# Filling The Gaps In The Kirromycin Biosynthetic Gene Cluster In Streptomyces Collinus Tü 365

**Robertsen, Helene Lunde; Musiol-Kroll, Ewa Maria ; Ding, Ling; Lee, Sang Yup; Weber, Tilmann**

*Publication date:*
2017

*Document Version*
Publisher's PDF, also known as Version of record

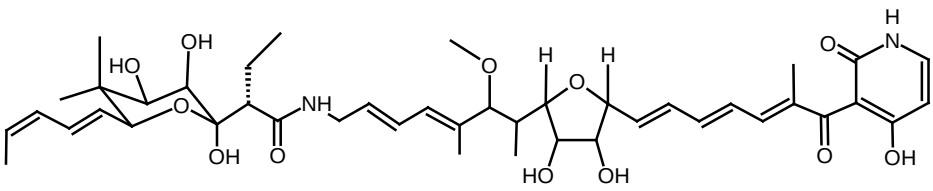
# Filling The Gaps In The Kirromycin Biosynthetic Gene Cluster In *Streptomyces Collinus* Tü 365

**Helene Lunde Robertsen[1], Ewa Maria Musiol-Kroll[2], Ling Ding[1], Sang Yup Lee[3] and Tilmann Weber[1*]**

[1]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet 220, 2800 Kongens Lyngby, Denmark
[2]Eberhard-Karls-Universität Tübingen, Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Microbiology/Biotechnology, Auf der Morgenstelle 28, 72076 Tübingen, Germany
[3]Department of Chemical and Biomolecular Engineering (BK21 Plus Program), Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

Kirromycin is a potent inhibitor of protein biosynthesis in prokaryotes as it binds to the elongation factor Tu, leading to conformational changes and ultimately stalling of the bacterial ribosome. The linear molecule is synthesized by a hybrid PKS-I/NRPS and the biosynthetic gene cluster comprises 26 genes spanning an 82 kb DNA region. A combination of *in silico* bioinformatic predictions and *in vitro* mutational studies have revealed the role of core genes involved in biosynthesis of kirromycin, however, genes involved in tailoring reactions remained to be fully characterized in order to fill the gaps in the pathway.
In this study, the role of the seven tailoring enzymes, including the putative methyltransferase KirM, the Dieckmann cyclase KirHI, two cytochrome P450 hydroxylases KirOI and KirOII, the dioxygenase kirHVI, and the two hypothetical proteins KirHIV and KirHV, were elucidated based on gene inactivations and complementations. High-resolution mass spectrometry (HRMS) and HR-MS/MS findings allowed for correctly assigning function to all seven genes and by that providing experimental evidence for the tailoring reactions involved in kirromycin biosynthesis. Another so far uncharacterized gene, *kirN*, encodes a putative crotonyl-CoA reductase/carboxylase (CCR) that is proposed to provide the ethylmalonyl-CoA extender unit required for kirromycin assembly. As there is a second copy of a CCR gene encoded in the genome, presumably involved in primary metabolism, the effect of a *kirN* deletion was investigated as well. Albeit expected to give rise to lowered kirromycin production, initial results from the *kirN* deletion revealed a production profile identical to that of kirHVI mutant. This led to further investigations in the operon-structure of the gene cluster, in which *kirHVI* is immediately downstream of *kirN*. Hence, deletion of *kirN* could in fact give rise to a non-functional *kirHVI*. To confirm this hypothesis, a gene complementation of the *kirN* mutant with *kirHVI* was carried out and with that the kirromycin production could be restored, albeit to only 30 % of wild type level.
This study has managed to fill out many of the gaps in the hypothetical pathway of kirromycin biosynthesis.

**Keywords:** antibiotic, kirromycin, tailoring enzymes



Structure of kirromycin