CHAPTER III

CHARACTERIZATION OF ENDO-XYLANASE ACTIVITY

FROM STREPTOMYCES FLAVOGRISEUS 45CD
INTRODUCTION

Xylan [(1→4)-β-D-linked polymer of xylose] is the main component of hemicellulose and forms in large amounts in agricultural wastes such as straw, hard wood and corn cobs. Enzymatic hydrolysis of (1→4)-β-D-xylan to xylose is accomplished by sequential reactions, the first is the conversion of xylan to xyloaccharides by the extracellular endo (1→4)-β-D-xylanase (1,4-β-D-xylan-β-xylanohydrolase, E.C. 3.2.1.8) followed by hydrolysis to xylose by the intracellular β-D-xylosidase (1,4-β-D-xylan-xylohydrolase E.C. 3.2.1.37).

Xylanases have been purified and characterized from various microorganisms (Woodward, 1984) including several Streptomyces (Nakajima et al., 1984; Morosoli et al., 1986).

The xylanolytic enzymes are usually inducible in nature and subject to end product inhibition (Gascoigne and Gascoigne, 1960; King and Fuller, 1968; Esteban et al., 1982).

Xylan hydrolysis could be of commercial significance since some industrial processes, for example, the pulp and paper industry release large quantities of xylan in their effluents. Alternatively fermentation to ethanol is possible either by intermediate formation of xylulose (Wang et al., 1980) or with some yeasts by direct fermentation (Jeffries, 1981; Schneider et al., 1981).

Thus for efficient enzymatic hydrolysis a strain would be recommended which would utilize xylan in native hemicellulose
state. Fungi are found to penetrate and colonize the wood but their large scale cultivation is often difficult because of slow generation time, coproduction of highly viscous polymers and poor oxygen transfer. Besides fungi, actinomycetes are also well associated with wood and its colonization of wood in soil and marine environment is reported (Crawford, 1978). Short term culture studies showed that members of genus Streptomyces could rapidly colonize and extensively penetrate wood tissue (Crawford and Sutherland, 1978).

Streptomyces seem to closely resemble their fungal counterparts in comparison to bacterial anaerobes, Streptomyces produce a large amount of extracellular xylanase activity and offer a potential advantage over fungi in being prōkaryote.

Substantial utilization of Streptomyces for hemicellulose conversion would require much knowledge about the way they decompose hemicellulose, the nature of enzymes involved and optimization of various steps leading to degradation. This chapter describes characterization of xylanase enzyme from *S. flavogriseus* 45CD with respect to main parameters such as optimum temperature, optimum pH, thermal inactivation, substrate stabilization, effect of inhibitors and product inhibition.

**MATERIALS AND METHODS**

*S. flavogriseus* was maintained in sporulated form on YEME agar slants, stored at 4°C and routinely transferred every 30 days.
DETECTION OF Xylanase Activity in Plates

Different Streptomyces strains available in laboratory were grown on zone clearing medium (ZCM) containing 0.5% xylan. Plates were incubated at 30°C for 5-7 days and the xylanolytic activity was detected as clearing zone around the Streptomyces colonies on opaque xylan medium, the sensitivity of this method is increased in presence of 0.2M sodium acetate buffer pH 5 which was added to plate after third day and zone of clearing was observed on fourth day (Sylvestre-Daigneault and Kluepfel, 1979). To further enhance the clearing, plates were flooded with aqueous solution of congo red (1 mg/ml) for 30 min, then washed with 1M NaCl for 15 min, the zone of hydrolysis appeared as yellow halo in red background (Teather and Wood, 1982). The zone of clearing is produced by degradation of β-1,4-glycosidic bonds of xylan.

Enzyme Preparation

Enzyme was prepared as described in Materials and Methods, Section IV.

Characterization of Enzyme

(a) Optimal temperature for enzymatic hydrolysis

Optimal temperature of enzyme was determined by measuring the initial rates at which reducing sugars are released at different temperatures ranging from 20-60°C.
The reaction mixtures were incubated at different temperatures for 30 min and the reducing sugar released was determined as described in Section VII, General material methods.

(b) Optimal pH determination

For determination of the pH range at which enzyme is active, five different buffers were made covering the range between 4-8. The enzyme activity was assayed at 30 and 60 min interval.

(c) Thermal inactivation

Samples of enzyme were preincubated at 40°, 50° and 60° respectively. At the given time interval i.e. 1 hr, 2 hrs, 3 hrs, 5 hrs, 12 hrs and 24 hrs aliquots were assayed for enzyme activity at optimum temperature for 30 min.

(d) Inducers for enzyme production

To determine whether S. flavogriseus would produce xylanolytic activity if grown on substrate other than xylan, cells were grown in ERM supplemented with one of the variety of sugars i.e. maltose, xylose, mannose, sucrose, fructose, glucose, cellobiose, lactose, arabinose, cellulose, avicel, carboxy methyl cellulose, galactose and glycerol to the final concentration of 1%. Supernatants were assayed for xylan degrading activity after time interval of 12, 60, 80 and 108 hrs.
(e) **Kinetic parameters**

Determination of $K_m$ of crude culture filtrate was carried out by incubating fixed amount of enzyme with increasing concentration of substrate ranging from 0.1 mg/ml to 3 mg/ml at temperature 50°C for 15 min followed by assay for enzyme activity.

(f) **Effect of detergents**

SDS and Triton X-100 were added to standard enzyme assay to final concentration 0.1% and 1% respectively to observe their effect on substrate degradation activity. This reaction mixture was incubated at optimum temperature and enzyme activity was measured.

(g) **Effect of inhibitors**

Various divalent cations and metal chelators were added to the standard xylanase assay to observe their effect on xylanolytic activity. The cations $\text{Ag}^{+2}$, $\text{K}^{+2}$, $\text{Ca}^{+2}$, $\text{Mg}^{+2}$ were added to the final concentration of 100 mM and 10 mM respectively, EDTA was added to the final concentration of 100 mM and 200 mM respectively.

**EFFECT OF INCREASING END PRODUCT CONCENTRATION**

Xylose added at various concentration from 0-5% allows the detection of concentration at which the catabolic repression occurs. ZCM plates were streaked with *S. flavogriseus* and incubated...
at 30°C for 3-15 days. Xylanolytic activity was measured by determining the magnitude of clear zone formed in medium. The zone of hydrolysis can be seen with naked eye but it was enhanced by staining with congo red (0.1%) for 30 min and the excess stain was washed with 1M NaCl.

This assay is a semi-quantitative way of determining the amount of enzyme produced (Sylvestre-Daigneault and Kluepfel, 1979).

POLYACRYLAMIDE GEL ELECTROPHORESIS was carried out as described in Section IX of General Materials and Methods.

RESULTS

In view of our objective to isolate highly xylanolytic Streptomyces sp. several strains available in laboratory were screened. They were as follows:

1. Streptomyces wedmorensis ATCC 21230
2. Streptomyces flavogriseus 45CD
3. Streptomyces graminfaciens ATCC 12705
4. Streptomyces lividans
5. Streptomyces sp. ATCC 21175

SELECTION OF S. FLAVOGRISEUS STRAIN: PRIMARY SCREENING

The above strains were screened for xylanolytic activity according to Sylvestre-Daigneault and Kluepfel (1979). S.flavogriseus
was selected as source of xylanase enzyme on the basis of extracellular nature of enzyme and high magnitude of zone of cleaning on xylan plate (Fig. 1).

SECONDARY SCREENING

The second basis was the ability of the strain to grown on solid media with xylan as sole carbon source on the basis of above two screenings S. flavogriseus was selected for further studies.

CHARACTERIZATION OF STREPTOMYCES IAF 45CD

S. flavogriseus produces grey aerial mycelium mass on all the media studied and sporulated abundantly on most of them. The spore chain morphology is typically rectiflexibilis and consists of upto 10 spores. The conidia have smooth surface as determined by electron microscopy. The strain grows optimally at 30\(^{\circ}\)C with temperature range of 20-37\(^{\circ}\)C. The reverse side pigment of mycelium is distinctively yellow on YEME but no soluble pigment such as melanin are produced.

XYLANOLYTIC ACTIVITY IN SUBMERGED CULTURES

Strain was grown in ERM\(^{+}1\%\) xylan and assayed for xylanase production. The strain were incubated at 30\(^{\circ}\)C for 3 days and samples were taken every 12 hrs.

Maximum activity in terms of enzyme unit i.e. 45 IU was produced in filtrates of strain after 2 days of incubation. In
72 hrs sample the enzyme activity was found to decrease slightly, though in 86 and 98 hrs sample it was relatively constant. The time course of xylanase production in *S. flavogriseus* is shown in Fig. 2. Xylanase activity in medium increased proportionally to cell growth, reached its maximum 48 hrs after inoculation and there after remained almost constant. Specific activity was found to be 27.5 IU per mg of protein.

The extracellular filtrates contained only trace amounts of β-xylosidase. The intracellular xylanase levels were very low as compared to extracellular levels. The cellulose system is inducible by xylan as primarily shown by Kluepfel and Ishaque (1982).

**INDUCERS OF XYLANASE ACTIVITY**

It was of interest to determine whether the strain would produce xylanolytic activity grown on carbon source other than xylan. Accordingly the strains were grown in ERM supplemented with one of the variety of sugars. Supernatants were assayed for xylanase activity at 12, 24, 36 and 72 hrs respectively. Among all inducers tested xylan, lactose, mannose and galactose were found to be the most effective ones. The highest xylanase activity was obtained on xylan and lactose, yeast extract was also effective for xylanase production. An initial pH 6.0-7.0 and temperature 30°C was maintained in all condition. There
was basal level of xylanase production when strain was grown in medium containing glucose as sole carbon source. After 48 hrs xylan and lactose were found to be the most effective inducers, mannose and galactose also induced activity but to lower extent, with cellulose the activity reached maximum after 60 hrs, while glycerol, sucrose, fructose, arabinose, xylose, cellobiose, maltose and glucose were found to be very poor inducers as there was no increase in activity with time.

The protein content was found to increase in first 12 hrs with xylan and lactose as inducer and there was considerable decrease after that, though later on it was almost found constant, maximum value of 1.1-1.2 mg/ml was found, as far as other inducers are concerned, protein concentration was seen ascending for 20 hrs and then over next 12 hrs there was a heavy decline which later was almost constant.

OPTIMAL TEMPERATURE FOR ENZYMATIC HYDROLYSIS

The optimal temperature in 30 min assay with soluble larch xylan as substrate at pH 6 was 50°C for S.flavogriseus (Fig.3).

OPTIMAL pH FOR ENZYMATIC HYDROLYSIS

For determination of pH range at which xylanase is active four different buffers were used covering the range between 4-8. xylanase activity was assayed at 30 and 60 min intervals. At
both time intervals maximum activity was obtained at pH 6 though enzyme retained 95% of its activity at pH 7. There was steep decline in activity on alkaline side (pH 8) (Fig. 4).

THERMAL INACTIVATION OF ENZYME

The stability of enzyme against thermal inactivation is shown in Fig. 5. Exposure at 30°C upto 48 hrs hardly affected the enzyme whereas at 40°C the half life was 24 hrs and at 50°C the half life was further reduced to 2.5 hrs.

SUBSTRATE STABILIZATION

Considerable substrate stabilization was observed at 50°C. In presence of substrate the enzyme was dramatically more stable, half life was extended from 2.5 hrs to 7 hrs. Activity levels in the presence of substrate were calculated on the basis of reducing sugar released between adjacent time points.

INHIBITION BY CATIONS, CHELATORS AND DETERGENT

Various divalent cations, metal chelators and detergents were added to standard xylanase assay to observe their effect on xylanolytic activity of strain supernatant. It was observed that the enzyme activity was inhibited significantly by 200 uM of Hg⁺², however, there was no inhibition by 20 uM, other cations at the concentration of 200 uM showed no significant inhibitory effect.
There was overall lack of inhibition by these metals except at very high concentration as given in Table 1. With EDTA no inhibition was observed at 1 mM concentrations whereas 50 mM and 100 mM resulted in only 5% and 12% inhibition respectively.

With detergents i.e. SDS and Triton X-100 at a concentration of 1% there was considerable inhibition (Table I).

**PRODUCT INHIBITION**

Inhibition of xylanase by addition of xylose was studied by adding varying concentration of xylose in ZCM + xylan (0.5%) plates. The strains were grown on them. It was possible to assess rapidly not only the degree of catabolic repression of xylanase production by xylose but also in semiquantitative way the amount of enzymes produced, xylose was added at varying concentration (0-2.5%). This variation allowed the detection of xylose concentration at which catabolic repression occurred. The petri plates were incubated of 30°C for 3-12 days and examined regularly for zone clearing around the colony.

<table>
<thead>
<tr>
<th>ZCM + Xylose (0.1%)</th>
<th>no clearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZCM + Xylose (0.5%) + Xylan (0.5%)</td>
<td>Large zone of clearing</td>
</tr>
<tr>
<td>ZCM + Xylose (1%) + Xylan (0.5%)</td>
<td>Zone of clearing half of the above</td>
</tr>
<tr>
<td>ZCM + Xylose (2.5%) + Xylan (0.5%)</td>
<td>no zone of clearing</td>
</tr>
<tr>
<td>ZCM + Glucose (0.5%)</td>
<td>no clearing</td>
</tr>
<tr>
<td>ZCM + Glucose (1%) + Xylan (0.5%)</td>
<td>narrow zone of clearing</td>
</tr>
<tr>
<td>ZCM + Glucose (2.5%) + Xylan (0.5%)</td>
<td>very narrow zone of clearing</td>
</tr>
</tbody>
</table>
Fig. 1: Detection of endoxylanase activity in *Streptomyces flavogriseus* on zone clearing medium containing xylan (0.5%). Plates were incubated at 30°C for 5-7 days and xylanolytic activity was detected as clearing zone by staining with Congo-red according to Teather and Wood (1982).
Fig. 2: xylan degrading activity in culture supernatant of *S. flavogriseus*. It was grown at 30°C in ERM containing xylan (1%) and initial pH 7. Production of xylanase activity of culture was followed for 120 hrs. Aliquots were taken at different culture times aseptically and assayed for xylanase activity.
Fig. 3: Optimal temperature for endoxylanase of *S. flavogriseus*.

It was determined by measuring the initial rates at which reducing sugars are released at different temperatures ranging from 20-60°C at pH 6 in 30 min assay. The reducing sugar released were measured according to Miller *et al.* (1980).
Fig. 4: Optimal pH for endoxylanase of *S. flavogriseus*. The enzyme reaction was carried out with different buffers covering the range between 4-8, at 50°C for 60 min assay. The reducing sugar released were assayed according to Miller *et al.* (1960).
1 hr incubation
Fig. 5: Thermal inactivation of S. flavogriseus endoxylanase. Samples of enzyme were preincubated at 40°C, 50°C and 60°C respectively at different time intervals. Aliquots were assayed for enzyme activity at optimum temp. 50°C and pH 6, in 30 min assay. Reducing sugar released were assayed according to Miller et al. (1960).
Fig. 6: Polyacrylamide gel electrophoresis (7.5%) of xylanase fraction of S. flavogriseus which was prepared as described in Chapter 2.
Lane 1 reveals zymogram of xylanase fraction of extracellular S. flavogriseus xylanase. Lane 2: detection of bands after Coomassie blue staining. S. flavogriseus revealed endoxylanase activity corresponding to 42,000, 29,000 and 18,000 dalton protein.
SDS PAGE ANALYSIS OF XYLANASE

A concentrated crude preparation of endoxylanase was prepared from culture supernatant of \textit{S. flavogriseus} by ammonium sulphate fractionation followed by redissolution in 0.05M sodium acetate buffer pH 5. It was electrophoretically separated on 7.5\% polyacrylamide gel containing SDS. 100 \textmu g of protein was loaded in two lanes on SDS PAGE. Half of the gel was stained for protein with Coomassie blue whereas the remaining half was used for sensitive zymogram technique by washing it free of SDS with 25\% isopropanol in 0.2M sodium acetate buffer pH 5 and then incubated on top of 2\% agar sheet containing 0.2\% xylan following incubation, substrate was washed and immersed in 0.1\% \textit{G}\textsuperscript{N}go red for 30 min followed by washing in 1M NaCl.

Region of enzymatic hydrolysis of xylan appeared as orange zone on a red background that matched in position to corresponding \(\beta\)-1,4-endo-xylanase band on Coomassie blue stained PAGE. Three distinct endo-\(\beta\)-(1,4)-xylanase activity band were observed in the zymogram stained portion corresponding to 42,000, 29,000 and 18,000 dalton protein in Coomassie blue stained gel (Fig. 6).

DISCUSSION

\textit{Xylanase} activity has been characterized in detail from several \textit{Streptomyces} strain (Ishaque and Kluepfel, 1985; Marui \textit{et al}., 1985; Kluepfel \textit{et al}., 1986). While screening a variety
of *Streptomyces* for xylanolytic activity by a previously described method (Sylvestre-Daigneault and Kluepfel, 1979). The strain was selected on the basis of higher magnitude of zone of clearing on agar xylan medium. *S. flavogriseus* produced clearing around the colonies indicating the synthesis of extracellular xylanase and led to further investigation of strain.

When the culture of *S. flavogriseus* was grown on ERM containing xylan (1%) in shake flasks it produced considerable amounts of extracellular xylanase. The extracellular proteins and xylanases were closely related and proportional. The growth was rapid reaching its maximum in 48 hrs, the optimal growth temperature was found to be 30°C, though the strain was able to grow at 17°C, maximal levels of xylanase over 45 IU were obtained after 48 hrs of incubation. Maximum yields of xylanase upto 200 IU by a basidiomycete Sclerotium rolfsii have been reported by Sadana et al. (1980). These values were obtained from 14 day old culture. Thus the 45 IU of xylanase activity produced in filtrates of *S. flavogriseus* after two days of incubation compare favourably with *Sclerotium rolfsii*.

The specific activity for xylanases of *S. flavogriseus* was found to be 27 LU per mg of protein which is comparable with results obtained from *S. lividans* (Morosoli et al., 1986).

Xylanase activity was localized and found extracellular in nature. Approximately 80% activity was released in the medium.
Intracellular xylanase levels were very low compared with extracellular activity.

Nutritional studies with \textit{S. flavogriseus} demonstrated the inducibility of xylan hydrolysing enzymes. The inducible nature of xylanase is in agreement with earlier reports (Gascoigne and Gascoigne, 1960; King and Fuller, 1968; Howard \textit{et al.}, 1960). This fact contrasts with some reports concerning \(\beta\)-D xylanases in eukaryotic microorganisms. Notario \textit{et al.} (1971) demonstrated the constitutive nature of those enzymes in \textit{Cryptococcus albidus} var. \textit{aereus}, enzyme is synthesized even in presence of glucose as carbon source.

The extracellular xylanase activity was obtained from culture filtrates of \textit{S. flavogriseus}. It exhibited versatility in utilizing different carbon sources as inducers for xylanase. Xylan and lactose were found to be the most effective inducers, mannose and galactose also induced activity but to lower extent. On avicel as substrate, the enzyme production was minimal for first 48 hrs then the activity increased during next 24 hrs to about 5 IU and levelled off thereafter. However when xylan served as carbon source considerably higher xylanase activity was obtained reaching 45 IU in 48 hrs. Glycerol, sucrose, fructose, arabinose, xylose, cellobiose, maltose and glucose were found to be poor inducers. Mannose and galactose also induced xylanolytic activity which is logical as D-mannose and D-galactopyranosyl residues are often found in varying amounts along with acetate and uronic acid residues in xylan (Timell, 1967).
Most of the enzyme activities were detected early during the growth cycle and reached maximum close to the midpoint of complete growth. Although the organism grew well in absence of added lignocellulosic materials (0.7 mg/ml), enzyme levels were just above the levels of detection i.e. 0.59 IU/ml.

Johnson et al. (1988) have reported that addition of oat spelts xylan to basal medium resulted in highest observed production of endoβ-1,4-xylanase i.e. 20.30 IU.

The fact that xylan is the best carbohydrate for β-D-xylanase induction is in agreement with the results published by Nakashiki et al. (1985) on β-D-xylanase synthesis by Streptomyces sp.

The optimal pH for xylanase activity was found to be 6, whereas in alkaline side there was heavy decline in activity.

The Streptomyecete xylanases have relatively high thermostability like those of other bacteria such as Bacillus while the temperature optimal is similar to that of fungal xylanases (Hagerdahl et al., 1978). S. flavogriseus was found to have half life of 24 hrs and this was shortened considerably to 2.5 hrs at 50°C.

The presence of substrate, however, extended the half life of enzyme from 2.5 to 7 hrs at 50°C, similarly at 4°C the half life was increased from 24 to 40 hrs. The similar effect has been reported earlier for S. flavogriseus cellulose enzyme (Ishaque and Kluepfel, 1980).
The Km value of *S. flavogriseus* endoxylanase was found to be 0.75 mg/ml which is comparable to *S. lividans* endoxylanases, Km 0.60 mg/ml. Km value is low compared to fungal and yeast xylanases which range from 4-20 mg of xylan per ml.

All the divalent cations added to standard xylanase assay, substantial inhibition occurred with addition of 200 µM of Hg$^{2+}$ whereas moderate inhibition occurred with 200 µM Ag$^{2+}$. Other cations i.e. Cu$^{2+}$ and Mg$^{2+}$ only caused substantial inhibition when present in concentrations as 10 and 100 mM. The overall lack of inhibition of xylanase degrading activity by these metals except at very high concentrations indicates that metal precipitation technique could possibly be used to help purify this activity. The addition of 50 mM and 100 mM EDTA to the assay resulted in only 5% and 12% inhibition suggesting that this compound was not chelating cations required by xylanase.

*S. flavogriseus* (Kluepfel and Ishaque, 1982) when grown on xylan induces both xylanase and cellulose enzymes. This seems to indicate that parts of xylanase gene exert some control on the expression of cellulose gene.

The product inhibition demonstrated by *S. flavogriseus* on adding xylose or glucose in medium containing xylan points towards a regulatory mechanism in which besides the necessity of inducer, xylose and glucose are also affecting the synthesis of B-D-xylanase. The question whether or not the effect is due
to catabolic repression can be answered only by studying effect of cAMP in glucose growing strain.

Zymogram of culture filtrate of S. flavogriseus revealed that it codes for multiple xylanases corresponding to Mr 42,000; 29,000 and 18,000 respectively. However 95% of the activity was localized in the fraction corresponding to 18,000 Mr.

Fractionation studies by Johnson et al. (1988) of partially purified extracellular preparations from S. flavogriseus gave some insight into the complexity of the xylanolytic enzyme system. Three distinct endo-β-(1,4) xylanases, one neutral and two anionic activities obtained by anion exchange chromatography however molecular weight was not characterized.

The molecular weights of several xylanases have been reported ranging from 15,000 to 30,000 daltons (Dekker and Richards, 1976). Xylanase of higher molecular weight are also known to exist. The xylanases from Talaromyces byssochlamydoides YH-50 have molecular weights ranging from 45,000 to 70,000 (Yoshioka et al., 1981).

Hemicelluloses bear characteristic substituents on the main polymer chain, as a result complete hemicellulose hydrolysis requires substituent hydrolyzing enzyme activities in addition to endo-β-(1,4) xylanase. Johnson et al. (1988) have reported that S. flavogriseus are generally well suited to such processes.
Although a number of Streptomyces are known to degrade lignocellulose efficiently only two mesophilic species, *Streptomyces flavogriseus* (Kluepfel and Ishaque, 1982), *S. lividans* (Kluepfel et al., 1986) and one thermophilic species *Thermomonospora mesophila* (McCarthy et al., 1986) have been objects of close study.

This organism can be suggested as a model for studying the synthesis and regulation of xylan hydrolytic enzyme when using a variety of inducers and also for studying in a comparative way the convergence and difference between prokaryotic and eukaryotic microorganisms in relation to utilization of complex polysaccharide.

This strain can be choice organisms for utilization of xylan in native form which *Streptomyces* are known to utilize as they produce cellulosics, hemicelluloses and lignolytic enzymes (Crawford, 1981; Kluepfel and Ishaque, 1982) which are all essential for complete degradation of lignocellulose biomass.
Table-I

Effect of cations chelating agents and detergents *S_*flavogriseus*.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Concentrations</th>
<th>% Inhibition</th>
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</thead>
<tbody>
<tr>
<td>Hg$^{2+}$</td>
<td>20 um</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>200 um</td>
<td>94</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>200 um</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 mM</td>
<td>25</td>
</tr>
<tr>
<td>Ag$^{2+}$</td>
<td>200 um</td>
<td>20</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>10 mM</td>
<td>7</td>
</tr>
<tr>
<td>EDTA</td>
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<td>5</td>
</tr>
<tr>
<td></td>
<td>100 mM</td>
<td>12</td>
</tr>
<tr>
<td>SDS</td>
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<td>33</td>
</tr>
<tr>
<td>Triton X-100</td>
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