The Effect of Mutations at R266 in *Lysinibacillus sphaericus* NSAR/OSBS

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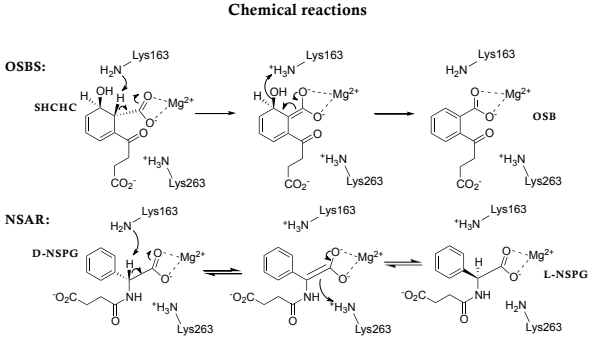
Abstract

Catalytic promiscuity is the ability of an enzyme to catalyze non-biological reactions in the same active site as its native biological reactions. Catalytic promiscuity plays a central role in the evolution of new enzyme functions. Studying the evolution of catalytically promiscuous enzymes can reveal mechanisms by which new functions evolve. This study focuses on various catalytically promiscuous enzymes from the N-succinylamino acid racemase/o-succinylbenzoate synthase (NSAR/OSBS) subfamily. The NSAR/OSBS subfamily is the branch of the OSBS family in which NSAR activity evolved, and to understand how this occurred we looked for amino acid substitutions that were present in the NSAR/OSBS subfamily but absent in the branches of the OSBS family that lack NSAR activity. Previously, we identified a conserved arginine in all the NSAR/OSBS enzymes. At the homologous position in non-promiscuous OSBS subfamilies there is usually a hydrophobic residue. Further studies showed that R266 was important in the evolution of NSAR activity in the promiscuous *Amycolatopsis* sp. T-1-60 NSAR/OSBS (AmyNSAR/OSBS). The R266Q mutation in AmyNSAR/OSBS profoundly reduces NSAR activity with only a moderate effect on OSBS activity. However, in *Lysinibacillus sphearicus* NSAR/OSBS, another member of the NSAR/OSBS subfamily, which shares 48% sequence identity to AmyNSAR/OSBS, the R266Q mutation had much less significant effects on NSAR and OSBS activities. Therefore, R266 was mutated to all other amino acids to observe the effect of those mutations on NSAR and OSBS activities. Mutations that decrease NSAR activity with a minimal effect on OSBS activity will be selected for further mechanistic studies.

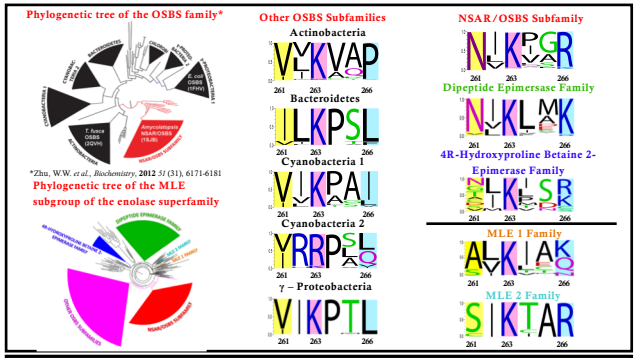
Promiscuous NSAR/OSBS Enzymes



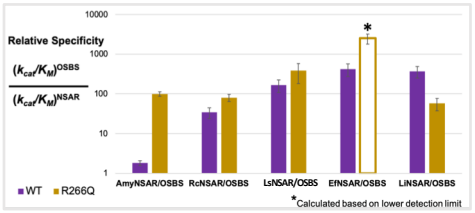
Goal

- How does the conserved second-shell residue R266 contribute to the NSAR reaction specificity in *Lysinibacillus sphaericus* NSAR/OSBS?

Conservation of R266 in the NSAR/OSBS subfamily



R266Q mutation decreases NSAR specificity in only AmyNSAR/OSBS



R266 was mutated to each other amino acid in LsNSAR/OSBS

Protein sequence alignment of all mutations:

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R266P     GVINIKIGPVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266K     GVINIKIGKVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266W     GVINIKIGWVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266V     GVINIKIGVVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
L.sphaeWT GVINIKIGRVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266L     GVINIKIGLVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266Y     GVINIKIGYVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266G     GVINIKIGGVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266C     GVINIKIGCVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266D     GVINIKIGDVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266F     GVINIKIGFVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266I     GVINIKIGIVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266N     GVINIKIGNVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266T     GVINIKIGTVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266A     GVINIKIGAVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266E     GVINIKIGEVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266M     GVINIKIGMVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266Q     GVINIKIGQVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266H     GVINIKIGHVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
R266S     GVINIKIGSVGGITEAKKIHDYCQQHAIPVWCGGMLEAGIGRAHNIALTALSNFVLPGDT
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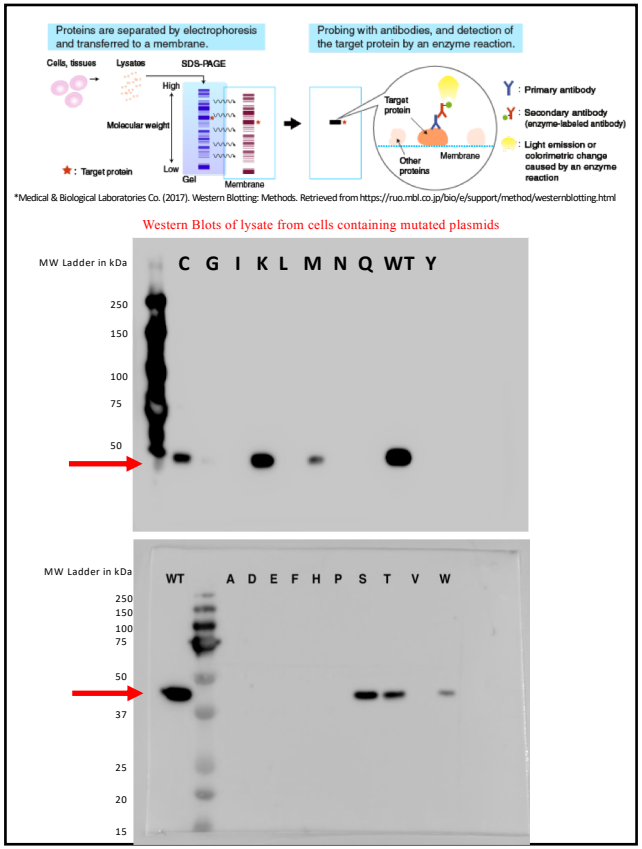
*Mutations at position 266 are indicated by the red

Expression tests showed which mutations produced the protein



Conclusions/Next Steps

- R266 is important enough that a mutation at this location can greatly affect the expression of the enzyme
- Next Steps:
  - Purify all the proteins that have expressed in large quantities
  - Assay the enzymes for NSAR and OSBS activity

Acknowledgments

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