Cloning of a *Thermomonospora fusca* Xylanase Gene and Its Expression in *Escherichia coli* and *Streptomyces lividans*

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*Thermomonospora fusca* chromosomal DNA was partially digested with EcoRI to obtain 4- to 14-kilobase fragments, which were used to construct a library of recombinant phage by ligation with EcoRI arms of λgtWES.AB. A recombinant phage coding for xylanase activity which contained a 14-kilobase insert was identified. The xylanase gene was localized to a 2.1-kilobase SalI fragment of the EcoRI insert by subcloning onto pBR322 and derivatives of pBR322 that can also replicate in *Streptomyces lividans*. The xylanase activity produced by *S. lividans* transformants was 10- to 20-fold higher than that produced by *Escherichia coli* transformants but only one-fourth the level produced by induced *T. fusca*. A 30-kilodalton peptide with activity against both Remazol brilliant blue xylan and xylan was produced in *S. lividans* transformants that carried the 2.1-kilobase SalI fragment of *T. fusca* DNA and was not produced by control transformants. *T. fusca* cultures were found to contain a xylanase of a similar size that was induced by growth on xylan or Solka Flox. Antiserum directed against supernatant proteins isolated from a Solka Flox-grown *T. fusca* culture inhibited the xylanase activity of *S. lividans* transformants. The cloned *T. fusca* xylanase gene was expressed at about the same level in *S. lividans* grown in minimal medium containing either glucose, cellobiose, or xylan. The xylanase bound to and hydrolyzed insoluble xylan. The cloned xylanase appeared to be the same as the major protein in xylan-induced *T. fusca* culture supernatants, which also contained at least three additional minor proteins with xylanase activity and having apparent molecular masses of 43, 23, and 20 kilodaltons.

Next to cellulose, hemicelluloses are the most abundant naturally occurring polymers, and they are present in association with lignin and cellulose in plant cell walls. A major component of hemicelluloses from monocots is xylan, a polymer which consists of a backbone of β-(1,4)-d-xylopyranoside residues. Xylans comprise only a minor fraction of dicot-hemcellulose. Xylans of different origins vary with respect to molecular weight, composition, number of side chains, and level of acetylation.

Many bacteria and fungi can utilize xylans as carbon sources; they do so by producing xylanolytic enzymes, which are either excreted into the medium or remain associated with the cell envelope. A number of activities such as endoxylanases, exoxylanases, β-xylosidases, α-glucuronidases, α-arabinofuranosidases, and esterases have been identified in xylanolytic microorganisms (6, 10, 36). Out of all the xylanolytic proteins the endoxylanases and β-xylosidases have been most widely studied, and xylanase genes from a wide variety of mesophilic bacteria and fungi have been cloned (4, 5, 15–17, 20, 27, 28, 31, 32, 37, 38). Studies of a xylanase gene from an extreme thermophile were presented in a preliminary report (P. A. Caughey, D. R. Love, and P. L. Bergquist, FEMS Symp. 1987, abstr. no. P4-07, p. 90), and recently a report on the cloning of a thermophilic xylanase appeared (13).

*Thermomonospora fusca* is a filamentous soil thermophile that produces cellulolytic, xylanolytic, and pectinolytic enzymes (1, 14, 33, 35; D. L. Ristroph, Ph.D. dissertation, University of Pennsylvania, 1981). The xylanases of *T. fusca* have been studied by several workers (1, 26, 29; Ristroph, Ph.D. dissertation).

In this report we describe the isolation by cloning into bacteriophage λ of a xylanase gene from *T. fusca* YX. The xylanase gene was expressed in both *Escherichia coli* and *Streptomyces lividans*. The *S. lividans* transformants expressed into the medium a 30-kilodalton (kDa) xylanase that bound to xylan and appeared to be identical to the major xylanase produced by xylan-induced cultures of *T. fusca*. In addition, we present initial studies on the regulation of xylanase production in both *T. fusca* and *S. lividans*.

**MATERIALS AND METHODS**

**Bacterial strains and vectors.** The bacterial strains and vectors used in this study are listed in Table 1. *E. coli* LE392 was obtained from the *E. coli* Genetic Stock Center, Department of Biology, Yale University, and was used as a host for λgtWES.AB and its derivatives (21). λgtWES.AB DNA and λgtWES.AB EcoRI arms were purchased from either Bethesda Research Laboratories, Inc., or Amersham Corp. λgtWES.AB SalI arms were prepared as described below. *S. lividans* TK24 and plasmid pIJ702 were kindly provided by D. A. Hopwood, John Innes Institute, Norwich, England.

**Growth of organisms.** Unless indicated otherwise, *T. fusca* YX was grown as described previously (12). *E. coli* strains were grown in Luria Bertani (LB) medium (24). When used as a host for a phage growth, LE392 was grown in 1% tryptone–2.5% sodium chloride–10 mM MgCl₂–0.2% maltose. *E. coli* strains carrying recombinant plasmids were grown in LB medium containing ampicillin (50 μg/ml).

The media for *S. lividans* protoplast preparation and regeneration were described previously (11, 12). Unless indicated otherwise, *S. lividans* cultures were grown on tryptone soya broth as previously described (12).

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TABLE 1. Bacterial strains and vectors used in this study

<table>
<thead>
<tr>
<th>Bacterial strain or vector</th>
<th>Genotype or phenotype</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> LE392</td>
<td>F⁻ hsdR514 (rK⁻ mK⁻) supE44 supF58 lacY1 galK2 galT2 metAB trpR55 λ⁻</td>
<td>24</td>
</tr>
<tr>
<td><em>H. bulbifera</em> HB101</td>
<td>F⁻ hsdS120 (rK⁻ mK⁻) supE44 recA13 ara-14 lacY1 proA2 galK2 rpsL20 xyl-5 mtl-1 λ⁻</td>
<td>9, 24</td>
</tr>
<tr>
<td><em>S. lividans</em> TK24</td>
<td>str-6</td>
<td>D. A. Hopwood</td>
</tr>
<tr>
<td><em>T. fusca</em> YX36</td>
<td>Prototroph</td>
<td>W. D. Bellamy</td>
</tr>
<tr>
<td><strong>Vectors</strong></td>
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<td></td>
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<tr>
<td>λgtWES.AB</td>
<td></td>
<td>21</td>
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<tr>
<td>λxyl-1</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>λxyl-2</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>pBR322</td>
<td>Amp’ Tet’</td>
<td>8</td>
</tr>
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<td>pTX101</td>
<td>Amp’ Tet’</td>
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</tr>
<tr>
<td>pU702</td>
<td>Tsr’ Mel’</td>
<td>18</td>
</tr>
<tr>
<td>pU702-pBR322</td>
<td>Tsr’ Mel’</td>
<td>12</td>
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<td>pGG82</td>
<td>Tsr’ Amp’</td>
<td>This study</td>
</tr>
<tr>
<td>pGG92</td>
<td>Tsr’ Amp’</td>
<td>This study</td>
</tr>
<tr>
<td>pGG93</td>
<td>Tsr’ Amp’</td>
<td>This study</td>
</tr>
</tbody>
</table>

Recombinant DNA techniques. *T. fusca* chromosomal DNA and λDNA were isolated as described previously (12, 24). Preparation of plasmid DNA was performed as described by Maniatis et al. (24) or by the procedure of Keiser (19).

All restriction endonucleases were purchased from Bethesda Research Laboratories, Amersham, or New England BioLabs, Inc., and restriction enzyme digests were performed as recommended by the manufacturer. DNA ligation was carried out as described by Maniatis et al. (24). DNA fragments were separated on 0.7% agarose gels in 0.089 M Tris-0.089 M boric acid-2 mM EDTA (pH 8.0). After visualization with ethidium bromide and UV light, a strip of DEAE membrane (S & S NA-45; Schleicher and Schuell Co.) was placed in an incision just ahead of the band. Another strip was placed just behind the band. Electrophoresis was continued until transfer was complete. The DNA on the membrane strip was eluted with 1.0 M NaCl-0.1 mM EDTA-20 mM Tris (pH 8.0). The eluted solution was extracted with 3 volumes of water-saturated N-butanol to remove the residual ethidium bromide, and the DNA fragments were precipitated with 2.5 volumes of cold ethanol.

*T. fusca* chromosomal DNA was partially digested with EcoRI, and fragments in the range of 4 to 14 kb were isolated by sucrose gradient centrifugation. These were ligated with λgtWES.AB EcoRI arms (purchased from Bethesda Research Laboratories). The ligation mixture was incubated at 4°C overnight and packaged with a λ in vitro packaging kit as described by the manufacturer (Amersham).

λgtWES.AB SalI arms were prepared by completely digesting 50 μg of λgtWESAB DNA with SalI. The digests were separated on a linear gradient of 10 to 40% sucrose (containing 1 M NaCl, 5 mM EDTA, 20 mM Tris [pH 8.0]) at 27,000 rpm in a Beckman SW27 rotor at 25°C for 36 h. The fractions near 27.6 kilobases (kb) (left arm) and 12.3 kb (right arm) were collected, dialyzed against 10 mM Tris-1 mM EDTA (pH 8.0), and precipitated with 2.5 volumes of cold ethanol. After dephosphorylation with calf alkaline phosphatase (Boehringer Mannheim Biochemicals) by the procedure of Maniatis et al. (24), the DNA was ligated with a partial Sall digest of the DNA to be subcloned. The ligated DNA was packaged with a λ packaging kit.

For subcloning the xylanase gene, the DNA from λxyl-1 phage was partially digested with SalI and extracted with phenol-chloroform; the fragments were ligated with λgtWES.AB SalI arms and packaged into λ phage. The insert from λxyl-2 was subcloned into pBR322 DNA that had been cut with SalI and dephosphorylated with calf intestine alkaline phosphatase. Transformation of *E. coli* was performed as described by Maniatis et al. (24).

Preparation of DNA from *S. lividans*, construction of recombinant plasmids, and protoplast transformation techniques were carried out as previously described (11).

Preparation of soluble xylan. Oat-spelt xylan (arabinogalactan-polysaccharide) was purchased from Sigma Chemical Co. Soluble xylan (25) was prepared as follows: 10 g of xylan was dissolved in 200 ml of H₂O, the pH was adjusted to 10 with 1 M NaOH, the mixture was incubated at room temperature for 1 h and centrifuged for 10 min at 10,000 × g, and the supernatant was neutralized with 1.0 M acetic acid and lyophilized. About 3 to 4 g of dried xylan was obtained.

Preparation of insoluble xylan. After the soluble xylan was removed as described above, the insoluble xylan was suspended in 200 ml of H₂O, the pH was adjusted to 7.0 with 1 M acetic acid, and the preparation was filtered on Whatman no. 1 filter paper and extensively washed with water. The white paste was carefully removed and dried by lyophilization.

Screening of plaques for xylanase activity with Congo red. Plates to be screened for xylanase activity were overlaid with 7 ml of xylan-agarose (1% agarose, 0.05% soluble xylan, 10 mM Tris [pH 6.5]) and incubated at 55°C for 6 h. After incubation, the plates were stained with 0.1% Congo red for 15 min. The dye solution was removed, and the plates were destained with 1 M NaCl. A yellow ring resulting from xylan hydrolysis appeared around positive plaques (27, 34).

Screening of colonies with RBB-xylan. Remazol brilliant blue (RBB)-xylan was prepared by the procedure of Biely et
FIG. 2. Construction of shuttle plasmids pGG92 (A) and pGG93 (B) that are capable of replicating in E. coli and S. lividans and carry T. fusca DNA coding for xylanase. Symbols: ( ) pBR322 sequence, ( ) sequences originating from pIJ702, ( ) Thermomonospora sequences carrying the xylanase gene. Amp, Ampicillin resistance gene expressed in E. coli; Tsr, thiostrepton resistance gene expressed in S. lividans. Only the sites that were important for this study are shown. Additional sites are shown in Fig. 1 and reference 8.

al. (7). Each plate was overlaid with 5 ml of 1% agarose containing 10 mM Tris (pH 6.5) and 0.07% RBB xylan and incubated at 55°C for over 4 h. If necessary, the plates were destained with a solution of 50 mM sodium acetate (pH 5.4) in 95% ethanol (1:2, vol/vol); positive plaques were surrounded by a clear ring.

TABLE 2. Expression of a T. fusca xylanase gene in E. coli and S. lividans

<table>
<thead>
<tr>
<th>Strain</th>
<th>Xylanase (U/ml)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell extracts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB101(pTX101)</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB101(pBR322)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>HB101(pGG92)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>HB101(pGG93)</td>
<td>0.22</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>TK24(pGG82)</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td></td>
</tr>
<tr>
<td>TK24(pGG92)</td>
<td>1.0 ± 0.5</td>
<td>7.0 ± 2.0</td>
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</table>

Enzyme assays. Xylanase was assayed by E. coli by growing a 100-ml overnight culture and harvesting it by centrifugation (10 min at 10,000 × g); the pellet was suspended in 3 ml of 0.05 M potassium phosphate buffer (pH 6.5) (buffer I), and an extract was prepared in a French press at 10,000 lb/in². The culture supernatant was concentrated to 3 ml with an Amicon UM10 membrane. Xylanase activity was determined by measuring the rate of release of reducing sugar from xylan. Cell extract or concentrated culture supernatant was mixed with buffer I to give a final volume of 0.3 ml, 0.10 ml of 1% soluble xylan solution made up in buffer I was added, and the mixture was incubated at 55°C for 30 min. After incubation, 0.75 ml of dinitrosalicylic acid reagent (35) was added, and the samples were incubated in a boiling water bath for 15 min. The samples were allowed to cool to room temperature, and then the A₆₅₀ was determined. One unit of xylanase is defined as the amount of enzyme that produces 1 μmol of reducing sugar per min in the above procedure.
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TABLE 3. Xylanase and carboxymethylcellulase activity of T. fusca grown on glucose, xylan, and cellulose*

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>CMCase</th>
<th>Xylanase</th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Oat spelt xylan</td>
<td>1.6</td>
<td>21.6</td>
</tr>
<tr>
<td>Solka floe</td>
<td>22.8</td>
<td>22.4</td>
</tr>
</tbody>
</table>

* A 2-day culture of T. fusca in LB medium was centrifuged and suspended in the same amount of LB medium. Then 2.0 ml of this suspension was transferred to a 250-ml flask containing 40 ml of Hagerdal medium (22) containing 0.5% (wt/vol) of the indicated carbon source and incubated in a shaking water bath for 2 days. The supernatants recovered by centrifugation was assayed for carboxymethylcellulose (CMCase) and xylanase activities.

Xylanase binding assays. Insoluble xylan (from 0 to 20 mg) was added to 1.5-ml microcentrifuge tubes and washed two times with 1.0 ml of water by centrifugation for 30 s and placed on ice. Then 0.5 ml of transformed S. lividans culture supernatant was added to each tube, tubes were incubated for 5 min and centrifuged, and the supernatant was assayed for xylanase. The activity lost from the supernatant was assumed to be the activity bound.

Western blotting. Western blotting (immunoblotting) was carried out as previously described (12). Briefly, proteins were separated on sodium dodecyl sulfate (SDS) gels (12.5% polyacrylamide), transferred to nitrocellulose, and reacted with the appropriate rabbit antiserum; primary antigen-antibody complexes were detected by reacting with an anti-rabbit immunoglobulin G-coupled alkaline phosphatase and visualized with nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate.

Other procedures. Electrophoresis of proteins on polyacrylamide gels or SDS-polyacrylamide gels were performed as described before (11). The zymogram technique for Beguin developed for cellulase was adapted to locate xylanase bands in SDS gels (2, 23). The bands were located by Congo red staining of xylan-agar and/or clearing of RBB-xylan agar replicas. Antibody inhibition assays were carried out as previously described (11).

RESULTS

Xylanase gene cloning. The transformation efficiency of the recombinant λgt phage T. fusca genome bank was 8 × 10^6 PFU/µg of DNA. Of 3,500 plaques screened for xylanase activity by both the Congo red and the RBB-xylan procedures, 2 showed xylanase activity. Based on restriction maps, the DNA of both phage contained the same ~14-kb EcoRI insert. This recombinant phage was designated λxyl-1 (Fig. 1).

Subcloning of the xylanase gene. Since there were no EcoRI sites in the insert DNA, λxyl-1 DNA was subcloned by using λgtWES.AB SalI arms as described in Materials and Methods. About 1,500 plaques were screened by the Congo red procedure, and 11% were xylanase positive. Seven positive plaques were purified, and their xylanase activities and restriction maps were determined. One phage had an insert DNA containing 3.4- and 2.1-kb SalI fragments, whereas all of the other phage contained both of these fragments along with at least one additional fragment. The xylanase-positive phage with the smallest insert was called λxyl-2, and its restriction map is shown in Fig. 1. λxyl-2 DNA was digested with SalI, and the 3.4- and 2.1-kb fragments were separated by gel electrophoresis and subcloned into pBR322. Transformants were screened for
xylanase activity by the Congo red procedure. All of the colonies from the 3.4-kb fragment ligation were negative, whereas 35% of the transformants from the 2.1-kb ligation were positive. Restriction maps of several transformants showed that they all contained only the 2.1-kb insert. This plasmid was designated pTX101, and its restriction map is also shown in Fig. 1.

Construction of *E. coli-S. lividans* shuttle plasmids containing the xylanase gene. The multicopy plasmid pIJ702 (18) commonly used for cloning in *S. lividans* contains five *Sall* restriction sites and therefore is not convenient for cloning *Sall* fragments. Instead, a derivative of pIJ702, pGG82, was constructed and used for expressing the *T. fusca* xylanase gene in *S. lividans*. In plasmid pGG82 the smaller *BglII-SphI* fragment of pIJ702 is replaced by a *BamHI-SphI* fragment of *E. coli* plasmid DNA. The structure of pGG82 appears in Fig. 2. The *EcoRI-SphI* fragment of pGG82 is from pBR322, and the *BamHI-EcoRI* fragment is derived from pUR222 (30). Plasmid pGG82, unlike plasmid pIJ702, no longer produces melanin (Mel- phenotype) and contains single *EcoRI* and *HindIII* sites. The *BglII* site of pIJ702 is inactivated in pGG82, due to its fusion to the *BamH-I* site of pUR222.

Further restriction analysis of pTX101 showed that it had no sites for *SstI* and *BglII*, one site each for *EcoRI*, *SphI*, *HindIII*, and *BamHI*, and three sites for *SacI*. The construction of shuttle plasmid pGG92 from pTX101 and pGG82 is depicted in Fig. 2A. The three *E. coli* HB101 transformants tested had the correct restriction pattern. This plasmid is stable in *E. coli* HB101. The construction of shuttle plasmid pGG93 from pGG82 and pTX101 is shown in Fig. 2B.

![FIG. 5. SDS-gel electrophoretic analysis of xylanase induction in *T. fusca*. Cells grown in LB medium were used to inoculate Hagerdal medium containing the indicated carbon sources (0.5%) at a dilution of 1:20 and incubated in a shaker at 55°C. After 24 h the supernatants were prepared by centrifugation (10,000 × g for 15 min), assayed, electrophoresed on an SDS-12.5% polyacrylamide gel, and stained with Coomassie brilliant blue. Each lane contains 16 μl of supernatant. Lanes: 1, glucose grown (0.4 U of xylanase per ml); 2, xylan grown (15 U of xylanase per ml); 3, cellulose grown (13 U of xylanase per ml).](http://jb.asm.org/)

<table>
<thead>
<tr>
<th>Plasmid contained</th>
<th>Carbon source</th>
<th>Xylanase in culture filtrate (U/ml) on day:</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGG82</td>
<td>Glucose</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Xylan</td>
<td>0.0</td>
</tr>
<tr>
<td>pGG92</td>
<td>Glucose</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Xylan</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* TABLE 4. Xylanase production in *S. lividans* gene in glucose and xylan*.

Transformed colonies were grown in tryptone soya broth containing thiostrepton. Two-day cultures (mycelial volume, about 10%) were centrifuged and taken up in modified Hagerdal medium (22) lacking a carbon source. Then 1 ml of this culture was transferred to 50 ml of the indicated medium containing 10 μg of thiostrepton per ml and 0.25 g (0.5%) of either glucose or xylan. The cultures were shaken at 30°C, and at the indicated times samples were removed, centrifuged, and assayed for xylanase activity.

12.8-kb plasmid DNA was isolated from six *E. coli* transformants, and all six had the orientation shown in Fig. 2.

Expression of shuttle plasmids in *E. coli*. Unlike plasmid pTX101, which had strong xylanase activity in *E. coli* HB101 (Table 2), shuttle plasmid pGG92 failed to confer a xylanase-positive phenotype on HB101 (Fig. 3). The tetracycline resistance promoter is deleted during the construction of pGG92 while leaving the *T. fusca* DNA sequence intact, and when this promoter was present as in pGG93, xylanase activity was present in the HB101 transformants.

Transformation of *S. lividans* and expression of the *T. fusca* xylanase gene. Plasmid DNA was isolated from *E. coli* transformed with pGG92 and pGG93 and was used to transform *S. lividans* protoplasts. pGG92 consistently gave more transformants than pGG93 when equal amounts of DNA were used. Furthermore, all of the pGG92 transformants were xylanase positive, whereas only a few of the pGG93 transformants were xylanase positive, possibly because most pGG93 transformants were unstable due to recombination between the duplicated regions of pBR322 present in pGG93. The xylanase activities of transformed and control strains are given in Table 2.

When supernatant proteins from *S. lividans* transformants were separated on an SDS gel and stained for xylanase activity, a 30-kDa protein was the major active band. This band was not present in control cultures (Fig. 4).

The xylanase in *S. lividans* transformants is similar to an induced xylanase in *T. fusca*. Table 3 shows the specific induction of extracellular xylanase activity in *T. fusca* cultures grown for 2 days on xylan. Xylan-grown cultures had low levels of cellulase and high levels of xylanase activity, whereas cellulose (Solka flocculent)-grown cultures had high levels of both activities. The level of xylanase was higher than the level of cellulase in glucose-grown cultures. The proteins present in the supernatants of cultures grown on the different carbon sources were separated by gel electrophoresis (Fig. 5). The major protein present in the supernatant of xylan-grown cells migrated in the same position as the cloned xylanase (Fig. 4). Four bands of xylanase activity were present in stained gels (43, 30, 23, and 20 kDa) run on the *T. fusca* sample. The larger two (43 and 30 kDa) are probably distinct gene products, whereas the smaller forms may be derived by proteolysis of the larger enzymes, especially as the lower-molecular weight bands are similar in size to the proteolysis products present in *S. lividans* transformants.

The xylanase activity of *S. lividans* transformants was inhibited by an antiserum prepared against proteins isolated...
fusca xylanase.

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cultures and Proteins soya xylanase xylan (Fig. 6).

The lack of xylanase activity at early time points in the supernatants of cultures grown on xylan could indicate that the activity binds to undegraded insoluble xylan. In fact, xylanase activity quickly disappeared from the solution after the addition of insoluble xylan, and the bound enzyme released soluble reducing sugars at 55°C in 50 mM KP (pH 6.5) (Fig. 7).

FIG. 6. Comparison of cloned xylanase and T. fusca proteins by activity staining (A) and Western blotting (B). Supernatants from T. fusca and S. lividans transformant cultures were diluted with loading dye and left in a boiling water bath for 5 min. The boiled samples were subjected to electrophoresis on an SDS-12.5% polyacrylamide gel to separate the peptides. After SDS was removed as described in the text, the gel was incubated with xylan agar replica for 30 min in a 55°C oven. The replica was stained with Congo red. Proteins in the acrylamide gel were electroblotted onto nitrocellulose (0.45-μm pore size) and probed with antiserum directed against Solka Floc-grown culture supernatant proteins. Lanes: 1, T. fusca culture grown in glucose; 2, T. fusca culture grown in xylan; 3, T. fusca culture grown in Solka floc; 4, S. lividans(pGG82) transformant culture; 5, S. lividans(pGG92) transformant culture. Std, protein molecular mass standards of 43, 25.7, 18, and 14 kDa.

FIG. 7. Release of soluble reducing sugars into the medium by cloned xylanase bound to insoluble xylan. Supernant (0.5 ml) from an S. lividans(pGG92) transformant culture (5 U of xylanase per ml) grown in tryptone soya broth was added to a 1.5-ml plastic tube on ice containing 20 mg of insoluble xylan. The sample was gently mixed for 5 min at 4°C and centrifuged to separate the xylan and supernatant. The xylanase activity in the supernatant was 0.6 U/ml (88% binding). The xylan was washed twice with 1 ml of cold H2O, mixed with 0.5 ml of 0.05 M potassium phosphate buffer (pH 6.5), and incubated at 55°C. A control S. lividans(pGG82) culture was treated similarly. At appropriate times the tubes were centrifuged to remove 10-μl samples for the determination of reducing sugar. Symbols: △, pGG92 transformant; ○, pGG82 transformant.

from the culture supernatant of cellulose-grown T. fusca. The activity was not inhibited by control serum or serum prepared against purified cellulases from T. fusca (data not shown). Immunoblotting of S. lividans transformants revealed a 30-kDa protein; control cultures lacked this band (Fig. 6).

Regulation of xylanase production of S. lividans transformants and its binding to xylan. S. lividans transformants containing the control plasmid (pGG82) did not produce host xylanase in medium lacking xylan (Table 4), whereas the plasmid-encoded T. fusca xylanase was produced in minimal medium containing glucose, cellobiose, or xylan and in tryptone soya broth. The activity of pGG92 cultures grown on xylan was about two- to threefold the activity of control cultures and contained both induced host activity and T. fusca xylanase.

The lack of xylanase activity at early time points in the supernatants of cultures grown on xylan could indicate that the activity binds to undegraded insoluble xylan. In fact, xylanase activity quickly disappeared from the solution after the addition of insoluble xylan, and the bound enzyme released soluble reducing sugars at 55°C in 50 mM KP (pH 6.5) (Fig. 7).

DISCUSSION

The cloned T. fusca xylanase gene was expressed at a low level in E. coli and at a higher level in S. lividans, which also efficiently excretes the activity into the culture medium. The results of expression in S. lividans indicate that the xylanase gene uses its own promoter. However, efficient expression in E. coli seems to require additional promoter activity that may be provided by the adjoining T. fusca or λ DNA on the λxyl-1 and λxyl-2 phages or the tet promoter in pTX101 or pGG93. The xylanase produced by the T. fusca gene present in plasmid pGG92 clearly differed from S. lividans xylanase activity in its regulation, electrophoretic mobility, and reaction with T. fusca antiserum.

S. lividans xylanase activity is induced by xylan; an S. lividans xylanase gene cloned into S. lividans on plasmid pIJ702 was reported to be inducible (27). However, the T. fusca xylanase gene described here was not induced by xylan in S. lividans, although it was induced in T. fusca.

A number of xylanase genes from different organisms have been expressed in E. coli; however, this report appears to be the first example of heterologous xylanase expression in S. lividans. These studies demonstrate the usefulness of the S. lividans system for expressing T. fusca genes (11, 12).

Streptomyces transformants that carry the T. fusca xylanase gene produce a 30-kDa protein that displays xylanase activity and appears in all respects to be the same as the major protein in xylan-induced T. fusca supernatants. The xylanase activity of Streptomyces transformants can be specifically and quantitatively inhibited by an antiserum raised against induced T. fusca extracellular protein. The availability of large amounts of xylanase protein in S. lividans culture supernatants free from other T. fusca proteins and the xylan-xylanase binding demonstrated in this
study should facilitate purification and structure-function studies of this protein.

ACKNOWLEDGMENTS

This work was supported by U.S. Department of Energy grant FG02-84ER13233 and by grant 85-CRCR-1-1880 from the U.S. Department of Agriculture.

We thank S. J. Lucania, Squibs Institute, Princeton, N.J., for the thiostrepton.

LITERATURE CITED