PEPTIDOGLYCAN DEACETYLASES FROM *BACILLUS SUBTILIS*. SPECIFICITY AND STRUCTURAL INSIGHTS FOR MurNAc AND GlcNAc DEACETYLATION

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Peptidoglycan (PG) is a net-like structure in the bacterial cell wall that envelops the cytoplasmatic membrane with a fundamental role to preserve and protect cell integrity. It is composed of a glycan chain of alternating β-1,4-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) units that are cross-linked by short peptides. PG is constantly remodeled during cell growth and division to incorporate new material. After PG biosynthesis, post-synthetic modifications tightly control the action of autolysins. Among them, de-*N*-acetylation of either GlcNAc or MurNAc residues regulate autolysis and provide resistance to host lysozymes to evade detection by the innate immune system.

PG deacetylases (PG DAs) are members of family 4 carbohydrate esterases (CE4) in the CAZY database, which operate by metal-assisted general acid/base catalysis [1]. Our objective is to understand the different specificities (GlcNAc *vs.* MurNAc PG deacetylases) and evaluate them as novel therapeutic targets for antimicrobials.

Here we analyze the six PG DAs annotated in the genome of *Bacillus subtilis*. PdaA was the first characterized MurNAc deacetylase and its X-ray structure solved [2], being the prototype of a canonical MurNAc deacetylase involved in sporulation. PdaB was isolated later, but with yet unknown substrate [3]. We characterized and solved the X-ray structure of PdaC [4], being a novel subclass of MurNAc deacetylases with dual activity, acting on MurNAc residues of PG but also on GlcNAc residues of chitin oligosaccharides (GlcNAc oligomers). We will report its mode of action and a mutational analysis to modulate specificity and catalytic activity. We also recombinantly expressed and preliminary characterized the other three annotated putative PG deacetylases, YheN, YxkH and YlxY, in the *B. subtilis* genome. YheN and YxkH also show activity on chitin oligosaccharides, whereas YlxY is inactive because it lacks the catalytic base and one metal-coordinating residue in the conserved MT1 motif (Figure 1). Their mode of action and structural and mutational analyses will be discussed.

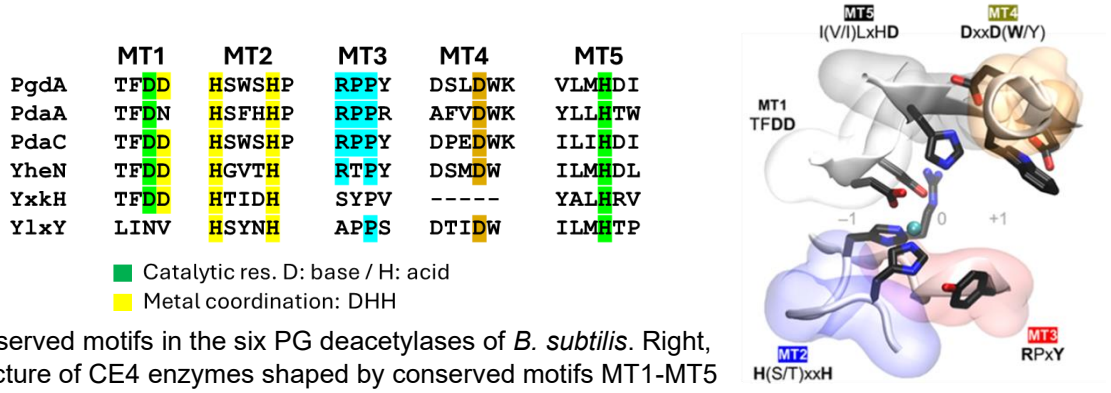

Figure 1. Conserved motifs in the six PG deacetylases of *B. subtilis*. Right, active site structure of CE4 enzymes shaped by conserved motifs MT1-MT5

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