(ST0966 and ST2187) from Sulfolobus tokodaii (St) were overexpressed in E. coli, purified, and crystallized. The crystal structure of Ap-ThrRS-1 has been successfully determined at 2.3 Å resolution, as the first example. Ap-ThrRS-1 is a dimeric enzyme, the two identical subunits being associated to each other. Each subunit is composed of the two domains for the catalytic reaction and for the anticodon-binding, as expected. Their structures are similar to those of Ec-ThrRS. The amino acid residues essential for the catalysis and for the anticodon recognition are highly conserved at the positions and in the orientations. The essential editing domain of ThrRS is completely missing in Ap-ThrRS-1 as expected, suggesting the necessity of the second enzyme ThrRS-2 for editing. Since the N-terminal sequence of Ap-ThrRS-2 is similar to the sequence of the editing domain of Pa-ThrRS, Ap-ThrRS-2 is expected to catalyze de-aminoacylation of the misacylated serine moiety at the CCA terminus.

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Keywords: aminoacylation; threonyl-tRNA synthetase; protein synthesis

FA1-MS03-O4

Crystallographic Snapshots of Iterative Substrate Translocations During Nicotianamine Synthesis in Archaea. Cyril Dreyfus[a], David Lemaire[b], David Pignol[a], Pascal Arnoux[a]. [a]*Laboratoire de Bioénergétique Cellulaire, CEA, DSV, IBEB, Saint-Paul-lez-Durance, F-13108, France.* [b]*Laboratoire des Interactions Protéine Métal, CEA, DSV, IBEB, Saint-Paul-lez-Durance, F-13108, France.*
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Nicotianamine is a ubiquitous metabolite in plants that is able to bind heavy metals both *in vitro* and *in vivo* [1]. It is the main precursor in phytosiderophore synthesis and also an important metal chelator allowing long distance iron transport and sequestration. The action of NA is not restricted to iron homeostasis but extends to that of other metal ions such as Cu^{2+}, Zn^{2+}, Mn^{2+} and Ni^{2+} [2].
Nicotianamine synthase (NAS) is the enzyme catalysing NA synthesis by the condensation of three aminopropyl moieties of S-adenosylmethionine (SAM) and the cyclization of one of them to form an azetidine ring (Fig. 1). An intriguing feature of NAS, when compared to other aminopropyltranferase enzymes, is that it uses three molecules of SAM without any other aminopropyl acceptor. By comparison, spermidine synthase and spermine synthase, two enzymes belonging to the aminopropyltransferase family, use putrescine or spermidine as their respective acceptors and are limited to only one aminopropyl transfer [3]. The NAS gene family has long been considered to be plant-specific. However, recent sequencing projects have revealed *nas*-like genes in the genome of various organisms including plants, fungi and archaea [4].
Here we report six crystal structures of an archaeal NAS from *Methanothermobacter thermautotrophicus* either free or in complex with its product(s) and substrate(s) [5]. These structures reveal a novel fold arrangement with a C-Terminal Rossman-fold domain topped by a NAS specific alpha helical N-Terminal domain. Combined together our work depict an original reaction mechanism taking place in a buried reaction chamber located between the N and C-Terminal domains. This reaction chamber is open to the solvent through a small inlet and a single active site allows the selective entrance of only one substrate at a time that is then processed and translocated stepwise.

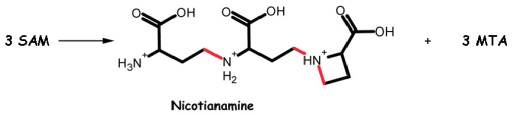

Figure 1: Biosynthesis of nicotianamine (novel bonds created during the reaction are coloured in red).

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FA1-MS03-O5

Substrate Recognition and Catalysis of Polysaccharide Lyases. Sine Larsen[a], Michael McDonough[a], Majbritt Thymark[a], Malene H. Jensen[a], Harm Otten[a], Leila Lo Leggio[a], Torben Borchert[b], Lars H. Christensen[b], Henrik Frisner[b], Carsten Sonksen[b]. [a]*Department of Chemistry, University of Copenhagen, Denmark.* [b]*Novozymes, Bagsværd, Denmark.*
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Lyases cleave the backbone of polysaccharides by a β-elimination mechanism, which results in a double bond in the non-reducing end of the cleaved substrate. Six out of the 21 families of polysaccharide lyases contain enzymes that assist in the degradation of the pectic network of the primary cell wall of plants [1]. We report recent results on investigations of enzymes assigned to Family 1 and 4. The pectate lyases in Family 1 act on the smooth region of pectin, homogalacturonan (HGA) a homopolymer of (1,4)-α linked D galaturonic acid (GalUA) residues. Pectin has "hairy" rhamnogalacturonan regions (RG-I) interspersed and the Family 4 lyases act on this part of pectin. The backbone of RG-I is composed of alternating rhamnose (Rha) and galacturonic acid (GalUA) residues with [,2-α-L-Rh-(1,4)-α-D-GalUA-(1,] as the repeating unit, the family 4 lysases cleave the (1,4)-α-glycosidic bond. Among the differences

between the two lyase families are the nature of the catalytic base, which is Arg in Family 1 and Lys in Family 4 in agreement with the lower pH optimum (~6.0) for the latter family.

We have investigated the structure of the thermostable Family 1 pectate lyase from *Thermotoga maritima.* It displays the parallel β-helix fold that was first seen at pectate lyases but in contrast to other lyases of Family 1 it crystallizes as a trimer. The structures have been determined of this enzyme at two different pH values (4.2 and 6.5) and of two substrate complexes of an inactive enzyme (mutation of the catalytic base). The Family 4 lyase from *Aspergillus aculeatus* display a completely different fold comprised of three different domains each dominated by β-structure [2]. An excellent picture of the substrate binding in this Family 4 lyase was obtained by the crystal structure of the inactive Lys150Ala mutant complexed with hexamer of rhamnogalacturonan.

The structural and kinetic studies of these two enzymes in combination with those of other pectate lyases has provided insight in catalysis of the lyases (the role of calcium ions and pH) and the factors that have an impact on the recognition of the complex substrates.

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