



General information	
PPP number	AF-15263
Title	Harness bacterial platform for mono- and diterpene production
Theme	Circular
Executive knowledge institution(s)	WUR-WPR, BU Bioscience
Research project leader (name + e-mail address)	Dirk Bosch (dirk.bosch@wur.nl)
Coordinator (on behalf of private parties)	Jaap Drenth namens Isobionics
Government contact person	Wijnie van Eck
Total project size (k€)	1200
Address projectwebsite	https://www.wur.nl/nl/Onderzoek-Resultaten/Topsectoren/show/Harness-bacterial-platform-for-mono-and-di-terpene-production-.htm
Start date	01-01-2016
End date	31-12-2020

Approval by the coordinator of the consortium	
The annual report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the annual report.	
Assessment of the report by the coordinator on behalf of the consortium:	<input checked="" type="checkbox"/> Approved <input type="checkbox"/> Not approved
Additional comments concerning the annual report:	

Summary of the project	
Problem definition	Isoprenoids for the F&F market have several issues. Availability from plant biomasses is unreadable, may contain traces of agrochemicals and prices are unstable. In addition, production and extraction is often not sustainable. Sometimes, rare plant species are used and extraction protocols can involve chemicals which can be a burden for the environment.
Project goals	Isobionics focuses to produce isoprenoids for the F&F market, by using a biobased production platform. This project aims to expand the products that can be made with this platform to novel product categories

Results	
Planned results 2019	<p>An important goal of the PPS is to engineer the bacterial platform (Rhodobacter) in such a way that it can produce novel product categories. Normally the platform is applied to make C15 terpenes (sesquiterpenes), but in this project we try to tweak it to produce C10 terpenes (monoterpenes) or C20 terpenes.</p> <p>Aim for 2019 is to</p> <ul style="list-style-type: none"> -further improve monoterpene titers through strain engineering and fermentation optimisation.

	-Optimise the platform for diterpene production, in particular in the context of diversification towards diterpene products.
Achieved results 2019	<p>Monoterpene production was further optimized as compared to the levels reported for 2018. Further attempt to reach even higher toxicity were not successful, probably because higher productivity leads to toxicity. This holds for different monoterpenes that we investigated and different strain-engineering approaches.</p> <p>We therefor involved the company DAB in the consortium which has developed a process for in situ removal of the product during fermentation, to prevent this toxicity. DAB applies extraction as ISPR method to accelerate production rates. DAB has investigated different organic phases and employed the Fast-100 fermentation set-up on a limonene producing strain. Due to specific properties of limonene, related to volatility, no significant improvement of limonene productivity could be observed compared to Isobionic's standard procedure. At the given product titre ranges, gas stripping should be the in-situ removal method. The biomass was successfully retained.</p> <p>Limited efforts could be devoted to diterpene platform improvement due to personal issues (mentioned below). Design for diterpene product diversification were made and will be implemented in 2020.</p> <p>Administrative: In the first half of 2019 and in consultation with the industrial partners, a significant amount of money (€100.000) has been transferred from the 2019 budget to 2020. This because of long term illness of a WPR employee. In relation with this the end date of the project has been moved to 31/12/2020. Both have been approved by TKI.</p>
Planned results 2020	In 2020 we will focus on diterpene related activities (including those that could not be executed in 2019). We will try to establish the production of other diterpenes than sclareol to enlarge the scope of diterpene products that can be produced, and thus increase the applicability of the platform.

Deliverables/products in 2019 (provide the titles and /or a brief description of the products/deliverables or a link to a website.)
<p><u>Scientific articles:</u></p> <p>An analysis of characterized plant sesquiterpene synthases. Durairaj J, Di Girolamo A, Bouwmeester HJ, de Ridder D, Beekwilder J, van Dijk AD. Phytochemistry. 2019 Feb;158:157-165. doi: 10.1016/j.phytochem.2018.10.020.</p> <p>Metabolic flux ratio analysis by parallel 13C labeling of isoprenoid biosynthesis in Rhodobacter sphaeroides. Orsi E, Beekwilder J, Peek S, Eggink G, Kengen SWM, Weusthuis RA. Metab Eng. 2020 Jan;57:228-238.</p> <p>Toward Developing a Yeast Cell Factory for the Production of Prenylated Flavonoids. Levisson M, Araya-Cloutier C, de Bruijn WJC, van der Heide M, Salvador López JM, Daran JM, Vincken JP, Beekwilder J. J Agric Food Chem. 2019 Dec 11;67(49):13478-13486.</p>
<u>External reports:</u>
<u>Articles in professional journals/magazines:</u>
<u>(Poster) presentations at workshops, seminars, or symposia.</u>
2019-09-23, 19th Int. CeBiTec Research Conference Bielefeld. Renewable feedstocks and synthetic pathways for production of fine chemicals.
<u>TV/ radio / social media / newspaper:</u>

Remaining deliverables (techniques, devices, methods, etc.):