present in microorganisms, plants and animals. From a biotechnological point of view, *Kluyveromyces lactis* β-galactosidase is suitable for many applications due to its neutral optimum pH and for the fact that *K. lactis* is a GRAS organism (Generally Recognized As Safe). Interestingly, β-galactosidases are being used in lactose intolerance treatments and in food industry. Moreover, yeast expressing this enzyme can be used to improve the valorization of the cheese whey, a cheese industry byproduct by coupling the degradation of lactose with ethanol production, biomass production, etc. [1].

On the basis of their sequence, β-galactosidases are classified within families 1, 2, 35 and 42 of glycosyl hydrolases in the CAZy database [2]. Those from eukaryotic organisms are grouped into family 35 with the only exceptions of *K. lactis* and *K. marxianus* β-galactosidases which belong to family 2, together with the prokaryotic β-galactosidases from *Escherichia coli* and *Arthrobacter* sp. Whereas the structures of these last two prokaryotic enzymes have been determined [3], [4], none of the eukaryotic β-galactosidase structures have been reported to date. Although their sequence similarity with the prokaryotic enzymes is significant (48% vs. *E. coli* and 47% vs. *Arthrobacter*) there are many differences, particularly some long insertions and deletions, which play an important role in protein stability and in substrate recognition and specificity.

Gaining insight into the structural features that determine its stability and understanding the specificity determinants and catalytic mechanism should lead to improvements of its biotechnological applications by rational protein engineering. In this study, we describe X-ray crystallographic studies and an analysis of *Kluyveromyces lactis* β-galactosidase structure.

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Keywords: yeast, glycosidase, biotechnology

MS93.P37

Acta Cryst. (2011) A**67,** C781

Structural studies of β–D-xylosidase from *Streptomyces thermoviolaceus* OPC-520

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Xylan is one of the major hemicellulose components of plant cells and is the second most abundant resource after cellulose. *Streptomyces thermoviolaceus* OPC-520 produces relatively high xylanase activity when grown in a medium containing xylan as a carbon source.

An intracellular β–D-xylosidase (BxlA) from *Streptomyces thermoviolaceus* OPC-520, together with a extra-cellular BxlE and the integral membrane proteins BxlF and BxlG, constitutes a xylanolytic system that participates in the intracellular transport of xylan degradation products and the production of xylose. To elucidate the hydrolytic mechanism of xylooligosaccarides to xylose at the atomic level, we attempted X-ray structural analysis of BxlA. The recombinant BxlA protein (molecular weight: 82kDa) was crystallized by the hanging-drop vapor-diffusion method at 293 K. The crystals belonged to the monoclinic space group *C*2, with unit-cell parameters *a*=141.0, *b*=129.5, *c*=100.4 Å, β=120.1°, and contained two molecules per asymmetric unit (V_M=2.47 Å^3/Da). Diffraction data were collected to a resolution to 2.2 Å on the BL26B1 beam line at the SPring-8. The brilliant light source present at SPring-8 was necessary to obtain useful data from these very weakly diffracting crystals. The initial structure of BxlA was determined by MAD method using the SeMet-labeled crystal. The structure of BxlA was built from typical catalytic domain and additional c-terminal domain. We now progress the refinement of structure to elucidate the detailed mechanism of glycoside hydrolysis of BxlA at the atomic level.

Keywords: xylosidase, MAD phasing, crystallographic structure

MS93.P38

Acta Cryst. (2011) A**67,** C781

Structural study of the mutants of FDTS from *T. maritima*

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Like thymidylate synthase (TS) in eukaryotes, the flavin-dependent thymidylate synthase (FDTS) is essential for cell survival of many prokaryotes for the *de novo* synthesis of thymidylate (dTMP). TS and FDTS inhibition stops DNA production, eventually leading to cell death. The two enzyme families present no structural or sequence homology. While both convert dUMP to dTMP, in contrast to the classical TS enzymes, the FDTS enzymes employ 5,10-methylene-5,6,7,8-tetrahydrofolate (CH2H4folate) as the methyl donor but not as reducing agent. Furthermore, recent studies showed that, in contrast to TS, FDTS doesn't use enzymatic nucleophile for the reaction and that the catalytic mechanism of TSs and FDTSs is substantially different [1]. These differences offer the possibility of developing specific FDTS inhibitors as antibiotic drugs with low toxicity.

We report the structural study of five mutants of FDTS from *Thermotoga maritima*. All of the mutants crystallize in the orthorhombic space group $P2_12_12_1$ with unit cell dimensions of a=~55Å, b= ~116Å, and c=~141Å. A comparison of the structures with the wild type enzyme and a comparison of the structures of the mutant enzymes with and without the substrate dUMP show several features that are important for the FDTS reaction. The structural information is also compared with the activity data to get a better understanding of the reaction mechanism.

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Keywords: flavin, thymidylate, synthase

MS93.P39

Acta Cryst. (2011) A**67,** C781-C782

Structure determination of levan fructotransferase

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Levan is fructan composed of linear and branched polymers of