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

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Details

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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.

Involved units

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