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Genomic modifications for enhanced antibiotic production in rifamycin derivative-producing *Amycolatopsis mediterranei* S699 strains: focusing on *rifQ* and *rifO* genes

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Rifamycin and its derivatives are natural products that belong to the class of antibiotic-active polyketides and have significant therapeutic relevance within the therapy scheme of tuberculosis, a worldwide infectious disease caused by *Mycobacterium tuberculosis*. Improving the oral bioavailability of rifamycin B was achieved through semisynthetic modifications, leading to clinically effective derivatives such as rifampicin. Genetic manipulation of the rifamycin polyketide synthase gene cluster responsible for the production of rifamycin B in the *Amycolatopsis mediterranei* strain S699 represents a promising tool to generate new rifamycins. These new rifamycins have the potential to be further derivatized into new, ideally more effective, clinically usable compounds. However, the resulting genetically engineered strains only produce these new derivatives in low yields. One example is the strain DCO36, in which *rif*AT6 was replaced by *rap*AT2, resulting in the production of rifamycin B and the new derivative 24-desmethyl rifamycin B. Here we describe the successful method adaptation of the PCR-targeting *Streptomyces* gene replacement approach to *Amycolatopsis mediterranei* S699 and further on the implementation of genetic modifications that enable an increased production of the derivative 24-desmethyl rifamycin B in the mutant strain DCO36. The described genetic modifications resulted in a mutant strain of DCO36 with *rifQ* deletion showing a 62% increase in 24-desmethyl rifamycin B production, while a mutant with *rifO* overexpression showed a 27% increase.

KEYWORDS

rifamycin, *Amycolatopsis mediterranei* S699, tuberculosis, increased antibiotic production, homologous recombination, *rifQ*, RifO