Heinrich Heine Universität Düsseldorf

Heinrich-Heine-Universität Düsseldorf 40204 Düsseldorf
Dekanat der Mathematisch-Naturwissenschaftlichen Fakultät

An alle
hauptamtlichen Professoren/innen
und Privatdozenten/innen
des Faches Biologie der
Mathematisch-Naturwissenschaftlichen Fakultät

Mathematisch-Naturwissenschaftliche Fakultät

Dekanat

Promotionsangelegenheiten
Universitätsstraße 1
40225 Düsseldorf
Telefon: +49 (0)211 81 15092
E-Mail: promotionmnf@hhu.de

30.04.2024

Promotionsverfahren von **Frau M.Sc. Nora Lisa Bitzenhofer**
Auslage der Dissertation und Gutachten sowie Termin der mündlichen Prüfung
Anlage: Einseitige Zusammenfassung der Dissertation

Sehr geehrte Damen und Herren,

in dem oben genannten Promotionsverfahren wird die Annahme der Dissertation

Harnessing the potential of *Pseudomonas putida* as a robust platform for the synthesis of bioactive natural products

von den Berichterstattenden Prof. Dr. K.-E. Jaeger, Prof. Dr. M. Pohl und Prof. Dr. rer. nat. habil. Wolfgang Liebl beantragt. Sie kann zusammen mit den Gutachten in der Zeit

vom 09.05.2024 bis 20.05.2024

eingesehen werden. Bitte wenden Sie sich zur Einsicht an das Promotionsbüro (promotionmnf@hhu.de).

Einsprüche gegen diese Dissertation können nur zwei Tage nach der vorgenannten Frist geltend gemacht werden. Erfolgt kein Einspruch, so gilt die Dissertation als angenommen (§ 7 Ziffer (5) PO).

Sofern die Dissertation angenommen wird, findet die mündliche Prüfung am

23.05.2024 um 11:00 Uhr

im **Hörsaal 6K** statt. Als Prüferinnen bzw. Prüfer sind vorgesehen:
Prof. Dr. M. Zurbriggen, Prof. Dr. G. Groth und Prof. Dr. S. Smits.

Die Öffentlichkeit ist bei der Befragung nicht zugelassen.

Mit freundlichen Grüßen
im Auftrag

Nora Lisa Bitzenhofer

Summary

Natural products (NPs) are a valuable source of potentially useful pharmaceutical compounds and have been a focus of scientific research for a long time. Obtaining these compounds from the native producer is often limited or even impossible, which highlights the significance of recombinant production in appropriate microbial hosts. As *Pseudomonas putida* has already demonstrated to be a promising heterologous host, this thesis aimed to establish strategies for harnessing the potential of *P. putida* as a robust production platform for several NPs.

First, the criteria identified by prior research for generating a robust *Pseudomonas chassis* were reviewed and applied in subsequent studies. For stable and straightforward strain generation, a fully modular genetic toolbox was established, which enables the random as well as site-specific chromosomal integration in different genomic loci. This toolbox could be used for the integration of genes encoding biosynthetic pathways and other bioprocess-relevant features in subsequent studies. Pathway engineering was then carried out using the established vector series to genomically introduce genes involved in precursor supply or entire additional precursor biosynthetic pathways.

Further, the release and extracellular storage of the produced NP was pursued with different strategies. For the first time, engineering the formation of outer membrane vesicles, a native stress response in Gram-negative bacteria, was explored to support NP biosynthesis in *P. putida*. Using a two-phase cultivation with polyurethane foam cubes as an adsorbent resulted in the extracellular accumulation of the major part of produced prodiginines and arcyriaflavin A, with over 95% of each compound recovered from the cubes. Finally, access to diverse NPs and their derivatives was enabled by combining biology with classical chemistry to build tailored biosynthetic pathways. Namely, production of new-to-nature hydroxylated and cyclic prodiginines was achieved through a hybrid synthesis route that involves adding chemically prepared building blocks as well as artificial biosynthetic pathway expansion or late-stage chemical conversion of the bioproduct, respectively. This showcase underlined the value of combining different biosynthetic concepts like (combinatorial) biosynthesis, muta-, and semisynthesis. By applying all mentioned strategies, the recombinant production of several NP classes was realized in *P. putida* KT2440, which include rhamnolipids, terpenes, and especially alkaloids such as prodiginines, violacein, and arcyriaflavin A.

In conclusion, genetic as well as biosynthetic tools were established, and engineering strategies were investigated to help to ensure strain stability, to access diverse NPs, and to enhance bioprocess productivity of the chosen host organism. These findings contribute to research aimed at harnessing the potential of *P. putida* as a recombinant production host and may significantly minimize the effort required to generate an optimal *chassis* organism hosting novel biosynthetic pathways in the future.