A novel non-heme Fe^{II} α-ketoglutarate-dependent chlorinating enzyme in syringomycin E biosynthesis

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The lipodepsi-nonapeptide syringomycin E (Figure 1A), elaborated as a phytotoxin by *Pseudomonas syringae* pv. *syringae* B301D contains a 4-Cl-L-Thr_9 moiety where failure to chlorinate results in a 30 fold drop in biological activity. The proteins SyrB1 and SyrB2 encoded by the biosynthetic cluster are shown to act as a substrate and enzyme pair in which the acyl enzyme L-Thr-S-SyrB1 is chlorinated by SyrB2 (Figure 1B). SyrB2 is a member of the non-heme Fe^{II} α-ketoglutarate-dependent enzyme superfamily, and requires O_2 and α-ketoglutarate as well as chloride ions to carry out monochlorination of the $-CH_3$ group of the L-Thr-S-SyrB1. Chlorination of the L-Thr-S-enzyme was validated by thioesterase-mediated release of L-Thr and 4-Cl-L-Thr, *N*-derivatization, HPLC separation and mass spectrometry, compared to authentic standards and with [^{14}C]-L-Thr and [$^{36}Cl^-$] as substrates. Enzymatic halogenation is a novel reaction type for non-heme Fe^{II}-dependent enzymes and may be the general mode for biosynthetic halogenation of aliphatic carbons of natural products.

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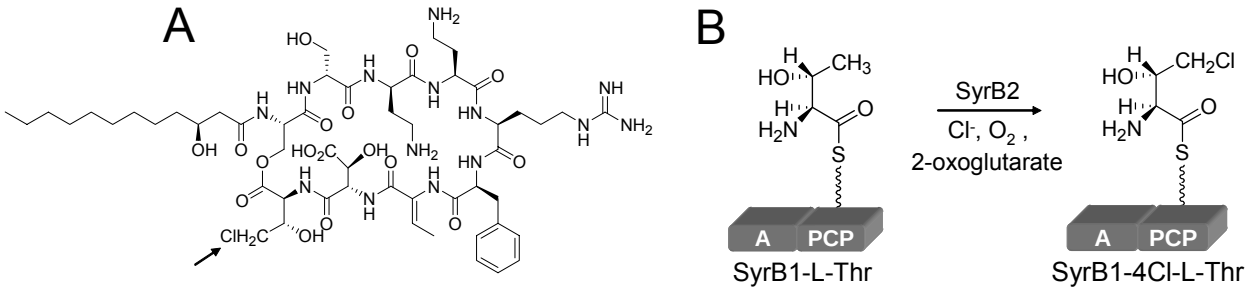

Figure 1 A. Structure of syringomycin E. B. Reaction catalyzed by SyrB2