

An Efficient Method To Generate Gene Deletion Mutants of the Rapamycin-Producing Bacterium *Streptomyces iranensis* HM 35.

Netzker T, Schroeckh V, Gregory MA, Flak M, Krespach MK, Leadlay PF, Brakhage AA (2016) An Efficient Method To Generate Gene Deletion Mutants of the Rapamycin-Producing Bacterium *Streptomyces iranensis* HM 35. *Appl Environ Microbiol* 82(12), 3481-3492.

Details



Abstract

Streptomyces iranensis HM 35 is an alternative rapamycin producer to *Streptomyces rapamycinicus*. Targeted genetic modification of rapamycin-producing actinomycetes is a powerful tool for the directed production of rapamycin derivatives, and it has also revealed some key features of the molecular biology of rapamycin formation in *S. rapamycinicus*. The approach depends upon efficient conjugational plasmid transfer from *Escherichia coli* to *Streptomyces*, and the failure of this step has frustrated its application to *Streptomyces iranensis* HM 35. Here, by systematically optimizing the process of conjugational plasmid transfer, including screening of various media, and by defining optimal temperatures and concentrations of antibiotics and Ca(2+) ions in the conjugation media, we have achieved exconjugant formation for each of a series of gene deletions in *S. iranensis* HM 35. Among them were rapK, which generates the starter unit for rapamycin biosynthesis, and hutF, encoding a histidine catabolizing enzyme. The protocol that we have developed may allow efficient generation of targeted gene knockout mutants of *Streptomyces*.

species that are genetically difficult to manipulate.

Beteiligte Forschungseinheiten

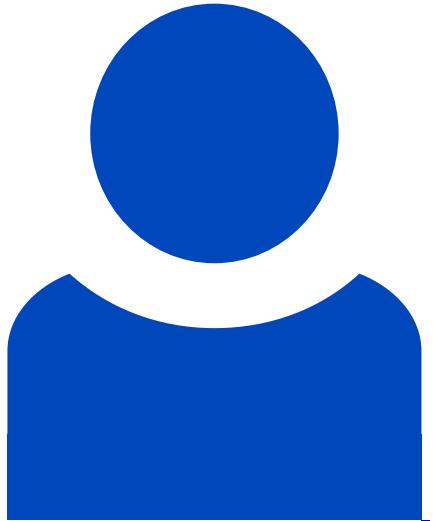
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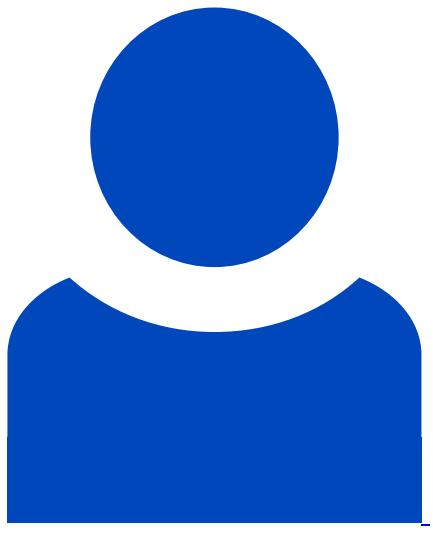
Axel A. Brakhage

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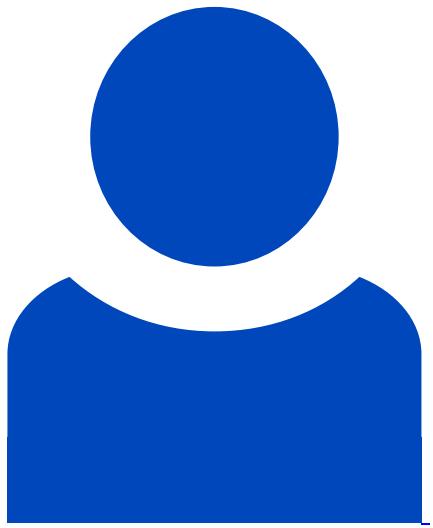
Michał Flak

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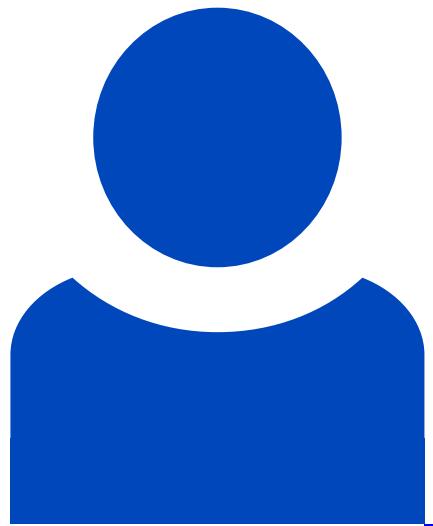
Mario Kespach

[Details](#)



Tina Netzker

[Details](#)



Volker Schroeckh

[Details](#)

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