



Microorganisms And Methods For Producing Reuterin

[View U.S. Patent Application Publication No. US-2024-0417762 in PDF format.](#)

WARF: P230254US02

Inventors: Jan Peter Van Pijkeren, Zhiying Wang

The Invention

UW-Madison researchers have developed a method to optimize the production of reuterin in bacterial cells that contain the reuterin production pathway (i.e., the pdu operon). The inventors compared the production of reuterin in their lab strain, *L. reuteri* ATCC PTA-6475, which contains two prophages, with their previously-developed isogenic mutant *L. reuteri* $\Delta\Phi1\Delta\Phi2$, in which the two prophages were deleted. The deletion of the two prophages abolished reuterin production, which was restored by complementation of the prophages; testing individual deletion mutants demonstrated that prophage $\Phi2$ (not $\Phi1$) drove reuterin production. By comparing the two prophage genomes, the inventors identified five unique genes on $\Phi2$ (orf3, orf6, orf14, orf16, and orf17), and individually cloned each into the inducible vector pSIP411 and then transfected into *L. reuteri* $\Delta\Phi1\Delta\Phi2$ to create *L. reuteri* $\Delta\Phi1\Delta\Phi2$ (pSIP-orf14). Overexpressing Orf14 in *L. reuteri* $\Delta\Phi1\Delta\Phi2$ not only rescued reuterin production in the *L. reuteri* $\Delta\Phi1\Delta\Phi2$ background, the inventors also observed a 3-fold increase ($P < 0.05$) in reuterin production compared to that in the wildtype strain following a 5 h incubation. Inactivation of Orf14 with an in-frame stop codon does not restore reuterin production, suggesting that the Orf14 protein product is involved in the effect. Taken together, the inventors have engineered a hyper-producer of reuterin by deleting prophages and overexpressing the phage-encoded gene Orf14. More broadly, the inventors have discovered a means of increasing reuterin production through the overexpression of Orf14 in strains that contain the reuterin production pathway (i.e., the pdu operon).

Additional Information

For More Information About the Inventors

- [Jan Peter Van Pijkeren](#)

Tech Fields

- [Animals, Agriculture & Food : Food ingredients & additives](#)
- [Research Tools : Biomanufacturing](#)
- [Research Tools : Microbial technologies](#)
- [Therapeutics & Vaccines : Anti-infectives \(antibacterials, antifungals, antivirals\)](#)

For current licensing status, please contact Rafael Diaz at rdiaz@warf.org or 608-960-9847