Appl Microbiol Biotechnol (1995) 43:808–814

ORIGINAL PAPER

M. E. Acuña-Argüelles · M. Gutiérrez-Rojas
G. Viniegra-González · E. Favela-Torres

Production and properties of three pectinolytic activities produced by *Aspergillus niger* in submerged and solid-state fermentation

Received: 5 August 1994/Received last revision: 16 January 1995/Accepted: 23 January 1995

Abstract Three extracellular pectinases were produced by *Aspergillus niger* CH4 by submerged and solid-state fermentation, and their physicochemical and kinetic properties were studied. The highest productivities of endo- and exo-pectinase and pectin lyase were obtained with solid-state fermentation. The kinetic and physicochemical properties of these enzymes were influenced by the type of culture method used. All activities were very different in terms of pH and temperature optima, stability at different pH and temperature values and affinity for the substrate (K_m values). In solid-state fermentation, all pectinase activities were more stable at extreme pH and temperature values but the K_m values of endo-pectinase and pectin lyase were higher with respect to those activities obtained by the submerged-culture technique. The pectin lyase activity obtained by the submerged-culture technique showed substrate inhibition but the enzyme obtained by solid-state fermentation did not. Electrophoresis, using sodium dodecyl sulphate/polyacrylamide gel with enzymatic extracts obtained for both culture methods, showed the same number of protein bands but some differences were found in their electrophoretic position. The results obtained in this work suggest that the culture method (submerged or solid-state) may be responsible for inducing changes in some of the pectinolytic enzymes produced by *A. niger*.

Introduction

Pectinases are a group of enzymes that hydrolyse pectin by different mechanisms (Fogarty and Kelly 1982) and are divided into those that attack methyl-esterified pectin (pectinic acid) and those that act on de-esterified pectin (pectic acids). For each class of enzymes there may be hydrolases and lyases that cleave randomly (endo-acting) or at the extremities (exo-acting) the appropriate polymers. The filamentous fungus *Aspergillus niger* produces several of these pectinolytic activities and such enzymes are currently used in fruit juice and wine industries as crude and usually ill-characterised mixtures. Aguilar and Huitrón (1987) have studied basic aspects of pectinase production by submerged fermentation (SmF), Lonsane and Ghildyal (1992) have reviewed the literature of enzymes production by solid-state fermentation (SSF) and Trejo-Hernández et al. (1991) and Solís-Pereira et al. (1993) have compared the pectinases yields and productivity of both techniques, suggesting that SSF is more productive than SmF. However, there are no reports comparing the enzyme profiles and kinetic features of the different types of pectinase activities (exo- and endo-pectinase and pectin lyase) when produced by the two different fermentation techniques mentioned above.

Studies on the production of enzymes by SSF are increasing because of the potential advantages (simplicity, high productivity and concentrated products) over submerged fermentations (Lonsane and Ramesh 1992; Lonsane and Ghildyal 1992; Trejo-Hernández et al. 1991). Characteristic differences in both microorganisms and enzymes, as a function of the kind of culture used, have been recently reviewed (Shankaranand et al. 1992; Lonsane and Ramesh 1992). Among these, Shankaranand et al. (1992) reported that, among 51 bacterial cultures studied, only 3 were able to produce α-amylase in both the SmF and SSF systems. He also found that cultures producing higher titres of the enzyme in the SSF systems were poor enzyme producers in the SmF process and vice versa. Similar results were obtained by our group with two classes of mutants of a wild strain of *A. niger* C28B25 which, after UV random mutation, were selected specifically for each kind

M.E. Acuña-Argüelles · M. Gutiérrez-Rojas
G. Viniegra-González · E. Favela-Torres (✉)
Departamento de Biotecnologia, Universidad Autonoma, Metropolitana, Unidad Iztapalapa, Ave. Michoacan y Purisima, D.F. CP 09340, México.
Fax: + 52-5-6120885

of culture system (Antier et al. 1993). Thus, there is some evidence that regulatory mechanisms of pectinase production by *A. niger* in solid and submerged culture are different, i.e. SSF pectinases synthesis is less affected by catabolic repression than SmF (Solís-Pereira et al. 1993). Similarly, Ramesh and Lonsane (1991) found that α-amylase produced by *Bacillus licheniformis* was less affected by catabolic repression and by final product concentration when produced by SSF than by SmF.

Another interesting and very sparsely studied topic is the physicochemical properties of enzymes produced by each kind of culture. Alazard and Raimbault (1981) and Deschamps and Huet (1987) characterised crude extracts of amylases and xylanases produced by *A. niger* by the SSF and SmF techniques. They found that the optimal temperature values of those enzymes activities were higher for the extracts produced by SSF than those produced by SmF. They also found that enzymes produced by SSF were more thermostable than those obtained by SmF. The optimal pH values for the activity of cellulases and xylanases produced by *Trichoderma* sp. and *Aspergillus terreus* also depended on the kind of culture employed (Brustavetskaya et al. 1992), but their thermostability was not modified. Although some evidence of the effect of the type of culture on metabolic or enzymatic activities has been reported more work is needed in order to characterize and clarify the mechanisms involved.

Therefore, the objective of this work was to study the production of three pectinolytic enzymes by *A. niger* CH4 grown by SSF and SmF techniques, analysing their kinetic and physicochemical properties as indicators of change in enzyme properties generated by the type of fermentation system used in their production.

Materials and methods

Microorganism

The *Aspergillus niger* CH4 strain, originally donated by Dr. C. Huitron from UNAM (Mexico), was used. It was propagated at 35 °C and maintained on potato/dextrose/agar slants at 4 °C.

Culture conditions

Solid-state fermentation experiments were carried out in a medium containing (g) pectin 1.5, sucrose 3.14, ammonium sulphate 1.26, potassium phosphate 0.65, urea 0.3, magnesium sulphate 0.02, ferrous sulphate 0.029, water 70 and sugar cane bagasse pith as support 23.1. Nutrients were dissolved in 80% of the total water, mixed with the sugar cane bagasse and were sterilised at 121 °C for 15 min. After cooling the medium was inoculated with a concentrated spore suspension (3-day-old spores) to 2×10^7 spores/g dry matter. Initial pH and moisture content were 4.5 and 70% respectively. Cultures were carried out at 35 °C in a system similar to that reported by Raimbault and Alazard (1980), using glass columns packed (0.3 g/cm^3) with 50 g solids and with an aeration rate of 60 ml saturated air/min.

Submerged-fermentation experiments were carried out in a medium containing (g) pectin 1.5, ammonium sulphate 0.29, potassium phosphate 0.30, urea 0.10, magnesium sulphate 0.0066, ferrous sulphate 0.0096, sugar cane bagasse pith 1.0 and distilled water up to 100. The initial pH was 4.5. Flasks (250 ml) containing 75 ml medium were sterilised at 121 °C for 15 min, inoculated with 2.25×10^7 spores/flask and incubated at 35 °C in a rotational shaker (180 rpm).

In order to compare enzymatic activities produced in liquid and solid-state fermentation, the activities were calculated as total activity produced per gram of initial inducer (substrate) present in the medium.

Enzymes extract and assays

For SSF, 50 g fermented material was mixed with 50 ml distilled water and immediately pressed at 87 kg/cm^2 in a hydraulic press. The liquid extract was filtered with a Millipore membrane (pore size 0.45 μm) and stored at 4 °C for further enzymatic assays. The culture broth from the SmF samples was centrifuged to remove biomass and subsequently filtered (Millipore, 0.45 μm). The supernatants were kept at 4 °C, for no more than 2 weeks, for further assays.

Pectinolytic activities were measured at 45 °C by viscometry for the endo-pectinase and by the release of reducing groups for the exo-pectinase. For viscometry, 1 ml suitably diluted sample was mixed with 18 ml 2% pectin in citrate/phosphate buffer, pH 5.5, and reduction in viscosity was followed with a rotational viscometer (Brookfield Engineering Laboratories, USA). One endo-pectinase unit (U) was defined as the amount of enzyme that reduced the viscosity of the solution by 50% per minute under the conditions mentioned above. The reducing groups were determinated as reported previously (Solís-Pereira et al. 1993). One exo-pectinase unit (U) was defined as the quantity of enzyme that liberated one micromole of galacturonic acid per minute under the assay conditions. Pectin lyase activity was determinated spectrophotometrically by monitoring the increase in absorbance at 235 nm of a reactive solution containing 2 ml 1% pectin in a pH 7 citrate/phosphate buffer with 0.5 ml dialysed enzymatic extract, adequately diluted, at room temperature (23 °C). The change of absorbance was followed in a Shimadzu UV-160 spectrophotometer for 20 min. One pectin lyase activity unit was defined as the quantity of enzyme needed to increase the absorbance by one unit per minute per millilitre of enzyme extract under the conditions described above.

Electrophoresis

Lyophilised and dialysed crude extracts were analysed by electrophoresis on sodium dodecyl sulphate (SDS) using 10% polyacrylamide slab gels (PAGE; Laemmli 1970). Molecular mass molecular markers MW-SDS-70L (Sigma Chemical Co. USA) were used. Proteins were stained with the Coomassie brilliant blue reagent. The identification of bands with pectinolytic activity was carried out on SDS-PAGE gels containing 0.1% pectin (Ried and Colmer 1985). The gels were washed for 2 h in 10 mM TRIS buffer, pH 7.0, then incubated in citrate/phosphate buffer pH 5 for 60 min at 30 °C. The pectinases were revealed as clear zones after staining with 0.1% ruthenium red.

Results

Comparison of pectinolytic activities produced by *A. niger* CH4 using SSF and SmF techniques

The culture media were optimised according to type and levels of enzymatic activities produced for both systems. The level of sugar cane bagasse added to the

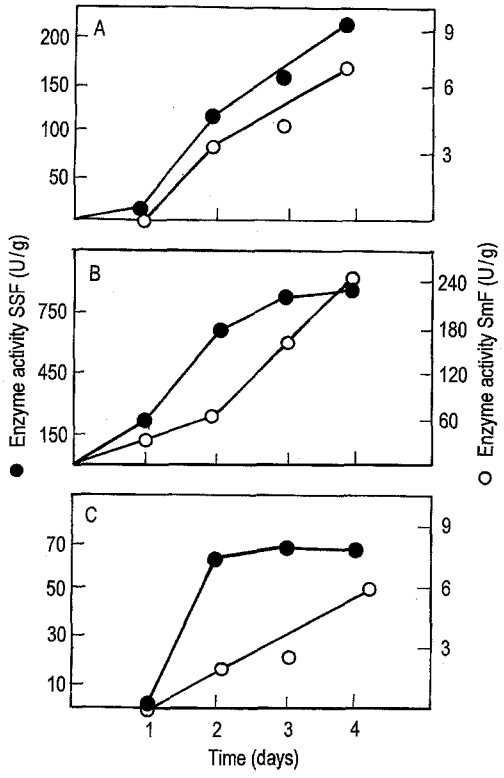

Fig. 1A–C Evolution with time of endo-pectinase (**A**), exo-pectinase (**B**) and pectin lyase (**C**) activities produced by *A. niger* CH4 using solid-state fermentation (*SSF*; ●) and submerged fermentation (*SmF*; ○) techniques

liquid medium did not affect the production of pectinases, nevertheless sucrose addition repressed pectin lyase production therefore it was not included (results not shown here). Pectinolytic activities are shown as a function of time in Fig. 1. SSF cultures showed higher pectinolytic activities as compared to those obtained by SmF (Fig. 1). Endo-pectinase activities produced by the two techniques appeared after 24 h of culture and had similar trends, but higher activity levels were found in SSF extracts (Fig. 1A). Maximal exo-pectinase was obtained after 72 h by SSF while exo-pectinase activity produced by SmF increased more slowly (Fig. 1B). Pectin lyase production obtained by SSF peaked at 48 h, while the activity produced by SmF peaked after 4 days (Fig. 1C). Enzyme productivity was estimated in terms enzyme units per millilitre of fermentation volume and per hour. The comparative ratios of productivities (SSF/SmF) obtained for endo-, exo-pectinase and pectin lyase (Table 1) were 6, 51 and 29 respectively. Showing that, overall, the SSF technique is more productive than SmF.

Effect of temperature on enzyme activity and stability

Pectinolytic activities produced by SSF were more thermostable than those produced by SmF (Fig. 2).

Table 1 Maximal productivity of the pectinases produced by *A. niger* CH4 in liquid and solid-state fermentations. Productivity is calculated as activity (reactor volume)$^{-1}$ (maximal production time)$^{-1}$

Activity	Productivity ($U\ ml^{-1}h^{-1}$)		Productivity ratio (solid/liquid)
	Solid	Liquid	
Endo-pectinase	0.06	0.01	6.05
Exo-pectinase	0.14	0.0002	51.47
Pectinlyase	0.008	0.0002	29.2

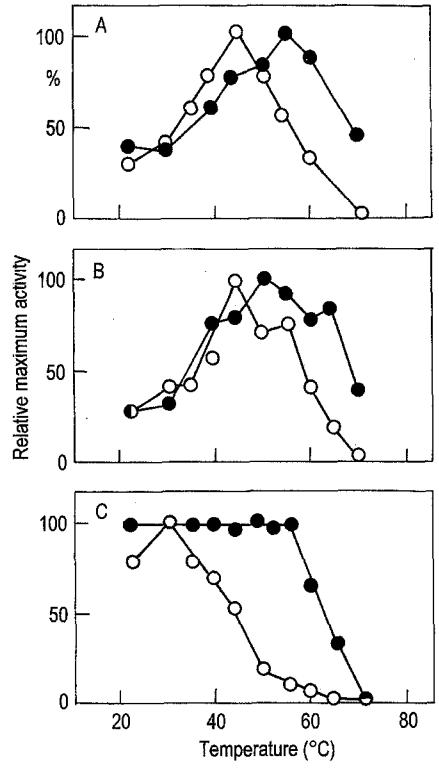

Fig. 2A–C Effect of temperature on endo-pectinase (**A**), exo-pectinase (**B**) and pectin lyase (**C**) activities produced by *A. niger* CH4 using SSF (●) and SmF (○) techniques

Maximal endo-pectinase activity for SSF was found at 60 °C whereas for SmF the maximum was found at 45 °C (Fig. 2A). Exo-pectinase activity obtained by SmF was more thermosensitive than that obtained by SSF; this was noted at temperatures above 45 °C (Fig. 2B). Differences in thermotolerance of pectin lyase activities produced by SSF and SmF were very pronounced, showing a sustained pectin lyase activity at 60 °C for the SSF technique but practically none for SmF (Fig. 2C).

Thermal inactivation was determinated by incubating the enzyme solutions at various temperatures for 30 min before assaying the activities under standard conditions. Inactivation temperatures varied from

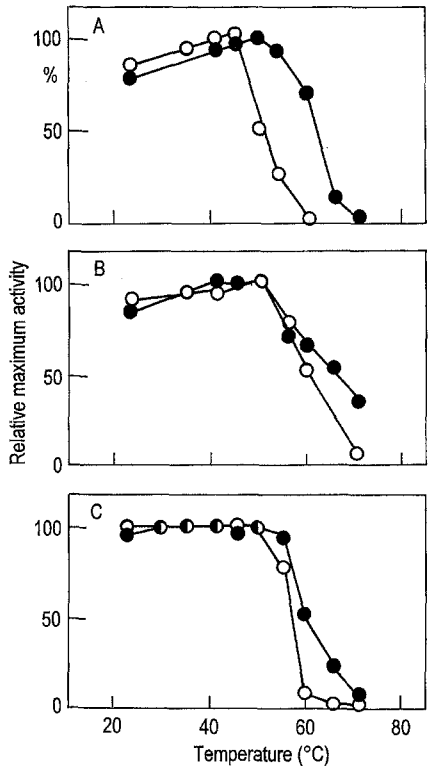

Fig. 3A–C Effect of incubation temperature (30 min) on the stability of endo-pectinase (**A**), exo-pectinase (**B**) and pectin lyase (**C**) activities produced by *A. niger* CH4 using SSF (●) and SmF (○) techniques

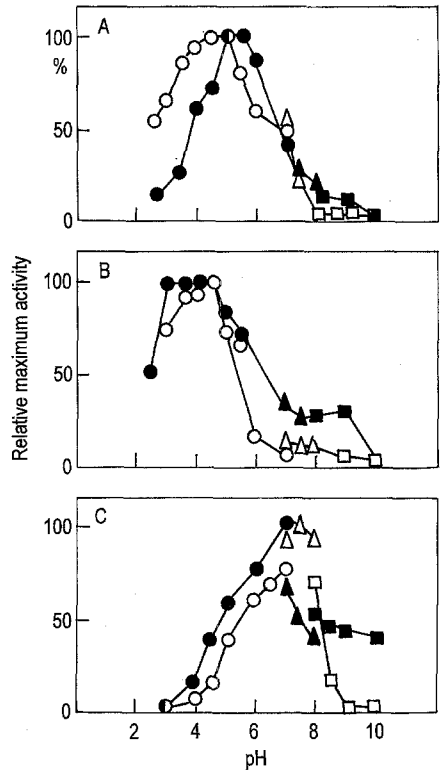

Fig. 4A–C Effect of pH on endo-pectinase (**A**), exo-pectinase (**B**) and pectin lyase (**C**) activities produced by *A. niger* CH4 using SSF (●▲■) and SmF (○△□) techniques. Buffer systems: ○ citrate/phosphate, △ phosphates, □ glycine/NaOH

25 °C to 70 °C (Fig. 3). Practically all pectinolytic activities remained unchanged if the incubation temperatures were lower than 50 °C. At higher temperatures, all activities decreased after the incubation time but this inactivation was more important for the activities produced by SmF as compared to those obtained by SSF.

Effect of pH on enzyme activity and stability

The effect of pH on the pectinolytic activities produced in both types of culture was determined by incubating the reaction mixtures at pH values between 2.6 and 10 (Fig. 4). Maximal endo-pectinase activities were produced by SSF when the pH was in the range 5.5–6.0, while endo-pectinase activities produced by SmF had maximal values for pH in the range 4.5–5.0 (Fig. 4A). For pH values lower than 4.5, endo-pectinase activities produced by SmF were higher than those obtained by SSF (Fig. 4A). Maximum exo-pectinase activity produced by SSF was observed over a wide range of pH values (3.5–5.0) while exo-pectinase obtained by SmF had a narrower pH range (3.5–5.0), as shown in Fig. 4B. Pectin lyase activities produced by SSF and SmF reached maximum values at pH 7.0 and 7.5 respectively (Fig. 4C). At extreme pH values (4 and 9) pectin lyase activity produced by SSF was higher than that produced by SmF (Fig. 4c). The stability of pectinolytic activities at different pH values was determined by incubating the enzyme extracts at different pH for periods of 72 h at 5 °C; results are shown in Fig. 5. Maximal endo-pectinase production by SSF was obtained between pH 2.6 and 6.0. For endo-pectinase produced by SmF the pH range for stability was narrower (6.0–7.0) as shown in Fig. 5A. The exo-pectinase produced by SSF was much more stable in relation to pH denaturation than that produced by SmF (Fig. 5B) since the latter was very sensitive to pH changes from the optimal range (4.0–4.5) whereas, exo-pectinase produced by SSF tolerated significant pH changes (Fig. 5B). Finally, pectin lyase activity was the more stable pectinolytic activity in terms of pH denaturation (Fig. 5C). Both types of culture produced pectin lyase activities that were stable in the pH range 2.5–8.0 (Fig. 5C).

Effect of the substrate concentration on enzyme activity

Pectinolytic activities produced by SSF and SmF were assayed at different pectin concentrations and initial velocities were measured. Exo-pectinase produced by the two types of culture and the endo-pectinase

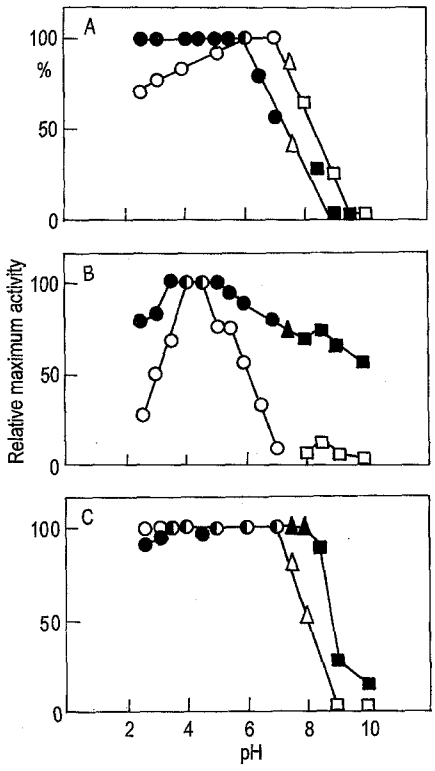

Fig. 5A–C Effect of pH of incubation on the stability of endo-pectinase (**A**), exo-pectinase (**B**) and pectin lyase (**C**) activities produced by *A. niger* CH4 using SSF (●▲■) and SmF (○△□) techniques. Buffer systems: ○ citrate/phosphate, △ phosphates, □ glycine/NaOH

Table 2 Values of apparent K_m of the pectinases produced by *A. niger* CH4 in liquid (*SmF*) and solid-state fermentation (*SSF*)

Enzyme activity	K_m(mg/ml)	
	SSF	SmF
Exo-pectinase	2.05	5.42
Endo-pectinase	270.4	69.4
Pectin lyase	12.8	2.28

produced by SmF showed the typical Michaelis-Menten profile but, in the case of endo-pectinase and pectin lyase produced by SSF, maximal activities were not reached because the extremely high viscosity of the reaction mixture prevented its accurate handling. Pectin lyase activities produced by SmF were strongly inhibited by pectin concentrations down to 10 mg/ml, whereas the activity produced by SSF did not show inhibition at pectin concentrations up to 20 mg/ml. Estimates of the apparent Michaelis constants (K_m) using Lineweaver-Burk plots ($1/v$ versus $1/S$) are shown in Table 2. For exo-pectinase activities the K_m value obtained by SSF (2 mg/ml) was slightly lower than but of the same order of magnitude as the K_m obtained by SmF (5 mg/ml). But for endo-pectinase and pectin lyase activities the K_m obtained by SSF was substantially higher (270 mg/ml and 13 mg/ml respectively) than the K_m obtained by SmF (69 and 2 mg/ml respectively).

Electrophoretic patterns of pectinases produced by SSF and SmF techniques

The electrophoresis on SDS-PAGE of extracellular proteins produced by *A. niger* CH4 cultured by SSF and SmF showed similar electrophoretic patterns in terms of the number of protein bands, but some differences in their localization were found (Fig. 6). In situ detection of the activities of both cultures in the gel showed the presence of four separate pectinases in SSF and three separate pectinases in SmF when the enzyme bands were revealed by ruthenium red. The pectinase activity in SSF appeared on the bands of 68, 46.5, 45 and 36 kDa, although for SmF the pectinase bands were found to have the following molecular masses: 68, 45 and 38 kDa (see arrows).

Discussion

Production and productivities of pectinases in SSF were higher than those produced by SmF. These results are similar to those obtained with endoglucanases and β-glucosidases (Grajek 1987a), xylanases (Deschamps and Huet 1987; Grajek 1987b) and α-amylases (Lonsane and Ramesh 1992) and confirmed an earlier report on pectinase production using the same strain of *A. niger* (Trejo-Hernández et al. 1991).

Optimal temperatures obtained for those pectinolytic activities of SmF were similar to those reported for other strains of *A. niger*. The increase of thermotolerance of the enzymes when produced by SSF rather than SmF indicated in our results on pectinases has also been observed for xylanases (Deschamps and Huet 1987) and amyloglucosidase (Alazard and Raimbault 1981) when produced by different strains of *A. niger*.

The observations related to thermal inactivation confirm that pectinolytic enzymes produced by SSF are more thermostable than those obtained by SmF. Similar conclusions were established by Deschamps and Huet (1987) and Alazard and Raimbault (1981) for xylanases and amylases respectively, although Brustovetskaya et al. (1992) did not find differences in thermal stability of the cellulases and xylanases of *Trichoderma* sp. and *Aspergillus terreus* obtained by both methods of cultivation.

It was observed that pectinases produced by SSF have more stable properties in relation to extreme pH values than those produced by SmF, because the pectinases produced by SSF had broader pH profiles of

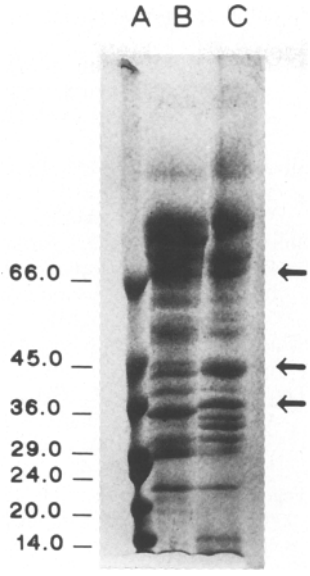

Fig. 6A–C Electrophoretic patterns of the extracellular proteins produced by *A. niger* and analysed in a gel with 0.1% SDS/10% PAGE. **C** SmF, **B** SSF. **A** Molecular mass standards (kDa)

enzyme activities and were denatured more slowly at pH values other than the optimal, as compared to pectinases produced by SmF. Alazard and Raimbault (1981) and Deschamps and Huet (1987) also found differences in the optimal pH values of amylases and xylanases produced by SSF and by SmF.

Results on the effect of substrate concentration on enzyme activity suggest that the pectinolytic enzymes produced by each kind of culture technique had different substrate affinities. Apparently endo-pectinase and pectin lyase activities produced by SSF had a much lower substrate affinity (higher K_m) when produced by SSF than when produced by SmF (lower K_m). The exo-pectinase activity might not be very different when produced by those two different culture techniques, as judged by the relative substrate affinities. The apparent K_m values for pectin lyase activity produced by *A. niger* CH4 (this work) and obtained by SmF were similar to those reported for *P. paxilli* (2.5 mg/ml) using the SmF technique (Szajer and Szajer 1982), although the K_m obtained by SSF was similar (15 mg/ml) to the value reported for strains of *Penicillum italicum* according to Alana et al. (1991) and also in relation to *Penicillum expansum* (9 mg/ml) as reported by Silva et al. (1993) when produced by SmF. There are very few reports comparing substrate affinities for enzymes produced by SSF and SmF. Alazard and Raimbault (1981) found that the affinity of the amyloglucosidase present in a crude extract produced by SmF was double that obtained by SSF. Such a result seems to agree well with values obtained in this work for endo-pectinase and pectin lyase activities, but not for the exo-pectinase activity.

According to the electrophoretic patterns presented in this work, the culture method appeared to induce differences in the mobility of pectinases. Such differences might be due to structural changes of the protein.

It has been reported that some physicochemical properties and electrophoretic patterns can be related to changes in the glycosylation level of enzymes (Neidleman 1990; Lis and Sharon 1993). In fact, it has been shown than the glycosylation level increases the thermostability of the enzymes (Vegeraud and Christensen 1975). It is also known that many fungi have the capacity to produce multiple of forms of extracellular hydrolytic enzymes (or isoenzymes) with differential glycosylation levels (Willick and Selegy 1985; Coughlan and Moloney 1988). Therefore, our preliminary results suggest the possibility that some of the differences in our pectinolytic activities could be related to different forms of the same polypeptides but modified by glycosylation. Future work using specific antibody labeling of enzymes, together with a quantitative glycosylation assay of each electrophoretic band, is necessary to confirm this hypothesis.

For the time being it is quite clear that SSF is not only an interesting way to produce high yields of pectinase activities but also a way to produce more stable enzyme activities for future industrial applications.

Acknowledgements This work was partially financed by Consejo Nacional de Ciencia y Tecnologia (Conacyt, Mexico), ORSTOM (Institut Français de Recherche Scientifique pour le Dévélopement en Cooperation) and Universidad Autónoma Metropolitana (Iztapalapa).

References

Aguilar G, Huitron C (1987) Stimulation of the production of extracellular pectinolytic activities of *Aspergillus* sp. by galacturonic acid and glucose addition. Enzyme Microb Technol 9:690–696

Alana A, Llama MJ, Serra JL (1991) Purification and some properties of the pectin lyase from *Penicillum italicum*. FEBS Lett 2:335–340

Alazard D, Raimbault M (1981) Comparative study of amylolytic enzyme production by *Aspergillus niger* in liquid and solid-state fermentation. Eur J Appl Microbiol Biotechnol 12:113–117

Antier P, Minjares A, Roussos S, Raimbault M, Viniegra G (1993) Pectinase-hyperproducing mutants of *Aspergillus niger* C28B25 for solid state fermentation of coffee pulp. Enzyme Microb Technol 15:254–260

Brustovetskaya TP, Okunev ON, Shulga AV (1992) Formation and properties of fungal cellulases and xylanases in a liquid medium and in the conditions of solid-phase fermentation. Appl Biochem Microbiol 27:438–443

Coughlan MP, Moloney AP (1988) Isolation of 1,4-β-D-glucan 4-glucanohydrolase of *Talaromyces emersonii*. Methods Enzymol 160:363–368

Deschamps F, Huet MC (1985) Xylanase production in solid-satate fermentation: a study of its properties. Appl Microbiol Biotechnol 22:177–180

Fogarty WM, Kelly CT (1982) Microbial enzymes and biotechnology. Applied Science, London, pp 131–182

Grajek W (1987a) Comparative studies on the production of cellulases by thermophilic fungi in submerged and solid-state fermentation. Appl Microbiol Biotechnol 26:126–129

Grajek W (1987b) Production of xylanases by thermophilic fungi using different methods of culture. 9:353–356

Laemmli UK (1970) Cleavege of estructural proteins during the assembly of the head of bacteriophage T4 Nature 227:680–685

Lis H, Sharon N (1993) Protein glycosylation: structural and functional aspects. Eur J Biochem 218:1–27

Lonsane BK, Ghildyal NP (1992) Exoenzymes. In: Solid substrate cultivation. Doelle HW, Mitchell DA, Rolz CE (eds) Elsevier Applied Science, London

Lonsane BK, Ramesh MV (1992) Production of bacterial termoestable enzymes by solid state fermentation: a potential tool for achieving economy in enzyme production. Adv Appl Microbiol 15:1–48

Neidelman SL (1990) Enzyme reactions under stress conditions. Crit Rev Biotechnol 9:273–286

Raimbault M, Alazard D (1980) Culture method to study fungal growth in solid state fermentation. Appl Microb Biotechnol 9:199–202

Ramesh MV, Lonsane BK (1991) Regulation of alpha amylase production in *Bacillus licheniformis* M27 by enzyme end-products in submerged fermentation and its overcoming in solid state fermentation system. Biotechnol Lett 19:355–360

Ried JL, Colmer A (1985) Activity stain for rapid characterization of pectic enzymes in isolectric focusing and sodium dodecyl sulphate polyacrylamide gels. Appl Environ Microbiol 50:615–622

Shankaranand VS, Ramesh MV, Lonsane BK (1992) Idiosyncrasies of solid state fermentation systems in the biosynthesis of metabolites by some bacterial and fungal culture. Process Biochem 27:33–36

Silva DO, Attwood MM, Tempest DW (1993) Partial purification and properties of pectin lyase from *Penicillum expansum*. World J Microbiol Biotechnol 9:574–578

Solís-Pereira S, Favela-Torres E, Viniegra-González G, Gutiérrez-Rojas M (1993) Effect of different carbon sources on the synthesis of pectinase by *Aspergillus niger* in submerged and solid state fermentations. Appl Microbiol Biotechnol 39:36–41

Szajer I, Szajer C (1982) Pectin lyase of *Penicillum paxilli*. Biotechnol Lett 4:549–552

Trejo-Hernandez MR, Oriol E, Lopez-Canales A, Roussos S, Viniegra G, Raimbault M (1991) Production of pectinases by *Aspergillus niger* by solid state fermentation on support. Micol Neotrop Apl 4:49–62

Vegeraud G, Christensen TB (1975) Glycosylation of proteins: a new method of enzyme stabilization. Biotechnol Bioeng 27:1391–1397

Willick EG, Seligy VL (1985) Multiplicity in cellulases of *Schizophyllum commune*: derivation partly from heterogeneity in transcription and glycosylation. Eur J Biochem 151:89–96