

(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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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. ^aLaboratoire de Bioénergétique Cellulaire, CEA, DSV, IBEB, Saint-Paul-lez-Durance, F-13108, France. ^bLaboratoire 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 Cu²⁺, Zn²⁺, Mn²⁺ and Ni²⁺ [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 azetidinium ring (Fig. 1). An intriguing feature of NAS, when compared to other aminopropyltransferase 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 thermoautotrophicus* either free or in complex with its product(s) and substrate(s) [5]. These structures reveal a novel fold arrangement with a C-Terminal Rossmann-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.

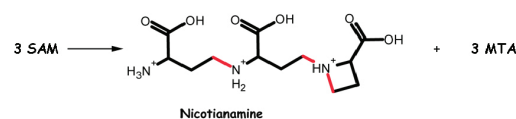


Figure 1: Biosynthesis of nicotianamine (novel bonds created during the reaction are coloured in red).

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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. ^aDepartment of Chemistry, University of Copenhagen, Denmark. ^bNovozymes, Bagsvaerd, 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 galacturonic 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 lyases cleave the (1,4)-α-glycosidic bond. Among the differences