ERA CoBioTech

BioTech Research & Innovation Hack

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ERA CoBioTech Funded Projects at A Glance: CoolWine

Model-guided evolution for balanced attenuation of wine ethanol content by developing non-GMO yeast strains and communities

CoolWine

Balancing the impact of climate change on the alcohol content of European wines

Researchers involved in CoolWine are developing improved non-GMO yeast strains and optimized fermentation procedures towards reducing alcohol levels in wines.

Project coordinator:

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CSIC, (Spain)

Consortium:

Spanish Council for Scientific Research (CSIC), (Spain)

Rovira i Virgili University (URV), (Spain)

Norwegian University of Science and Technology (NTNU), (Norway)

European Molecular Biology Laboratory (EMBL), (Germany)

University of Gothenburg (GU), (Sweden)

Bodegas Roda S.A., (Spain)

Project duration:

01 May 2018 - 31 May 2022

Total budget: 1.5 €M

Rewiring yeast metabolism towards lower ethanol yields

Rising temperatures in European wine-producing regions are having a negative impact on this key economic sector. Climate change causes an imbalance between the technological and phenolic maturity of wine grapes and, therefore, an increase in alcohol in wines. This trend is of great concern for the European wine sector since it has a negative impact on wine quality, becomes an obstacle to international trade and undermines the compatibility of moderate wine consumption with a healthy lifestyle.
Since the alcohol in wine is the direct result of the action of yeast cells on grape sugar, CoolWine aims to reduce the efficiency of yeast in converting sugar into ethanol. The researchers involved in the project will develop new yeast strains, new combinations of strains and new fermentation procedures so that less sugar is converted into alcohol during wine fermentation (but allowing full sugar consumption to maintain wine quality).
Key aspects of the project are the focus on non-GMO approaches to the genetic improvement of wine yeasts; and the use of respiratory metabolism as a clean way to ensure sugar consumption while avoiding the production of ethanol or any other compounds that would eventually put wine quality at risk.
The project design assumes that the reduced alcohol wines produced using the biotechnological approaches developed in the project are adapted to the wider social context in which these wines will ultimately be produced, sold and consumed. However, CoolWine researchers do not take this for granted and a social impact assessment has been conducted to identify stakeholders and map the potential impact of the biotechnological intervention in the community –understanding it from a sociological and anthropological point of view–. The SIA has been focused on how ideas and attitudes on wine culture and wine consumption are constructed and the response of consumers, non-consumers, producers, distributors, wine experts and health professionals towards CoolWine.

Model-guided experimental evolution

In order to contribute biotechnological improvements that would actually reach the industry, reduction of ethanol yields reached in CoolWine must not be dependent on genetic engineering of wine yeasts. The consortium takes advantage of the recent developments in Systems Biology in order to reach the right metabolic characteristics without resorting to GMOs. A powerful tool for the non-GMO genetic improvement of wine yeasts is experimental evolution. Further, the combination of microbial metabolic pathways resulting from the development of microbial consortia is also expected to result in lower ethanol yield during alcoholic fermentation. In CoolWine, the design of yeast combinations and the conditions for experimental evolution will be designed based on Systems Biology tools supported by yeast metabolic models. In addition to ethanol yield and respiratory metabolism, prediction tools will be used to anticipate undesired outcomes of the experimental evolution or microbial interactions (including known off-flavours and mainly acetic acid overproduction).

Main results

Characterization of the Core CoolWine Collection (a wine yeast strains collection gathered for CoolWine) provided some interesting wine yeast strains. One strain of *S. cerevisiae* showed a moderate ethanol reduction in aerated fermentations (to an extent close to the objectives of the CoolWine). This allows developing one-step fermentation procedures tuned for low alcohol production (the use of other yeast species would require two-step processes). The procedure has been scaled up to 2000 litres and further volume increases are on the way. In addition, several *M. pulcherrima* strains show very low ethanol yield under aerobic conditions. Mixed starter cultures with those and *S. cerevisiae* are now being optimized before scaling up the process.

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Different mixed inoculation strategies have been tested at lab scale, being sequential inoculations more efficient for lowering ethanol than co-inoculations.
CoolWine researchers have also developed a powerful pipeline for fungal genome-scale metabolic model reconstruction. The models are currently being challenged with high quality physiological data (using omics analyses). Those are helping dissect the aerobic and anaerobic metabolism in grape must of different wine yeast species. They have also used massive parallel experimental evolution to genetically improve strains of several wine yeast species; and are currently analysing over 8000 yeast evolved populations in order to obtain useful strains for lower ethanol winemaking. In addition, by experimental evolution in liquid medium researchers managed to reduce volatile acidity of some *S. cerevisiae* strains under the conditions required for alcohol level reduction.
Researchers in CoolWine involve not only biotechnologists, but also anthropologists and philosophers. They have performed a deep analysis of the societal implications of the technologies involved in the project and contributed to a better awareness of the science-society interface by project partners.

Future prospect

The participation in CoolWine of an international consortium of leading research groups in the fields of systems biology and wine biotechnology has attracted the attention of industrial partners to push the technologies developed in CoolWine to the industrial level. Agreements are currently underway to scale up the processes with the first interesting strains obtained in the project. But the advantage is that this effort will pave the way for the rapid adoption of the new strains already in the CoolWine project.
Instituto de Ciencias de la Vid y del Vino (Institute for Wine and Grapevine Sciences)-CSIC has filed a patent application for the use of one of the first strains obtained in this project for lower ethanol yield during wine fermentation. A peer reviewed scientific article (doi: 10.1016/j.fm.2021.103893) has also been published describing the steps that led to the selection of this strain and the optimization of the fermentation conditions to produce lower alcohol wines. A preprint demonstrating the successful use of modelling and systems biology in improving wine fermentation is deposited (Predictive evolution of metabolic phenotypes using model-designed selection niches; doi: 10.1101/2021.05.14.443989). The paper will be submitted to a peer-review journal in the near future.
CoolWine team has developed methodological approaches to microbial interactions that will now be applied to the understanding of yeast-yeast interactions in winemaking (doi: 10.1111/1751-7915.13614; doi: 10.1101/2021.08.04.455033).
CoolWine strategy relies in the use of non-*Saccharomyces* yeasts in sequential or co-inoculation with *S. cerevisiae*. Several works from URV partner have addressed parameters and methodologies to optimize the process of low alcohol fermentation (doi: 10.3390/FOODS10030623; doi: 10.3390/FOODS9101373; doi: 10.3390/microorganisms8050658; doi: 10.3390/microorganisms8020157).
Philosophers from NTNU have presented their views related with CoolWine topics in Eurosafe 2021 (https://events.unifr.ch/eursafe2021/en/).Anthropologists from URV have presented their conclusions to the consortium, based on focus groups, interviews, and questionnaires during sensory evaluation sessions; and participated in Responsive Research and Innovation Sessions for ERA CoBioTech. They are also preparing a video to communicate activities in CoolWine, as well as a scientific article based on free listings analysis.

Figure 1: CoolWine Kick-off meeting. Roda Cellar. La Rioja. Spain
Bodegas Roda S.A. is the industrial partner in the consortium. The kick-off meeting was organized at their cellar in La Rioja, producing some of the most prestigious Spanish wines.

Project website: https://coolwineproject.blogspot.com/
Twitter: @CoolWineProject

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