

1. PUBLISHABLE SUMMARY

Summary of the context and overall objectives of the project (For the final period, include the conclusions of the action)

Before industrial biotechnology platforms can deliver molecules that are market ready, several hurdles need to be overcome. In this project, existing technology platforms will be challenged to meet specific market needs. Tailor-made molecules with promising market potential are being developed in close cooperation with relevant major market players. This ensures that scientific developments follow a market-driven approach and guarantees that maximum results for the technology platforms will be achieved. In this project, the full exploitation of two very promising types of specialty biochemicals with high market interest will be addressed. These are glycolipid biosurfactants (new types of sophorolipids, rhamnolipids, mannosylerythritol lipids (MELs) and xylolipids) and specialty carbohydrates.

The overall objectives of the CarboSurf project are:

- Improved metabolic engineering and synthetic biology methodologies to shorten development times and time to market
- Availability of robust production strains to obtain well-defined molecules
- A competitive production system able to compete with currently used (petrochemically-based) alternatives
- Prove of efficacy in commercial applications
- Regulatory approval for the new molecules

If these objectives are met, the new biochemicals will enter the market at the end or after the project, which will have several beneficial impacts on society. Firstly, moving these innovative products close to the market will boost economic development and employment in Europe. Secondly, as the biochemicals are biodegradable and non-ecotoxic and are being produced from (second generation) renewable resources, this will have a positive effect on the environment. Thirdly a positive social impact is expected as (some of) the biochemical have high potential in pharmaceutical and nutraceutical applications and as a antibiotic alternative all improving human quality of life.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far (For the final period please include an overview of the results and their exploitation and dissemination)

The conversion of agricultural waste products (i.e. lignocellulosic material) into fermentable sugars was optimized, which resulted in higher purity and a lower price of these second generation (2G) sugars. It was shown that these 2G sugars can efficiently be fermentatively converted into biosurfactants, which will even improve the environmental profile of the latter. New molecular tools were and are being developed for a range of microbial (bacterial, yeasts and fungi) strains included in the CarboSurf project. These tools dramatically facilitate(d) the metabolic engineering of several glycolipid and carbohydrate producing strains. A set of microbial strains efficiently producing (new types) of glycolipids (new-to-nature sophorolipids, rhamnolipids and xylolipids) and producing specialty carbohydrates were thus created. Tool development for tailored MEL production is currently still ongoing and new strains are expected in the next year. The described (new-to-nature) biomolecules hold great intrinsic potential to be applied in a range of sectors like food, cosmetics,

nutraceuticals, etc. To investigate their potential, a set of physicochemical parameters were determined and for some molecules, more specific application tests were (or will be in the near future) performed by the industrial partners of the CarboSurf project and for some very promising results were already obtained e.g. xylolipids as biological preservatives in beverages and rhamnolipids as green and mild performers in personal hygiene products. To perform these application tests, considerable quantities of the products of high purity are required and to reach these goals, suitable and scalable production processes for the new strains/products had to be developed. For the new types of sophorolipids, the processes for promising strains were developed at the lab scale and one process was recently scaled up to the 100 L scale, giving range to kg amounts of the new product(s) (symmetrical bola sophorolipids), which will soon be evaluated for a range of applications. For the rhamnolipids, the production process was debottlenecked at the ton scale and possible commercial scale issues (100 ton scale) were identified. Also, for xylolipids, pilot scale production runs were performed after developing a suitable fermentation and purification process at the lab scale. For the fourth type of glycolipid (mannosylerythritol lipids (MELs)), small scale fermentations were performed to investigate the purification and produce some product for application tests. Once new strains become available, these will also be applied in large scale fermentations. The latter is also true for the specialty carbohydrate production strains. Strain were developed, but are not fit enough yet and metabolic engineering is still ongoing to obtain a strain with acceptable productivities/titers.

Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)

The most important progress point beyond the state of the art of the CarboSurf project are:

- Availability of a new process for the efficient and economical production of 2G sugars.
- Production of biosurfactants from the optimized 2G sugars, dramatically improving their environmental profile.
- New molecular tools available for *S. bombicola*, *S. cerevisiae*, *E. coli* and under development for *Pseudozyma* strains.
- Availability of microbial strains efficiently producing (new-to-nature) sophorolipids, rhamnolipids, xylolipids and specialty carbohydrates.
- New production processes for the new strains/biomolecules (symmetrical bola SL, rhamnolipids, xylolipids).
- Application data available for new biochemical (xylolipids and rhamnolipids) and ongoing/planned for new to nature sophorolipids and MELs.

The results obtained so far point towards the market entry of several new biochemicals in Europe. This will boost economic development and employment in Europe, have a positive effect on the environment and generally improve quality of life.

Address (URL) of the project's public website

<http://www.carbosurf.eu>