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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** `Structure of the extended spectrum beta lactamase BES-1`	**Experiment number**: `MX-633`
Beamline: `ID23 1`	**Date of experiment**: from: `15-MAR-2007 at 16:00` to: `16-MAR-2007 at 8:00`	**Date of report**: 18/09/07
Shifts: 2	**Local contact(s)**: `Dr. Raimond B.G. RAVELLI`	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Microbial resistance to β-lactams is one of the major problems facing health care institution, because of the wide use of these molecules. Structural data about extended-spectrum β-lactamases (enzymes able to cleave the beta-lactam ring by an acylation and hydrolytic deacylation catalytic mechanism) are particularly important for the conception of novel β-lactam antibiotics.

BES-1 is an atypical ESBL, which only share 47 % identity with CTX-M enzymes, which are the most closely related ESBL (Bonnet et al., 2000). BES-1 exhibits an unusual high activity against the two major wide spectrum β-lactams (ceftotaxime and ceftazidime) and a low susceptibility to the β-lactamase inhibitor tazobactam.

To investigate the structural features involved in the atypical activity of BES-1, we crystallized BES-1 and determined its three-dimensional structure at resolution of 1.5 Å (Figure 1). Crystal structure revealed insights into the structure-function relationships of BES-1. BES-1 general structure was close to those of other β-lactamases, especially a conserved disposition of the catalytic residues (Ser70, Ser130, Glu166). However, BES-1 also presented specificities. The Ω loop located at the entrance of the active site had an atypical positioning. In the Ω loop, Cα atoms of residues 167 to 179, which is probably implicated in the substrate recognition, shifted 0.6 to 1.5 Å in comparison with the structure of β-lactamases TEM-1 and CTX-M-9 (Figure 2). We also noticed

a 0.8 Å shift of the β3 strand (Figure 3). On the other site of the active site, the loop between residues 101 to 106 presented an original conformation and induced a 1.2 to 2 Å shift of Ala104 away from the active site (Figure 4). This important and very atypical conformation is probably explained by the present of the residue Ala106, instead Asn106 or Ser106, which are conserved in beta-lactamases (Figure 5). Overall, thee key elements of the active site exhibited a positioning, which lead to an enlargement of the active site. This enlargement can explain the activity of BES-1 against the bulky wide-spectrum β-lactams.

At last, the residue Arg220 establishes hydrogen bonds with the hydroxyl group of residues 236 and 245 and with the lateral chain of Thr237 (Figure 6). This conformation of Thr237 could favor accommodation of carboxylic group of cephalosporins.

This first structure of BES-1 provided very interesting data which explain the atypical activity of BES-1. To confirm, these preliminary results Arg220Ala and Ala106Asn BES-1 mutants have been constructed and have been analyzed by enzymatic method. The two mutants exhibited a decrease of activity against wide-spectrum β-lactams and the mutant Arg220Ala loosed the resistance to the β-lactamase inhibitor tazobactam. We are planning to investigate these mutants by X-ray diffraction to show the structural consequences of these substitutions and make the “story” complete.

Figure 1

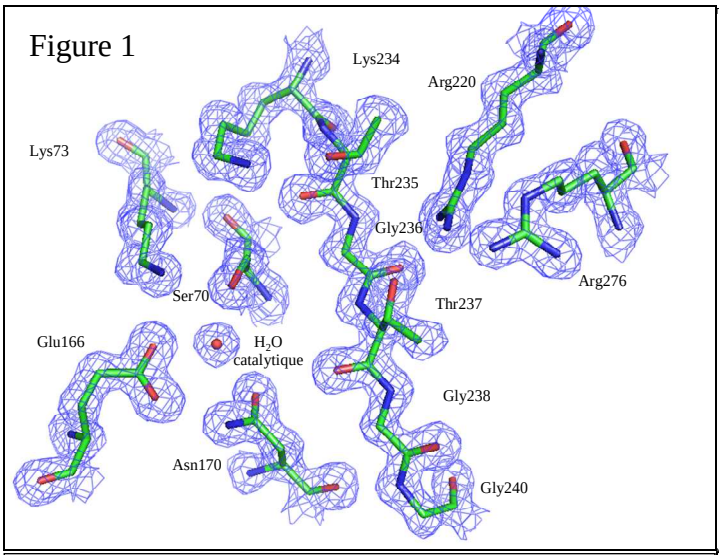

Figure 2

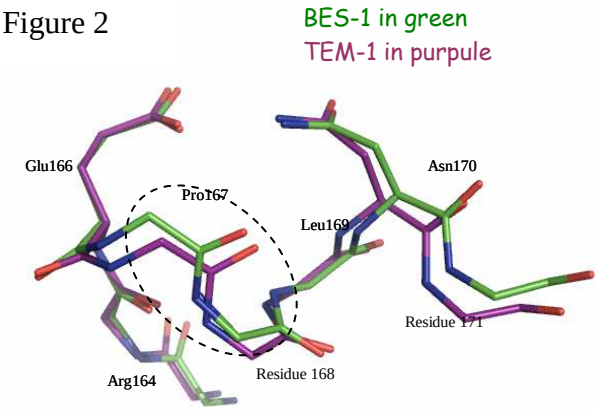

Figure 3

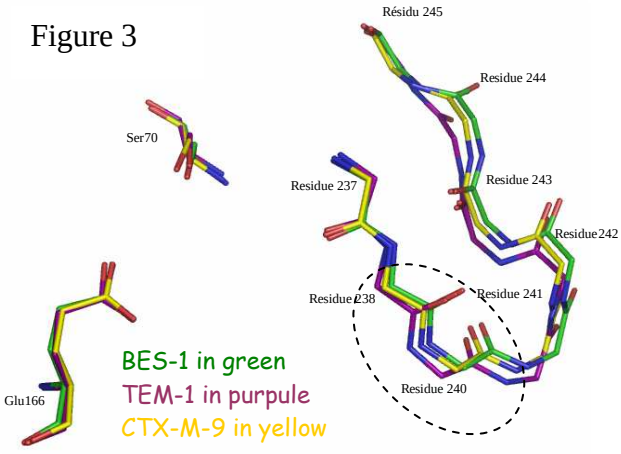

Figure 4

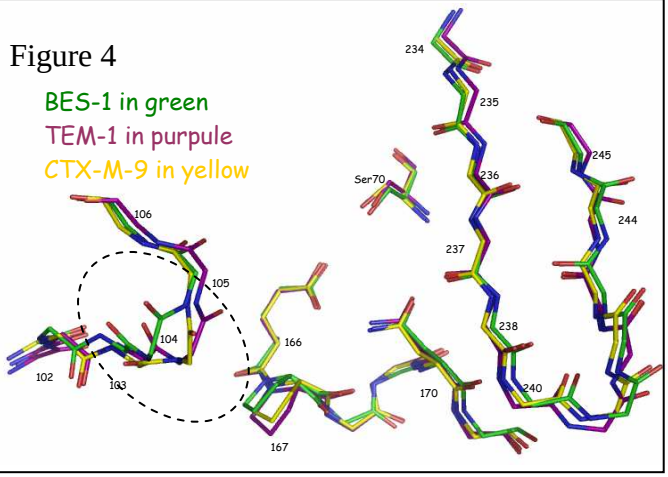

Figure 5

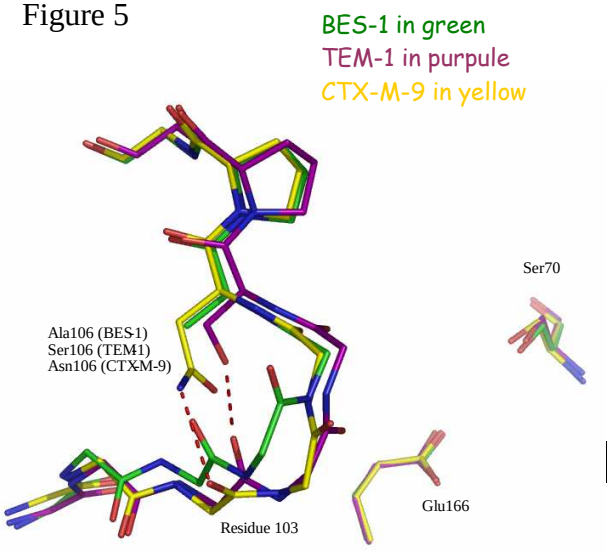

Figure 6