EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Deinococcus radiodurans cells irradiation for proteomics experiments	**Experiment number**: SC-1457
Beamline:	**Date of experiment**: from: 20/02/2004 to: 22/02/2004	**Date of report**: 07/02/2005
Shifts:	**Local contact(s)**: Elke BRAEUER-KRISCH	*Received at ESRF:*

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ESRF

Report:

Aims

The aim of this experiment was to produce samples of irradiated cells of *Deinococcus radiodurans* strain R1 (DEIRA), a non-pathogenic, extremophile, gram-positive soil bacterium which can survive exposure to acute doses of ionizing radiation (over 15,000 Gy).

These samples were needed to then carry out the analysis of the proteome (i.e., the array of proteins produced under certain conditions, at a given time, by a certain type of cells, tissue or organism) of DEIRA exposed to different doses of radiation and identify proteins which are necessary for its survival by means of 2D gel electrophoresis. This is a technique that separates the proteins in the proteome both by charge and molecular weight. By comparison of proteomes under different conditions (i.e. of different gels), the gene products that are down- or up-regulated are detected. Mass spectrometry then allows the identification of the proteins in question.

Requirements of the BL

The experiment required:

1. a high dose-rate so that the considerable dose of 15kGy could be delivered in a negligible time compared to the cell division time of DEIRA (~1h)
2. a set-up able to deliver the desired dose homogeneously on the sample
3. an accurate estimate of the absorbed dose
4. proximity to biochemistry laboratory to allow prompt manipulation of the sample after irradiation

ID17 fulfills all these requirements.

Experimental method

Before the irradiation experiment took place, a growth curve of DEIRA's cells under our conditions (medium 53, 30°C) was experimentally determined (Fig 1) in order to know to which optical density at λ=600nm (OD_{600}) corresponded the mid-log phase, the early stationary phase and the stationary phase. We also estimated the amount of culture needed at each phase in order to have enough material to run one 2D gel.
As this first experiment was meant for screening the effects of different doses on DEIRA's proteome, a large range of doses (100Gy, 500Gy, 1,000Gy, 5,000Gy, 10,000Gy and 15,000Gy) was chosen.
For each dose the experiment was repeated four times to check for reproducibility. Therefore twenty four DEIRA cultures were grown at 30ºC in 240ml of medium 53 to an OD_{600}=1.0, corresponding to the mid-log phase in the growth curve. Cells were harvested by centrifugation in two batches of 120ml, one to be irradiated and one to keep as control, resuspended in 2ml of fresh medium, stored in 2ml cryo-tubes (Ø = 0.8 cm) to fit to the beamsize (0.5x1.2cm^2), fast frozen in liquid nitrogen and stored at -80ºC until the irradiation experiment could take place.
Just before irradiation the two corresponding tubes were de-frozen and put on ice to avoid any further cell growth. One of the tubes was then mounted on the ID17 using an especially engineered sample holder, kept at 4ºC and irradiated with the requested dose of x-ray radiation at a dose-rate of 17,200 Gy/s. The tube was rotated and translated into the beam in order to irradiate homogeneously the sample.
Soon after the irradiation, back in the biochemistry laboratory, both the irradiated and non-irradiated cells were diluted to the original volume (120ml) with fresh medium 53 and incubated at 30ºC . A 20ml sample of both types of cells was collected 5 minutes, 30 minutes, 1 hour, 3 hours, 6 hours and 24 hours after irradiation, harvested by centrifugation, fast frozen in liquid nitrogen and stored at -80ºC. This procedure was repeated for all the six doses and for all samples.

Results

The experiment was extremely successful and produced 288 samples (144 irradiated/144controls) to be used in the following proteomics analysis.

As for now over 150 2D gels have been run (NOTE: it takes a full week to run 6 gels, which correspond to half time-course for one dose with controls, when everything goes all right with the apparatus). The full time-courses of doses 5kGy and 10kGy have been analysed (Fig. 2 and 3) to identify the main differences. The corresponding spots were identified by mass spectrometry fingerprinting, we have the firsts results and they are consistent with literature, which indicates that the study is going in the right direction. To investigate the more subtle changes in cellular activity a more complete analysis is required – this means using large amounts of cells in order to observe proteins of low abundance. The gels for the 100Gy (low dose) time- course have been run and analysis is under way. The gels for the 500Gy and 1,000Gy time-courses have been run, but not all of them are good enough for analysis so they need to be re-run.
Results from the 10kGy experiment are being validated and will form the basis for the first publication from these experiments.

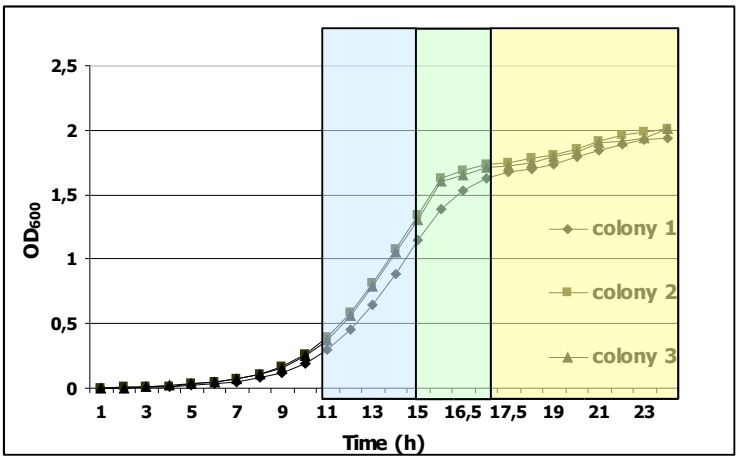

Fig. 1 Growth curve of DEIRA in medium 53 at 30°C. OD_{600} was sampled every hour. In blue it is shown the log-phase, in green the early stationary phase and in yellow the stationary phase.

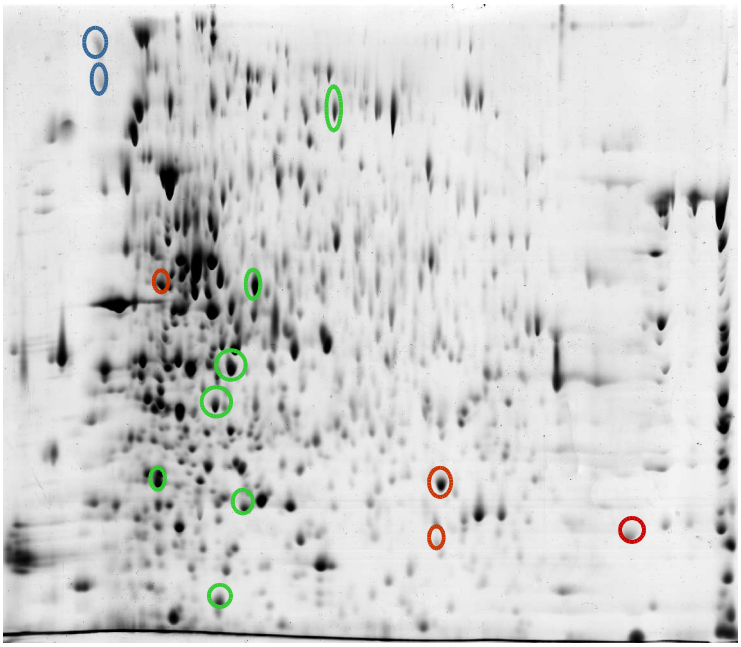

Fig. 2 Example of 2D gel : each spot corresponds to a different type of protein with different pI (3-10) and different mass. The intensity of the spot is proportional to the quantity of protein expressed.
In particular, this gel shows the proteome of DEIRA cells one hour after irradiation with a dose of 5kGy. The circles are highlighting some of the proteins which exhibit changes in abundance following irradiation: circled in red are proteins that appear after irradiation, in green proteins that are more abundant and in blue the ones which are produced in smaller quantities.

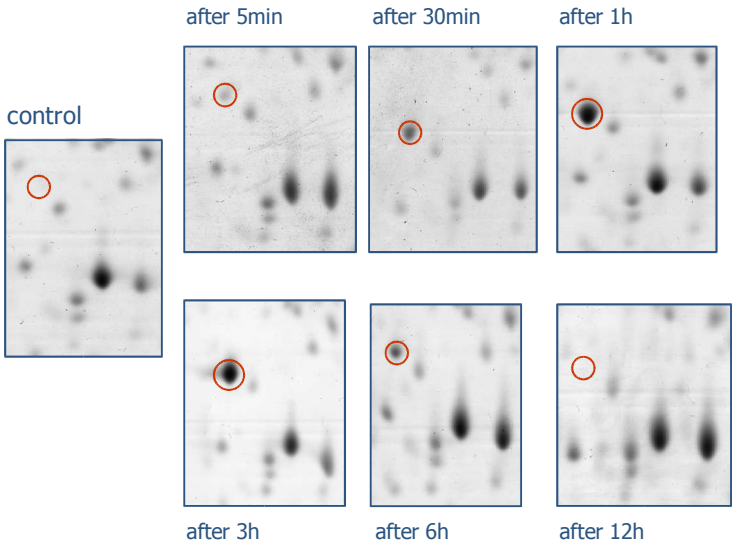

Fig. 3 Example of changes within time: the protein circled in red was not present before irradiation (10kGy). It appears 5 minutes after irradiation and is expressed more and more to reach a maximum of expression between 1 an 3 hours after irradiation. 12 hours after irradiation it has disappeared again. This is listed on the DEIRA known genome as a hypothetical protein, which means that the corresponding DNA sequence looks like coding for a protein, but this sequence is not similar to any other which is known to be coding for a protein in the genome of other organisms. In fact now we know that it is a protein and that it is produced in generous quantities after irradiation.