

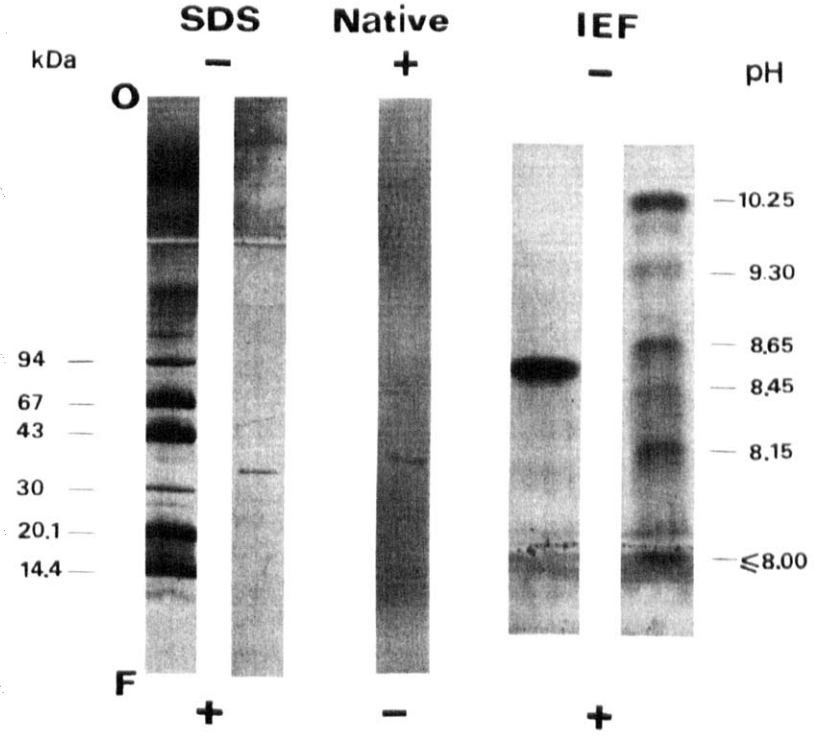


Fig. 1. Analysis of pectin lyase from *P. italicum* by SDS-electrophoresis in polyacrylamide gradient gels (SDS), by native-electrophoresis in homogeneous gels (native), and by isoelectric focusing (IEF). Samples, containing 75 ng (SDS) or 150 ng (native and IEF) of protein, were stained using silver staining. Gels on the left- and right-hand sides are molecular mass and pI markers, respectively. O and F represent the origin and front, respectively.

Table II

Amino acid composition of the pectin lyase from *P. italicum*

| Amino acid | % mol |
|---|---|
| Lys | 12.1 |
| Asp | 10.3 |
| Ala | 9.7 |
| Gly | 9.0 |
| Val | 7.7 |
| Ser | 7.1 |
| Thr | 6.9 |
| Met | 6.9 |
| Ile | 6.7 |
| Glu | 5.4 |
| Tyr | 4.1 |
| Leu | 4.1 |
| Phe | 3.0 |
| Pro | 2.6 |
| His | 2.3 |
| Arg | 2.1 |

Figures represent the percentages calculated with respect to the measured amino acids (Asn, Cys, Gln and Trp were not determined).

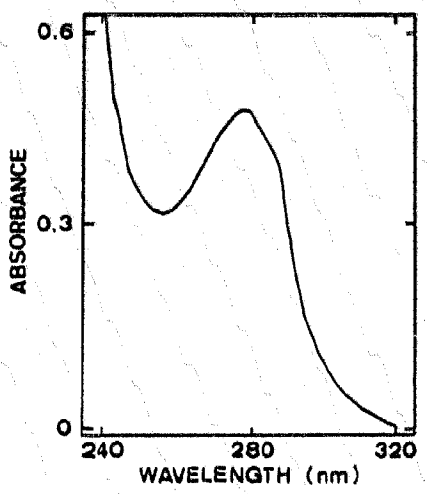


Fig. 2. UV absorption spectrum of the purified pectin lyase from *Penicillium italicum* in 0.05 M Tris-HCl, pH 8.0, and at room temperature.

The absorption spectrum of the purified enzyme from *P. italicum* showed a typical protein spectrum containing no-chromogenic prosthetic groups (no absorption between 320 and 750 nm) and an absorption maximum and minimum at 277 and 255 nm, respectively (Fig. 2). The molar absorption coefficients were 24.2 $\times$ $10^4$ $M^{-1} \cdot cm^{-1}$ (at 277 nm) and 18.7 $\times$ $10^4$ $M^{-1} \cdot cm^{-1}$ (at 255 nm). This spectrum was very similar to that reported for the *Aspergillus japonicus* enzyme [10].

*P. italicum* pectin lyase catalyzed the cleavage of pectins from different sources (citrus and apple) with a wide range of DE (from 37% to 86%) by a transeliminative process. The pure enzyme showed no pectate lyase activity when 0.5% w/v of polygalacturonic acid and 0.8 mM calcium chloride were used to measure the increase of absorbance at 235 nm and pH 9.0.

A $K_m$ for citrus pectin (DE 86%) of 15.0 mg/ml was calculated from a Hanes plot of the initial velocities of increase in $A_{235}$ at different substrate concentrations and at pH 9.0. The obtained $V$ was 26.8 U/ml with a catalytic yield ($V/K_m$) of 1.8 U/mg of pectin. Citrus pectin resulted as a more efficient substrate than apple pectin and the more esterified both of these polysaccharides were, the higher activity values were obtained.

The effect of temperature from 35 to 55°C on pectin lyase activity was examined. The activity increased up to 50°C and the activation energy calculated from an Arrhenius plot was 16 kJ/mol.

Pectin lyase from *P. italicum* was fully thermostable until 45°C and 20% of the activity remained after heating the enzyme for 10 min at 65°C. The enzyme was completely inactivated after 10 min at 80°C. The thermal inactivation was a first-order reaction with an activation energy of 181 kJ/mol.

The pH dependence of the pectin lyase activity was studied in citrate-phosphate (3.0-7.0), potassium phosphate (6.0-8.0), Tris-HCl (7.0-9.0), and 1,3-diaminopropane-HCl (8.0-12.0) buffers. The optimum pH of pectin lyase for pectin transelimination was 9.0, the profile of activity *versus* pH being affected by the substrate concentration (from pH 5.0 to 9.0 with citrate-phosphate and Tris-HCl) and not by the DE (from 6.0 to 10.0 with citrate-phosphate and 1,3-diaminopropane-HCl).

The pH stability of pectin lyase was noteworthy as only 39% of the activity was lost after maintaining the enzyme for 313 h at 4°C and pH 3.0. When the same treatment was performed at pH 10.5, the remaining enzyme activity was 93% of the initial, showing a high stability at basic pH values. Pectin lyase was completely stable in 50 mM Tris-HCl buffer (pH 8.0) for 24 h at room temperature, for several weeks at 4°C and for months at −20°C, in the absence of thiol or chelating reagents.

## 4. DISCUSSION

After the pectin lyase discovery by Albersheim et al. [11] and although several authors have reported the isolation of preparations nearly homogeneous after SDS-PAGE or IEF [4], pectin lyase has only been purified to homogeneity and characterized from the fungal genera *Aspergillus, Dothidea, Ganoderma* and *Rhizoctonia*, and from the bacteria *Erwinia* and *Pseudomonas* [8,12–16].

In the case of *Penicillium* spp., pectin lyase has been detected in crude preparations of *Penicillium citrinum, P. italicum* and *Penicillium paxilli* [17–19] and several efforts have been made to obtain enriched fractions of

the enzyme in *P. digitatum*, *Penicillium expansum* and *P. italicum* [20–22]. Nevertheless, neither a purification scheme capable of yielding an homogeneous pectin lyase preparation after SDS-PAGE or IEF nor a physico-chemical and catalytic characterization of the enzyme from this generus have been developed up to date.

The purification scheme here reported describes, for the first time, the application of a hydrophobic interaction chromatography to obtain a homogeneous pectin lyase preparation from a fungus source. This preparation showed a specific activity of 3934 U/mg protein at 40°C (pH 6.0) with citrus pectin (DE 70%) which corresponded to 7650 U/mg protein at the same temperature (pH 9.0) with citrus pectin (DE 86%). The former value is similar to that reported in *Dothidea ribesia* (3790 U/mg protein) [12] but much higher than those shown by enriched pectin lyase preparations of *Penicillium* spp. (22–48 U/mg protein) [20,22] and other fungi (30–355 U/mg protein) [8,10,23,25]. In bacteria, pectin lyase specific activity values of 433 (*Erwinia chrysanthemi* EC 183) [25] and 1907 U/mg protein (*Ps. fluorescens*) [16] have been reported. The lack of standardization in the assays (mainly in temperature and substrate conditions) is supposed to be the responsible, to some extent, for the diversity of values.

The achieved degree of purification of *P. italicum* pectin lyase (more than 1500-fold), represents the highest value obtained not only in fungi but also in bacteria [4]. In addition, the final yield of activity (41%) is within the range of reported works (11–55%) and much higher than those obtained from previous purification attempts developed in *Penicillium* spp., always with yields lower than 10% [20,22].

Unlike many phytopathogenic microorganisms [8,13,23,24,26], *P. italicum* produces only one pectin lyase, which consists of a single polypeptidic chain of 34 kDa as determined by SDS-electrophoresis in acrylamide gradient gels. A molecular mass of 22 kDa for the pectin lyase of *P. italicum* was observed after gel filtration chromatography. Albersheim and Killias [6], and Bugbee [14] reported retardation of the pectin lyase when it was applied to gel filtration columns using distilled water and 0.2 M sodium chloride in 0.05 M Tris-HCl, pH 8.3, respectively, as the eluants. This interaction between the enzyme and the gel gave false low molecular weights. As a result of this evidence, and because this method is not generally as accepted as the SDS-PAGE one, 34 kDa seems to be the most reliable value for the molecular mass of pectin lyase. This value is consistent with every pectin lyase reported, all of them having a monomeric structure and similar molecular masses (31–35 kDa).

About the isoelectric point (8.6), the *P. italicum* enzyme is within a group of basic pectin lyases from fungi (isoelectric point > 8.) only described in culture filtrates of *Colletotrichum lindemuthianum* (two isoenzymes with pI of 8.2 and 9.7) [26] and purified from *D. ribesia* (8,9) [12] and *Rhizoctonia solani* [14]. Basic pectin lyases have usually been described in bacteria [16,25,27].

Pectin lyase from *P. italicum* is more active with citrus pectin as substrate than using apple pectin, this behaviour being also reported for the purified enzyme from *Ps. fluorescens* [16]. One common feature of pectin lyases is the increasing of the activity when pectin esterification increases [10,12]. This fact has also been observed in our preparations when both pectins (citrus and apple) were used as substrate, the *P. italicum* pectin lyase being able to cleave pectins with a DE from 37% to 86%. Nevertheless, a partially purified pectin lyase which is more effective in degrading low esterified pectins has been described in *Aureobasidium pullulans* [24].

The lack of standardization in the substrate utilised and in the temperature and pH conditions during the assay make it difficult to compare the $K_m$ values of pectin lyases from different microorganisms. With this consideration, $K_m$ values of 1.8 mg/ml for citrus pectin (DE 65%) in enriched pectin lyase preparations of *A. niger* [6] and 3.2 mg/ml in *D. ribesia* [12] have been obtained in fungi. In bacteria, $K_m$ values for highly methoxylated pectins from 0.5 to 9.3 mg/ml have been described in *E. chrysanthemi* EC 183, *E. chrysanthemi* CIR 2002, and *Ps. fluorescens* [15,16,25]. Despite the low affinity of the pectin lyase from *P. italicum* to citrus pectin (DE 86%) when it is assayed at pH 9.0 ($K_m$ = 15.0 mg/ml), this enzyme shows its highest activity under these conditions. The low affinity could be explained by means of an electrical repulsion between the substrate and the enzyme (both negatively charged at pH 9.0). Preliminary studies developed in our laboratory with *P. italicum* pectin lyase have yielded lower $K_m$ values for citrus pectin (DE 86%) when the activity is assayed at pH 6.0. Those results support the hypothetical electrical repulsion which would not happen at pH 6.0 (the enzyme is now positively charged). The results show that the difficulty in achieving the formation of the complex enzyme/substrate (low affinity) at pH 9.0 is followed by a remarkable reactive capacity (high activity).

The optimum pH shown by *P. italicum* pectin lyase (9.0) includes this enzyme within a minority group of fungal (only a 10% of those reported) which show a pectin lyase with an optimum pH equal or higher than 9.0 [26,28]. The optimum pH for some pectin lyases has been described as buffer composition- and buffer and substrate concentration-dependent [8]. As we have also observed this substrate concentration-dependence in our experiments, we thought that this fact could explain the different optimum pH values described in the literature for the same microorganism.

The effect of pH on the stability of pectin lyase from *P. italicum* showed that the enzyme is more stable at

basic pH (up to 10.5) and in a wider range of pH (from 3.0 to 10.5) than many pectin lyases from other microorganisms [10,16,23,24]. This enzyme, besides being resistant to freezing and thawing processes at pH 8.0, is stable even at concentrations as low as 6.5 μg protein/ml while other pectin lyases, as that from *A. japonicus*, lose their activities at concentrations lower than 20 μg protein/ml [10]. In the bacterium *Ps. fluorescens*, the freeze/thawing process produces a 20% loss of pectin lyase activity [16].

The temperature effect on *P. italicum* pectin lyase was similar to that reported for the fungal and bacterial enzymes, whose optimum temperatures ranged from 33 to 55°C were reported [10,12,24,25]. The $E_a$ values described for the fungal enzyme ranged from 6.2 to 35.0 kJ/mol, although these values must be affected by the substrate source, the pH and the temperature range studied.

Finally, pectin lyase from *P. italicum* showed higher thermostability than some pectin lyases described not only in fungi [10,12,23] but also in bacteria [16,29], except two isoenzymes partially purified from *Au. pullulans* which displayed similar thermostability to the *P. italicum* pectin lyase [24]. Especially thermounstable is the pectin lyase from the fungus *D. ribesia* which loses 100% of activity in the range of 40–50°C [12] and the enzyme from the bacterium *Ps. fluorescens* whose activity is inactivated for 30% after 10 min at 36°C [16].

The relevant role played by *P. italicum* in spoilage of citrus products and the importance of pectin lyase in phytopathological and industrial processes justify current and future efforts to get a more convenient understanding of the enzyme from this microorganism. Studies on the mechanism of action, the alteration of vegetable tissues by this biocatalyst, and enzyme immobilization will give us the opportunity to complete this understanding.

*Acknowledgements:* A.A. was the recipient of a scholarship (PFPI) from the Spanish Ministry of Education and Science. This work was partially supported by grants from Diputación Foral de Vizcaya (47-1185) and Universidad del País Vasco/Euskal Herriko Unibertsitatea (310-0122/88).

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