ESRF	**Experiment title:** Cellular localization of iron in whole cell models of human disease using synchrotron radiation X-ray microfluorescence	**Experiment number**: LS-2133
Beamline: ID-21	**Date of experiment**: from: June 6, 2002 to: June 11, 2002	**Date of report**: August 26, 2002
Shifts: 15	**Local contact(s)**: Barbara Fayard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Ortega Richard, LCNAB, CNRS, Université de Bordeaux 1, Gradignan, France

*Devès Guillaume, LCNAB, CNRS, Université de Bordeaux 1, Gradignan, France
*Font Jean, LCNAB, CNRS, Université de Bordeaux 1, Gradignan, France
*Marcon Thomas, LCNAB, CNRS, Université de Bordeaux 1, Gradignan, France
Valentine Joan, UCLA, Los Angeles, CA, USA
Gralla Edith, UCLA, Los Angeles, CA, USA

Report:

The aim of this experiment was to determine the intracellular coordination state of iron in yeast *Sacharromyces cerevisiae* models of the human disease amyotrophic lateral sclerosis (ALS), using the scanning X-ray microscope at ESRF beam line ID-21.

Superoxide dismutase mutations have been observed in familal cases of ALS. In this neurological disorder disease motoneurons in the spinal cord degenerate causing rapid muscle degeneration and paralysis. It has been suggested that the superoxide anion could release iron from iron-sulfur proteins leading to oxidative damage through redox-cycling in motoneurons from ALS patients.

The yeast *Saccharomyces cerevisiae*, although unicellular, is a model of predilection to study the molecular mechanisms of several human pathologies. In this experiment, mutant yeast cells presenting either a deletion of the Cu,Zn superoxyde dismutase gene (Δsod1), a deletion of Mn superoxide dismutase (Δsod2), or a double deletion (Δsod1,sod2), were compared to wild type cells. A modification of iron coordination, revealed by XANES spectroscopy around Fe absorption edge, would confirm the involvement of altered iron homeostasis in SOD1 deficient cells, and possibly in ALS.

XANES spectroscopy around Fe K-edge was performed on bulk frozen hydrated specimens. The Fe K-edge X-ray absorption spectra were measured in fluorescence mode, with an energy resolution of about 0.5 keV using a Si(2,2,0) crystal as monochromator. X-ray fluorescence spectra were collected with a Ge solid state detector. A liquid nitrogen cryojet was used to maintained cells in their frozen hydrated state.

The analysis of XANES pattern (Fig. 1) shows no difference between samples in pre-edge positions and intensities, as well as in main-edge positions, suggesting a similar mean iron oxidation state in yeast cell mutants. More interestingly, a significant decrease of the main edge intensity is observed for EG118, the Cu,Zn superoxide dismutase deleted yeast strain, compared to wild type cells (EG103). This change in XANES pattern may indicate a modification of Fe coordination chemistry, possibly a decrease in the mean number of ligands. Similar features have been observed for iron proteins such as tyrosine hydroxylase. In a recent report, a decreased in main edge intensity was linked to a change from coordination 6 to 5 after removal of a water molecule ligand in tyrosine hydroxylase.

All together these results indicate that the mean coordination number of iron in Cu,Zn superoxide dismutase deleted cells could be lower than in wild type cells, while the iron electronic structure, oxidation state, remains basically unchanged. It also suggests a possible role for iron altered homoeostasis in ALS etiology. This experiment report is however still preliminary. A careful examination of data and comparison with XANES spectra from characterized iron proteins will give additional information in order to detail the nature of iron coordination, and the mean number of ligands (between 5 and 6 ?), as well as the mean iron oxidation state in yeast cells.

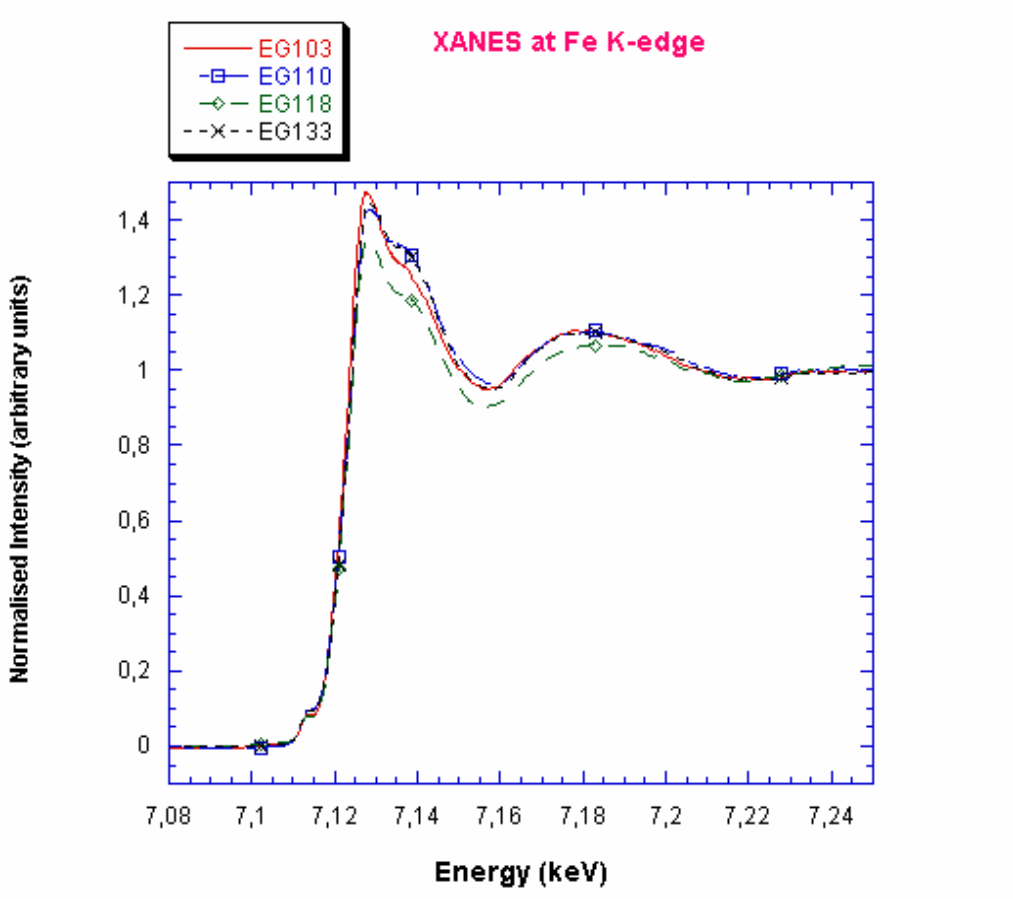

Fig. 1. Normalized Fe K-edge spectra from *Saccharomyces cerevisiae* strains, EG103 = wild type, EG110 = mutant cells lacking Mn superoxide dismutase (Δsod2), EG118 = mutant cells lacking Cu,Zn superoxide dismutase (Δsod1), and EG133 = double mutants Δsod1, Δsod2.