ESRF	**Experiment title:** Acetylxylan esterase from *Bacillus pumilus*	**Experiment number**: LS1824
Beamline: Id14-4	**Date of experiment**: from: 23rd November 2000 to: 25th November 2000	**Date of report**: 26/07 2002
Shifts: 1	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

S. Benini[1]*, G. Degrassi[1], I. Krastanova[2], D.Lamba[1,3]* and V. Venturi[1]

[1]International Centre for Genetic Engineering and Biotechnology, Area Science Park, Padriciano 99, I-34012 Trieste, Italy

[2]International School for Advanced Studies, Via Beirut 2/4, I-34014 Trieste, Italy

[3]Istituto di Strutturistica Chimica "G. Giacomello", Sez. di Trieste, Area Science Park, Basovizza, S.S. 14 Km 163,5, I-34012 Trieste, Italy

Report:

Xylan is the major constituent of hardwood and represents the second most abundant biopolymer on earth after cellulose. It is present in plant cell wall as a polymer of linear chains of xylose joined by β−1,4 glycosidic linkages. The xylose units in the backbone are modified with different kind of side chain substitutions. Most common substituents are represented by arabinose, 4-*O*-methylglucuronic acid and acetyl moieties.

To achieve the complete degradation of such a complex molecule a set of enzymes is required. The hydrolysis of β−1,4 D-xylan is accomplished by xylanhydrolases (E.C. 3.2.1.8) which break the β−1,4 glycosidic bonds between xylopyranosyl moieties. The products of the hydrolysis are short xylo-olygosaccharides, usually of a size between two and six sugar units. The complete degradation of the polymeric chain to simple sugar moieties is carried out by an additional endo-β−1,3 xylanase (E.C. 3.2.1.32) and β-xylosidases (E.C. 3.2.1.37). The substituents side chain groups are hydrolysed by α-**L**-arabinofuranosidases (E.C. 3.2.1.55), α-glucuronidases (E.C. 3.2.1.139) and acetyl xylan esterases (E.C. 3.1.1.72).

An acetyl xylan esterase from *Bacillus pumilus* (AXE) belonging to the esterase family 7 (Coutinho & Henrissat, 1999) has been recently characterised and over-expressed in *Escherichia coli* (Degrassi *et al.*, 1998, Degrassi *et al.*, 2000). The protein molecular weight is 40kDa as determined by SDS-PAGE, while the native molecular weight has been estimated to be 190kDa by gel filtration chromatography thus suggesting either an homo tetrameric or pentameric enzyme (Degrassi *et al*., 1998). The recombinant AXE showed no difference in activity when compared to the native protein indicating a correct expression of the gene in the host strain and a correct fold of the enzyme.

In *B. pumilus* the xylanolytic system has been partially investigated revealing that a complete xylan degradation pathway must be present as suggested by the presence of a xylanase and a β-xylosidase, indicating that

AXE is one of its components. Furthermore the production of the enzyme is induced by the presence of xylan and corncob in the growth medium. *B. pumilus* AXE reveals activity on a broad range of acetylated compounds such as acetylated xylan, xylose tetraacetate, glucose pentaacetate, *p*-nitrophenyl acetate and cephalosporin C.

The high sequence identity with *Bacillus subtilis* cephalosporin C acetylhydrolase (CAH) suggests a possible pharmaceutical application of *B. pumilus* AXE in the antibiotics production *e.g.* in the deacetylation of cephalosporin C (Mitsushima *et al.* 1995).

To date no structural information it is available for bacterial AXEs, while the crystal structures of AXE II from the fungus *Penicillium purpurogenum* (Ghosh *et al.*, 1999; Ghosh *et al.*, 2001) and the catalytic core of acetylxylan esterase from the fungus *Trichoderma reesei* (Hakulinen *et al.*, 2000) have recently been determined. Both structures reveal the presence of the catalytic triad Ser-His-Asp, typical of many esterases, lipases and serine proteases and of a doubly wound α/β sandwich fold with a central parallel β-sheet flanked by two parallel α-helices on each side.

In order to ascertain the role (AXE or CAH) and the catalytic mechanism of *B. pumilus* AXE, we began a study aimed at the X-ray crystal structure determination of this enzyme.

Crystals were grown from 2 M ammonium sulphate (AMS) Tris-HCl pH 8.0. Diffraction data were collected on a single crystal at 100 K using as cryoprotectant a solution of the precipitant in which the salt concentration was increased to 2.4 M AMS and to which glycerol was added to a final concentration of 20%. The crystals (0.05 x 0.05 x 0.05 mm^3), diffracted up to a resolution of 2.0 Å at ESRF, Grenoble (France) on ID14-EH4 beam line (Experiment n° : LS1824 – Nov 24th 2000) . A summary of the X-ray diffraction data is given in Table 1. Assuming a molecular weight of 36.012 kDa (as deduced by the amino acid sequence) and one monomer in the asymmetric unit, the value of the crystal packing parameter, V_M, is 2.36 $Å^3$ Da^{-1} with a solvent content of 48 %. This observation together with the native molecular weight (approx. 190 kDa) suggest that AXE may be arranged in the crystal as a multimer of six identical subunits (Benini *et al.*, 2001).

A search for heavy atom derivatives is currently underway in order to solve the three-dimensional structure of *B. Pumilus* AXE by SIR/MIR as well as expression of Se-Met protein in order to get experimental phases by MAD.

Table 1

Crystal parameters, data collection and processing statistics.
(Values in parentheses are for the highest resolution shell.)

X-ray source	ESRF ID14-EH4
Wavelength (Å)	0.976
Detector	Quantum4 CCD
Space group	R32
Unit-cell parameters (Hexagonal setting)	
a ,b (Å)	107.58
c (Å)	152.94
Mosaicity (°)	0.8
Resolution range (Å)	37.0 - 2.0 (2.1 – 2.0)
No. of measurements	319784
No. of observed reflection $I \geq 0$	132755
No. of unique reflections $I \geq 0$	21596 (1970)
Completeness (%)	91.4 (84.0)
Redundancy	6.1 (3.6)
$<I/\sigma(I)>$ of measured data	12.7 (2.7)
R_{sym} † (%)	10.4 (27.7)

† $R_{sym}(I) = \Sigma_{hkl}\Sigma_i | I_{hkl,i} - <I_{hkl}> | / \Sigma_{hkl}\Sigma_i | I_{hkl,i} |$ with $<I_{hkl}>$ mean intensity of the multiple $I_{hkl,i}$ observations from symmetry-related reflections.

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Carbohydrate-Active Enzyme server (http://afmb.cnrs-mrs.fr/~cazy/CAZY/)

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