

Saccharification of Straw by Actinomycete Enzymes

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(Received 17 December 1987; revised 18 March 1988)

Over 200 strains of actinomycetes, representing nine distinct genera, were screened directly for the ability to release reducing sugar from ball-milled wheat straw, using a microtitre plate assay system. Xylanase activity was detected in nearly all of the strains examined while activities against purified cellulosic substrates were less widespread and relatively low. Straw saccharification resulted from cooperative enzyme action and sugar yields were not simply correlated with substrate particle size. Straw-saccharifying activity was further characterized in selected strains comprising five representatives of the genera *Thermomonospora* and *Streptomyces*, one *Micromonospora* strain and the type strain of *Microbispora bispora*. Common features included optimal saccharification of straw in the pH range 6.0–9.0 and xylose and its oligomers as the principal products, although low concentrations of glucose were also detected. Optimal activity and increased stability at 70 °C was a feature of enzyme preparations from *Thermomonospora* and thermophilic *Streptomyces* strains. β -Xylosidase and β -glucosidase activities were largely intracellular, but significant amounts of extracellular β -xylosidase activity were also found in two strains. Other enzymes involved in straw saccharification include acetylesterase and arabinofuranosidase, and these activities were detected in all strains. Acetylesterase and arabinofuranosidase activities were largely extracellular, but in some strains significant amounts of intracellular activity were also detected.

INTRODUCTION

Agricultural residues such as grass lignocelluloses represent large renewable resources for which enzymic generation of fermentable sugars is one of a number of alternative strategies currently under investigation. Wheat straw is a widely available substrate and its disposal also presents an environmental problem. The suitability of wheat straw for bioconversion processes is further enhanced by its relatively low lignin content (<20%, w/w) and high content (up to 30%, w/w) of readily degradable hemicellulose (arabinoxylan) (Ladisich *et al.*, 1983). Actinomycetes are widely distributed in natural environments, such as soils and composts, where they make an important contribution to nutrient recycling and humification. They are therefore a potentially useful source of plant biomass-degrading enzymes, and activity against the major components (lignin, hemicellulose and cellulose) has been identified in many strains (McCarthy, 1987). However, the enzymology of lignocellulose degradation by actinomycetes remains poorly understood. It is likely that a concerted attack against all components of the substrate, probably involving synergistic action between enzymes, takes place as has been described for fungal cellulases (Wood & McCrae, 1978) and hemicellulases (Biely *et al.*, 1986).

Actinomycete cellulases and xylanases are inducible extracellular enzymes which are often produced simultaneously (Kluepfel & Ishaque, 1982; Van Zyl, 1985). Endoglucanase and exoglucanase components of the cellulase activity have been separated in several species including *Thermomonospora fusca* (Hägerdal *et al.*, 1978; Calza *et al.*, 1985) and *Microbispora bispora* (Bartley *et al.*, 1984). Analogous studies on actinomycete xylanase activity are fewer but a number of workers have successfully purified endoxyylanases from *Streptomyces* strains

Abbreviation: CMC, carboxymethylcellulose.

(Nakajima *et al.*, 1984; Kusakabe *et al.*, 1977; Morosoli *et al.*, 1986). Finally, genes coding for endoglucanase activity in *T. fusca* (Collmer & Wilson, 1983; Ghangas & Wilson, 1987) and endoxylanase activity in a *Streptomyces* sp. (Iwasaka *et al.*, 1986) have been cloned as an important step towards understanding the enzymology of lignocellulose saccharification and obtaining improved enzyme production. Disaccharidases (β -glucosidase and β -xylosidase) are cell associated in those actinomycetes studied to date (McCarthy, 1987) but it is possible to generate monomeric xylose directly from lignocellulosic substrates by treatment with culture supernatants (McCarthy *et al.*, 1985; Van Zyl, 1985). The objective of this study was to screen and directly compare the straw-saccharifying activity of actinomycete strains from a range of genera, including both mesophiles and thermophiles, and to select representative strains for further biochemical characterization.

METHODS

Strains and growth conditions. A collection of over 200 strains, including 60 fresh isolates from compost samples, obtained by the sedimentation chamber/Andersen sampler method (McCarthy & Broda, 1984), were maintained as suspensions of spores and hyphal fragments in 20% (v/v) glycerol at -70°C . Strains were routinely cultured on L-agar plates (Hopwood *et al.*, 1985) and distilled water suspensions of sporulating growth used to inoculate shake flasks containing basal salts medium plus 0.1% (w/v) yeast extract (McCarthy & Broda, 1984), supplemented with 0.2% (w/v) ball-milled wheat straw. Cultures were incubated for 72 h at 200 r.p.m. at 30°C for mesophilic strains or 50°C for thermophiles.

Enzyme preparations. Cultures were centrifuged at 10000 g for 10 min at 4°C and the supernatants appropriately diluted in 0.1 M-potassium phosphate buffer (pH 7.0) for enzyme assays. The harvested pellets were used to determine intracellular protein concentration. Where necessary, residual reducing sugar was removed by dialysis against 0.1 M-potassium phosphate buffer (pH 7.0). Enzyme preparations were stored with added sodium azide (0.03%, w/v) at -20°C . Intracellular enzyme activities were assayed in culture extracts prepared by sonication in an ice bath (3×30 s at 18 μm peak to peak) of washed culture pellets.

Assays. Enzyme preparations were assayed for the release of reducing sugar from the following substrates: ball-milled wheat straw, oat spelt xylan (Sigma), carboxymethylcellulose (CMC; low viscosity, BDH) and cellulose powder (CF11, Whatman). Some enzyme preparations were also assayed for release of reducing sugar from vibratory ball-milled straw (kindly supplied by Dr W. Zimmerman, Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester, UK). The reaction mixture for reducing sugar assays contained (total volume 200 μl): 160 μl enzyme preparation and 40 μl 0.1 M-potassium phosphate buffer (pH 7.0) containing 25 mg substrate ml^{-1} in each well of a microtitre plate. The total protein concentration in the enzyme preparations was in the range 46–240 $\mu\text{g ml}^{-1}$. Controls were routinely included in which enzyme preparation or substrate was omitted. Initially, results were compared with standard reducing sugar assays in test tubes (McCarthy *et al.*, 1985) to demonstrate correlation with the microtitre plate assay. Assays on enzyme preparations from thermophiles were incubated at 65°C and those from mesophiles at 55°C . After 15 min, the contents of the wells were removed, held in an ice-bath for 30 s and centrifuged for 1 min to remove insoluble material. Reducing sugar levels in the supernatant were determined by the dinitrosalicylic acid method (Miller, 1959). Enzyme activities were calculated as μmol reducing sugar released per min (IU) per mg intracellular protein.

Intracellular protein was determined by boiling harvested pellets for 20 min with 1 M-NaOH prior to protein estimation by the Lowry method. Intracellular protein was used as an index of growth, having first established a correlation with determinations of biomass by dry weight measurements and the absence of interference due to the presence of straw. Extracellular protein was also determined by the Lowry method. β -Glucosidase, β -xylosidase, acetylesterase and arabinofuranosidase activities were assayed using 4-nitrophenol β -D-glucoside (PNPG, Koch-Light), 4-nitrophenol β -D-xyloside (PNPX, Koch-Light), 4-nitrophenylacetate (NPA, BDH) and 4-nitrophenyl α -L-arabinofuranoside (PNPAF, Sigma), respectively, as substrates. The methods described by Hägerdal *et al.* (1979) (β -glucosidase), Ristroph & Humphrey (1985b) (β -xylosidase and arabinofuranosidase) and Biely *et al.* (1985) (acetylesterase) were adapted for use in microtitre plates, as described above.

Thin-layer chromatography (TLC) of degradation products. Conical flasks (25 ml) containing 6.4 ml undiluted, dialysed enzyme preparation, 1.6 ml 0.1 M-potassium phosphate buffer (pH 7.0) and 50 mg ball-milled wheat straw were incubated with shaking at 65°C (thermophiles) or 55°C (mesophiles). Samples were removed at intervals of up to 24 h, clarified by centrifugation and stored at -20°C . The products of enzyme hydrolysis of ball-milled straw, xylan and CMC were separated by ascending TLC on pre-coated cellulose plates (Merck; layer thickness 0.1 mm) in the solvent system formic acid/butanone/*tert*-butanol/water (15:30:40:15, by vol.). Samples (20 μl) and reference standards (0.1 M; 5 μl) were applied to chromatograms, which were developed twice and sprayed with a solution of phthalic acid (3.25 g) in 100 ml water-saturated butanol plus 2 ml aniline. After heating

at 90 °C for 5 min, spots stained brown (hexose) or pink (pentose) were located and R_f values relative to D-glucose were determined. The concentration of D-glucose in saccharified straw samples was also estimated using the glucose oxidase method described by Kunst *et al.* (1984).

Temperature and pH relationships. The pH optima for straw saccharification were determined by replacing phosphate buffer in the reaction mixture with Britton and Robinson Universal buffer (Johnson & Lindsey, 1939). Optimum pH conditions were determined in the range 3.0–11.0. Assays for the release of reducing sugar from straw and xylan after 15 min at temperatures in the range of 30–90 °C were used to determine the temperature optima. The thermostability of straw-saccharifying enzymes was assessed by pre-incubation of culture supernatants for 5 min at temperatures in the range 55–95 °C. After incubation, samples were assayed at 55 °C for the release of reducing sugar from ball-milled straw. Control experiments were also done in which supernatants were held in an ice-bath for 5 min, after heat treatment and prior to assaying for enzyme activity.

RESULTS

Saccharification of straw and related substrates by actinomycete extracellular enzyme preparations

The microtitre plate assay technique enabled the rapid screening of over 200 actinomycete strains for the production of enzymes capable of releasing reducing sugar from ball-milled wheat straw. The microtitre assay and standard assay procedure gave similar calibration curves and comparable enzyme activities, indicating the validity of this rapid method. A summary of the results of enzyme assays is presented in Table 1. *Thermomonospora* and *Streptomyces* strains were the most active against all substrates but there was considerable variation between individual strains. Activities against crystalline cellulose were found to be low [< 1 unit (mg intracellular protein)⁻¹] in all strains examined. The yields of reducing sugar were consistently higher from xylan but enzyme preparations from some strains of *T. chromogena* and *Micromonospora* were unusual in that activities against straw were high relative to their activity against xylan. Expression of enzyme activity as a function of intracellular protein concentration compensated for any differences in enzyme activity solely due to differences in growth yield. Our general conclusion is that an efficient actinomycete system for lignocellulose degradation may require combinations of properties exhibited by different strains.

Mechanical pre-treatment of lignocellulosic substrates such as straw results in a greater degree of saccharification due to an increase in the surface area available for enzymic attack (McCarthy *et al.*, 1985). The effect of pre-treatment on saccharification by actinomycete enzyme preparations was investigated by comparing straw milled in a conventional rolling ball mill with

Table 1. Enzyme activity [units (mg intracellular protein)⁻¹] of culture supernatants from actinomycetes

Results from each strain are the means of three replicates. (T) denotes thermophilic strains.

Actinomycete	No. of strains tested	Range of enzyme activities detected against:		
		Xylan	CMC	Straw
<i>Thermomonospora</i>				
<i>T. fusca</i> (T)	27	1–54	0–3	0–4
<i>T. curvata</i> (T)	17	1–5	0–3	0–2
<i>T. chromogena</i> (T)	20	0–7	0–3	0–2
<i>T. alba</i>	7	1–11	0–1	0–3
<i>Micromonospora</i>	10	0–3	0–2	0–4
<i>Streptomyces</i>				
<i>mesophiles</i>	61	0–66	0–3	0–4
<i>thermophiles</i> (T)	14	1–54	0–5	0–4
<i>Nocardia</i>	6	0–1	0	0
<i>Saccharomonospora viridis</i> (T)	10	0–3	0–1	0–1
<i>Thermoactinomyces</i> (T)	12	1–4	0–1	0–1

Table 2. Enzyme activity [units (mg intracellular protein)⁻¹] of culture supernatants from selected actinomycete strains

Results from each strain are the means of three replicates. Standard deviations were all within 15% of the mean values presented. Enzyme activity per ml culture supernatant is given in parenthesis. (T) denotes thermophilic strains.

Actinomycete	Strain	Enzyme activity detected against:			
		Xylan	CMC	Cellulose	Straw
<i>T. fusca</i> (T)	MT100	53.5 (8.3)	3.0 (0.5)	1.1 (0.2)	3.7 (0.6)
<i>T. chromogena</i> (T)	MT808	3.3 (0.4)	1.7 (0.2)	<0.1 (<0.1)	3.0 (0.3)
<i>Micromonospora</i>	LL23	2.9 (0.1)	1.5 (<0.1)	<0.1 (<0.1)	3.3 (0.2)
<i>Streptomyces</i> (T)	EC22	53.6 (9.4)	4.6 (0.8)	0.5 (0.1)	4.6 (0.8)
<i>Streptomyces</i>	EC3	67.0 (11.8)	2.2 (0.4)	0.8 (0.1)	3.6 (0.6)
<i>Streptomyces</i>	EC1	69.1 (12.4)	1.0 (0.2)	0.8 (0.1)	3.3 (0.6)
<i>Microbispora bispora</i> (T)	DSM 43038	8.9 (1.3)	3.1 (0.5)	4.0 (0.6)	4.9 (0.7)

Table 3. Activities of β -glucosidase, β -xylosidase, arabinofuranosidase and acetylerase in culture supernatants and cell extracts of the selected strains

Results are expressed in μ mol 4-nitrophenol released min⁻¹ (mg intracellular protein)⁻¹ and are the means of three determinations. Standard deviations were all within 15% of the mean values presented. I denotes cell extract activities; E denotes extracellular activities.

Actinomycete	Strain	Enzyme activity							
		β -Glucosidase		β -Xylosidase		Arabino-furanosidase		Acetyl-esterase	
		I	E	I	E	I	E	I	E
<i>Streptomyces</i>	EC1	32	2	99	16	73	250	44	114
<i>Streptomyces</i>	EC22	37	4	28	0	20	245	24	166
<i>Streptomyces</i>	EC3	31	2	50	0	58	250	27	123
<i>T. fusca</i>	MT100	36	2	88	38	35	120	123	143
<i>Micromonospora</i>	LL23	29	4	40	0	65	97	89	200
<i>T. chromogena</i>	MT808	18	2	25	0	16	67	45	100
<i>Microbispora bispora</i>	DSM 43038	20	1	20	0	27	151	79	74

that prepared in a vibratory ball mill. Although both substrates were identical in gross morphological appearance, the vibratory-ball-milled straw was always more readily saccharified. In 48 strains compared in this way, the activity against ball-milled straw was 50% (\pm SD 8%) of that on vibratory ball-milled straw. Scanning electron microscopy suggested that this difference in degradability was not due to decreased particle size, but due to the production of straw particles of a more amorphous and less crystalline appearance by the vibratory action of the ball mill. By comparison, the yield of reducing sugar from finely chopped straw prior to ball milling was 24% (\pm SD 7%) of that from vibratory ball-milled straw.

Characterization of straw saccharification by extracellular enzyme preparations from selected strains

The seven strains selected for further study included the most active mesophilic and thermophilic streptomycetes, the most active *T. fusca* strain and strains of *T. chromogena* and *Micromonospora* which were active against straw despite their limited xylanolytic activity. The type strain of *Microbispora bispora* was included due to its relatively high activity against fibrous cellulose. The enzyme activity profiles of these strains, expressed as a function of total protein are presented in Table 2. Comparison of these activities with those given in units (ml culture supernatant)⁻¹ (Table 2) clearly shows that the enzyme activity of slow-growing strains, e.g.

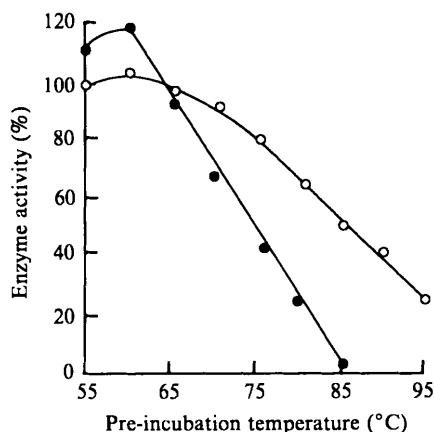


Fig. 1. Thermostability curves for straw saccharification by enzyme preparations from *Streptomyces* strains EC1 (●) and EC22 (○). Activity is expressed as a percentage of the value for untreated enzyme. All samples were assayed at 55 °C.

Micromonospora strain LL23, is underestimated when comparative growth yields (intracellular protein) are not considered. The seven selected strains were also examined for the presence of other enzymes likely to be involved in straw saccharification (Table 3). In all strains β -glucosidase activity was mainly associated with culture solids, with less than 10% of the total activity present in extracellular fractions. Maximum β -glucosidase activities were found in *Streptomyces* strains EC1 and EC3 and *T. fusca* strain MT100. β -Xylosidase activities were highest in *Streptomyces* strains EC1 and EC3, and *Thermomonospora* strain MT100. The association of disaccharide-hydrolysing activity with culture solids was even more pronounced for β -xylosidase, with no activity detectable in the culture supernatants of five strains. However, appreciable levels of β -xylosidase activity were detected in culture supernatants of *T. fusca* MT100 (33% of total) and *Streptomyces* sp. EC1 (12% of total). In each strain, cell-associated and extracellular β -xylosidase activities exhibited identical thermostability properties, suggesting leakage of intracellular enzyme. In contrast, arabinofuranosidase and acetylsterase activities were largely extracellular, with the partition more pronounced for arabinofuranosidase. Indeed, intracellular levels of acetylsterase were comparable to those detected in culture supernatants of *Microbispora bispora* and *T. fusca* strains. Again, for all of these enzyme activities the results obtained using the microtitre plate assay were comparable to those obtained using the standard assay procedure.

pH and temperature relationships of straw-saccharifying activity

The pH optima for straw saccharification were in the range pH 7.0–9.0 for all enzyme preparations examined. *Microbispora bispora* DSM 43038, *T. fusca* MT100 and the three *Streptomyces* strains (EC1, EC3, EC22) all produced enzyme activity with similar pH profiles; 50% of maximum activity (pH 8) occurred at pH 5.5 and pH 11 (approx.). *T. chromogena* MT808 and *Micromonospora* LL23 enzyme preparations had similar profiles to one another and distinct from the other strains. Enzyme activity was high over a wider range of pH values, with maximum activity at pH 9 (approx.) and 50% at pH 4.5 and pH 11.5 (approx.).

Maximum straw saccharification (measured over a 15 min period) by enzyme preparations from the thermophilic strains (MT100, MT808, EC22, DSM 43038) occurred at approximately 70 °C, compared to approximately 60 °C for the mesophilic strains (LL23, EC1, EC3). This was reflected in the thermal inactivation curves of enzyme preparations in which the mesophilic and thermophilic strains formed two homogeneous and distinct groups. Representative curves are presented in Fig. 1. The data show, for example, that pre-incubation at 85 °C for 5 min is sufficient to completely inactivate enzymes from mesophiles, while those of thermophiles retain 50% (approx.) of their activity. When these experiments were repeated with inclusion of a

cooling step prior to assay, identical results were obtained, suggesting that inactivation was due to irreversible denaturation rather than temperature-induced dissociation of enzyme subunits. In addition, the enzyme preparations exhibited some degree of thermal activation, a phenomenon which has been described previously for *T. curvata* endoglucanase (Stutzenberger & Lupo, 1986). These thermal activation effects were also observed when heat-treated enzyme preparations were cooled prior to assay.

Products of straw saccharification

Hydrolysis of ball-milled wheat straw by the selected mesophilic and thermophilic actinomycete culture supernatants yielded a number of products. On TLC plates the mobility of the glucose and xylose oligomers is inversely proportional to their M_r (Uchino & Nakane, 1981) and R_f values were calculated relative to glucose (R_f 100). Spots located at R_f 54 (xylobiose) and R_f 20 (oligomeric xylose) were the dominant products of straw saccharification by culture supernatants of all seven strains. These products appeared after incubation for 3 h and the intensity of the spots continued to increase for a further 3 h. Xylose (R_f 146) was detectable after 3 h in *T. fusca* strain MT100 and *Streptomyces* strains EC1 and EC3 and continued to increase for a further 3 h. After 24 h xylose was detectable in straw saccharifications by enzyme preparations from all strains. Since hexoses were not detected, the TLC results indicate that straw saccharification was primarily due to enzymic attack of the hemicellulose component. However, glucose was detected by glucose oxidase assay (at a concentration of 6–9% of the total reducing sugar present), and as neither glucose nor its oligomers have been found in cereal straw hemicellulose fractions (Whistler & Richards, 1970), this confirms that hydrolysis of the cellulose component had also occurred.

DISCUSSION

Studies on lignocellulose saccharification by enzyme preparations from actinomycetes have concentrated on the use of commercially available substrates applicable to investigations of the enzymology of cellulose and xylan utilization. In this paper, we have attempted to circumvent this approach by screening directly for the ability to saccharify ball-milled straw as a prerequisite for further enzymological studies. Modification of the reducing sugar assay (Miller, 1959) for use with microtitre plates enabled rapid quantitative screening on a relatively large scale and was found to be sufficiently valid and reproducible for further experiments on enzyme properties. The microtitre assay was also applicable to the assay of β -glucosidase, β -xylosidase, acetylsterase and arabinofuranosidase activities and can be used to identify endoxylanase, endoglucanase, β -glucosidase and β -xylosidase activity in fractions generated by FPLC (S. Bachmann, personal communication). Results from the screening programme revealed considerable variation in the ability of strains to saccharify different substrates. Generally reducing sugar release from xylan was greater (approx. 10-fold) than that from ball-milled wheat straw. Although wheat straw hemicellulose is largely arabinoxylan, it is covalently bound to lignin to form a ligno-carbohydrate complex. This, together with the insolubility of ball-milled straw, will limit the degree of hydrolysis attainable and may explain the limited variation in sugar yields from straw compared to the considerable variation in xylanolytic and cellulolytic activities (Tables 1 and 2).

Comparison of reducing sugar yields from vibratory and non-vibratory ball-milled straw together with scanning electron microscopic analysis demonstrated that particle size is not the only determinant of saccharification efficiency in mechanically pre-treated lignocellulose. These results are in agreement with other data illustrating the limitations of particle size as an indicator of potential conversion (Rivers & Emert, 1987) and support the hypothesis that vibratory action disrupts the ligno-carbohydrate matrix in addition to reducing particle size (Paterson *et al.*, 1984). Previous studies have demonstrated the ability of actinomycete culture filtrates to generate xylose directly from grass lignocelluloses (McCarthy *et al.*, 1985; Van Zyl, 1985) but although some xylose was produced from straw by the strains studied here, xylobiose and oligomeric xylose were the dominant products, probably resulting from endoxylanase

activity. Improved xylose yields could possibly be obtained by addition of β -xylosidase in the form of culture solids or extracts, analogous to the approach used to improve glucose yields from cellulose, to relieve end-product inhibition (Ferchak *et al.*, 1980). Neither xylose nor glucose had any inhibitory effects on straw saccharification by the enzyme preparations studied here, and it is presumed that oligomeric sugars were responsible for the end-product inhibition effects which necessitated dilution of enzyme preparations prior to assay. Enzyme preparations from all the selected strains contained cellulase activity but straw cellulose degradation products could not be detected by TLC. It is clear, therefore, that even if cellulase activity is increased or more highly cellulolytic actinomycetes such as *Microbispora bispora* DSM 43038 used as a source of enzyme, xylose will be a major end-product of straw saccharification, necessitating the development of pentose fermentation technology (Ladisch *et al.*, 1983).

It is becoming increasingly appreciated that efficient hydrolysis of native lignocellulose to yield utilizable quantities of pentoses and glucose requires the cooperative action of a range of enzymes, in addition to endoglucanases and endoxylanases. β -Glucosidases and β -xylosidases are responsible for the final steps in saccharification yielding glucose and xylose, and we have shown that these appear to be intracellular enzymes in actinomycetes in general. Efficient degradation of native cellulose requires the synergistic action of exoglucanase (cellobiohydrolase) and although we have yet to demonstrate specifically the presence of this activity in enzyme preparations from actinomycetes, this enzyme has been described previously in *Microbispora bispora* (Bartley *et al.*, 1984). Other enzymes involved in native arabinoxylan degradation include α -glucuronidases, acetylsterases and arabinofuranosidases (Biely, 1985). Activity of the latter two has been shown to enhance xylan degradation (Biely *et al.*, 1986; Greve *et al.*, 1984) and these enzymes are produced by actinomycetes in considerable amounts (Table 3). As expected, these are largely extracellular activities which are presumably deacetylating straw xylan and hydrolysing arabinose branch points to increase the amount of substrate accessible to endoxylanase.

Actinomycete cellulases and xylanases generally act optimally at pH values close to neutrality (McCarthy, 1987) and the pH optima reported here for straw-saccharifying activity conform to this pattern. Activity and stability at high temperatures ($>60^\circ\text{C}$) are also features of enzyme preparations from thermophilic strains, confirmed by the results of this study. Studies on pH and temperature relationships of actinomycete cellulases and xylanases have been concentrated on thermophilic thermomonosporas (McCarthy *et al.*, 1985; Stutzenberger, 1972; Hägerdal *et al.*, 1980; Ristroph & Humphrey, 1985a) and mesophilic streptomycetes (Kluepfel *et al.*, 1986; MacKenzie *et al.*, 1984; Van Zyl, 1985; Nakajima *et al.*, 1984). In this paper we have also described a thermophilic *Streptomyces* strain (EC22) whose straw-saccharifying enzymes exhibit similar pH and temperature relationships to those of thermomonosporas. Thus, the pH and temperature profiles of enzyme preparations from thermophilic actinomycetes in general may have an application in the saccharification of alkali-treated lignocellulose coupled to fermentation and ethanol recovery by direct distillation at high temperature (Sonnleitner & Fiechter, 1983).

It is clear that, while the properties of enzymes involved in straw saccharification by different actinomycetes have much in common, there are also significant differences in enzyme profiles between strains. Further characterization of the enzymes involved could lead to enhancement of straw saccharification for the production of fermentable substrates.

The technical assistance of Joanne Harrison and Paul Fieldsend is gratefully acknowledged. The authors also wish to thank Dr G. Sharples, Biology Department, Liverpool Polytechnic, for Scanning Electron Microscopy, Dr M. J. Penninckx and colleagues of the Microbiology Department, University of Brussels, for providing compost samples and some of the strains, and Dr W. Amner of this department for compost isolates. This research was funded by the EEC Non-Nuclear Energy Programme.

REFERENCES

- BARTLEY, T., WALDRON, C., & EVELEIGH, D. (1984). A cellobiohydrolase from a thermophilic actinomycete, *Microbispora bispora*. *Applied Biochemistry and Biotechnology* **9**, 337-338.

- BIELY, P., PULS, J. & SCHNEIDER, H. (1985). Acetyl xylan esterase in fungal cellulolytic systems. *FEBS Letters* **186**, 80–84.
- BIELY, P. (1985). Microbial xylanolytic systems. *Trends in Biotechnology* **3**, 286–290.
- BIELY, P., MACKENZIE, C. R., PULS, J. & SCHNEIDER, H. (1986). Co-operativity of esterases and xylanases in the enzymatic degradation of acetyl xylan. *Biotechnology* **4**, 731–733.
- CALZA, R. E., IRWIN, D. C. & WILSON, D. B. (1985). Purification and characterisation of two β -1,4-endoglucanases from *Thermomonospora fusca*. *Biochemistry* **24**, 7797–7804.
- COLLMER, A. & WILSON, D. B. (1983). Cloning and expression of a *Thermomonospora* YX endocellulase gene in *E. coli*. *Biotechnology* **1**, 594–601.
- FERCHAK, J. D., HÄGERDAL, B. & PYE, E. K. (1980). Saccharification of cellulose by the cellulolytic system of *Thermomonospora* sp. II. Hydrolysis of cellulosic substrates. *Biotechnology and Bioengineering* **22**, 1527–1542.
- GHANGAS, G. S. & WILSON, D. B. (1987). Expression of a *Thermomonospora fusca* cellulase gene in *Streptomyces lividans* and *Bacillus subtilis*. *Applied and Environmental Microbiology* **53**, 1470–1475.
- GREVE, L. C., LABAVITCH, J. M. & HUNGATE, R. E. (1984). α -L-Arabinofuranosidase from *Ruminococcus albus* 8: purification and possible role in hydrolysis of Alfafa cell wall. *Applied and Environmental Microbiology* **47**, 1135–1140.
- HÄGERDAL, B. G. R., FERCHAK, J. D. & PYE, E. K. (1978). Cellulolytic enzyme system of *Thermoactinomyces* sp. grown on microcrystalline cellulose. *Applied and Environmental Microbiology* **36**, 606–612.
- HÄGERDAL, B., HARRIS, H. & PYE, E. K. (1979). Association of β -glucosidase with intact cells of *Thermoactinomyces*. *Biotechnology and Bioengineering* **21**, 345–355.
- HÄGERDAL, B., FERCHAK, J. D. & PYE, E. K. (1980). Saccharification of cellulose by the cellulolytic enzyme system of *Thermomonospora* sp. I. Stability of cellulolytic activities with respect to time, temperature and pH. *Biotechnology and Bioengineering* **22**, 1516–1526.
- HOPWOOD, D. A., BIBB, M. J., CHATER, K. F., KIESER, T., BRUTON, C. J., KEISER, H. M., LYDIATE, D. J., SMITH, C. P., WARD, J. M. & SCHREMPF, H. (1985). *Genetic Manipulation of Streptomyces. A Laboratory Manual*. The John Innes Foundation: Norwich.
- IWASAKI, A., KISHIDA, H. & OKANISHI, M. (1986). Molecular cloning of a xylanase gene from *Streptomyces* sp. no. 36a and its expression in streptomycetes. *Journal of Antibiotics* **39**, 985–993.
- JOHNSON, W. C. & LINDSEY, A. J. (1939). An improved universal buffer. *Analyst* **64**, 490–492.
- KLUEPFEL, D. & ISHAQUE, M. (1982). Xylan-induced cellulolytic enzymes in *Streptomyces flavogriseus*. *Developments in Industrial Microbiology* **23**, 389–395.
- KLUEPFEL, D., SHARECK, F., MONDOU, F. & MOROSOLI, R. (1986). Characterisation of cellulase and xylanase activities of *Streptomyces lividans*. *Applied Microbiology and Biotechnology* **24**, 230–234.
- KUNST, A., DRAEGER, B. & ZIEGENHORN, J. (1984). Colorimetric methods with glucose oxidase and peroxidase, In *Methods of Enzymatic Analysis*, 3rd edn, vol. VI, pp. 178–185. Edited by H. U. Bergmeyer, J. Bergmeyer & M. Grassl. Weinheim: Verlag Chemie.
- KUSAKABE, I., KAWAGUCHI, M., YASUI, T. & KOBAYASHI, T. (1977). Studies on xylanase system of *Streptomyces*. VII. Purification and some properties of extracellular xylanase from *Streptomyces* sp. E-86. *Nippon Noei Kagaku Kaishi* **51**, 429–437.
- LADISCH, M. R., LIN, K. W., VOLOCH, M. & TSAO, G. T. (1983). Process considerations in the enzymatic hydrolysis of biomass. *Enzyme and Microbial Technology* **5**, 82–102.
- MACKENZIE, C. R., BILOUS, D. & JOHNSON, K. G. (1984). *Streptomyces flavogriseus* cellulase: evaluation under various hydrolysis conditions. *Biotechnology and Bioengineering* **26**, 590–594.
- MCCARTHY, A. J. (1987). Lignocellulose-degrading actinomycetes. *FEMS Microbiology Reviews* **46**, 145–163.
- MCCARTHY, A. J. & BRODA, P. (1984). Screening for lignin-degrading actinomycetes and characterization of their activity against [14 C]lignin-labelled wheat lignocellulose. *Journal of General Microbiology* **130**, 2905–2913.
- MCCARTHY, A. J., PEACE, E. & BRODA, P. (1985). Studies on the extracellular xylanase activity of some thermophilic actinomycetes. *Applied Microbiology and Biotechnology* **21**, 238–244.
- MILLER, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* **31**, 426–428.
- MOROSOLI, R., BERTRAND, J. L., MONDOU, F., SHARECK, F. & KLUEPFEL, D. (1986). Purification and properties of a xylanase from *Streptomyces lividans*. *Biochemical Journal* **239**, 587–592.
- NAKAJIMA, T., TSUKAMOTO, K., WATANABE, T., KAINUMA, K. & MATSUDA, K. (1984). Purification and some properties of an endo-1,4- β -D-xylanase from *Streptomyces* sp. *Journal of Fermentation Technology* **62**, 269–276.
- PATERSON, A., MCCARTHY, A. J. & BRODA, P. (1984). The application of molecular biology to lignin degradation, In *Microbiological Methods for Environmental Biotechnology*, pp. 33–68. Edited by J. M. Grainger & J. M. Lynch. London: Academic Press.
- RISTROPH, D. L. & HUMPHREY, A. E. (1985a). Kinetic characterisation of the extracellular xylanases of *Thermomonospora* sp. *Biotechnology and Bioengineering* **27**, 832–836.
- RISTROPH, D. L. & HUMPHREY, A. E. (1985b). The β -xylosidase of *Thermomonospora*. *Biotechnology and Bioengineering* **27**, 909–913.
- RIVERS, D. B. & EMERT, G. H. (1987). Lignocellulose pretreatment: A comparison of wet and dry ball attrition. *Biotechnology Letters* **9**, 365–368.
- SONNLEITNER, B. & FIECHTER, A. (1983). Advantages of using thermophiles in biotechnological processes: expectations and reality. *Trends in Biotechnology* **1**, 74–80.
- STUTZENBERGER, F. J. (1972). Cellulolytic activity of *Thermomonospora curvata*: optimal assay conditions, partial purification, and product of the cellulase. *Applied Microbiology* **24**, 83–90.
- STUTZENBERGER, F. J. & LUPO, D. (1986). pH dependent thermal activation of endo-1-4- β -glucanase in *Thermomonospora curvata*. *Enzyme and Microbial Technology* **8**, 205–208.

- UCHINO F. & NAKANE, T. (1981). A thermostable xylanase from a thermophilic acidophilic *Bacillus* sp. *Agricultural and Biological Chemistry* **45**, 1121-1127.
- VAN ZYL, W. H. (1985). A study of the cellulases produced by three mesophilic actinomycetes grown on bagasse as substrate. *Biotechnology and Bioengineering* **27**, 1367-1373.
- WHISTLER, R. L. A. & RICHARDS, E. L. (1970). Hemicellulose. In *The Carbohydrates*, pp. 447-469. Edited by W. Pigman & D. Horton. London: Academic Press.
- WOOD, T. M. & MCCRAE, S. I. (1978). The cellulase of *Trichoderma konningii*. *Biochemical Journal* **171**, 61-72.