	Experiment title: A Number of Proteins from Bacteria to Eukarya and from Antarctic to Volcanic Areas	**Experiment number**: MX-71
Beamline: ID29	**Date of experiment**: from: 7 DEC. 2002 to: 9 DEC. 2002	**Date of report**: 24/02/03
Shifts:	**Local contact(s)**: Dr. Steffi ARZT	*Received at ESRF:*

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Report:

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.

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.

The *axe* gene of *B. pumilus* was expressed in *E. coli* as previously reported (Degrassi *et al.* 2000). The protein was purified by standard chromatographic techniques according to the published procedure (Degrassi *et al.* 1998).

Crystals were grown from 2 M ammonium sulphate (AMS), Tris-HCl pH 8.0 at 4°C.

However N-terminal sequencing and EIS-MS analysis carried out on the protein following the crystals dissolution revealed that the obtained crystals were not of the expected enzyme but of *E.coli* 5-keto-4-deoxyuronate isomerase (KDUI) protein instead. This enzyme represented at most 4% impurity in the AXE preparation.

P. Dunten *et al.* (1998) in an attempt to crystallize *Rhodobacter capsulatus* S-adenosylhomocysteine hydrolase also face the crystallization of the very same KDUI impurity. No crystal structure determination of KDUI has been reported up to date and no homologous 3D structures are known.

Based on the number of cysteines (5) and methionines (12) representing overall 6.1% of the amino acids composition (278 residues) we planned to address the structure solution by performing a SAD experiment on the sulphur edge at ID29 (λ=1.731Å). We also hoped to collect an high resolution native data set as well.

During the effective time available to us, on December 7th 2002 (10.00am - 2.00pm), diffraction data on five crystals were collected 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 0.1 x 0.1 mm^3), diffracted up to a resolution of 3.5-3.0 Å. Lattice parameters of one of the best diffracting crystals are given in Table 1.

A number of preliminary tests on few crystals were performed in order to optimize the best attenuation of the beam (graphite + aluminum) and minimize the exposure time (3 sec) due to the radiation damage observed. Eventually we were not able to collect more than 30 frames (1° oscillation /frame) on each of the five crystals. Therefore we did not succeeded in collecting a high redundant and complete data set, despite the high symmetry space group, that is strictly required for solving the structure *via* SAD.
Moreover due to the severe radiation damage, scaling and merging of the five data sets respectively was not successful too.

Table 1
Crystal parameters

X-ray source	ESRF ID-29
Wavelength (Å)	1.731
Detector	Quantum 210
Space group	R32
Unit-cell parameters (Hexagonal setting)	
a ,b (Å)	103.9
c (Å)	180.2
Mosaicity (°)	1.3
Resolution range (Å)	30.0 - 3.0

References

Degrassi, G., Okeke, B., C., Bruschi, C., V. & Venturi, V. (1998). *Appl. Environ. Microbiol.* **64**, 789-792.

Degrassi, G., Kojic, M., Ljubijankic, G., & Venturi, V. (2000). *Microbiology* **146**, 1585-1591.

Dunten, P., Jaffe, H., Aksamit R.R., (1998) *Acta Cryst*. **D54**, 678-680.

Mitsushima, K., Takimoto, A., Sonoyama, T. & Yagi, S. (1995).*Appl. Environ. Microbiol* **61**, 2224-2229.