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Identification of volatiles released by fruit-associated yeasts for the sustainable control of *Drosophila suzukii*

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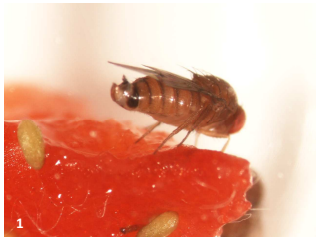
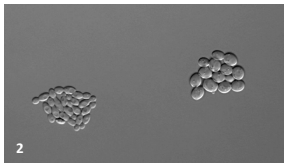

Adult female of *Drosophila suzukii* with extroflected ovipositor on strawberry fruit (photo by I. Castellan)

Research background

Chemical control of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) using synthetic insecticides is particularly challenging as it is difficult to respect pre-harvest intervals. Therefore, a different approach to tackle the problems caused by *D. suzukii* shall be developed. First detected in California in 2008, this pest has spread in at least 41 States in the United States, and many European countries including Italy.

Research aims

- We aim to develop a new control strategy that will reduce the residues of chemical insecticides on fruits, using targeting yeasts to attract as selectively as possible the oviposing females of *D. suzukii*
- The control method shall be applicable on a wide scale
- Applications within chemical ecology shall help us to modify the insect's behaviour in the field.


Hanseniaspora uvarum (left) and *Saccharomyces cerevisiae* (right) cells growing. Photo courtesy by D. Nordmann (2015), Genetik Universität Osnabrück.

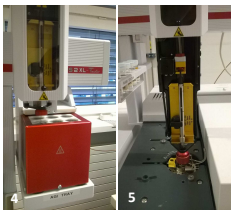
Experiment 1 – Comparison of two media on the release of yeast-associated volatiles

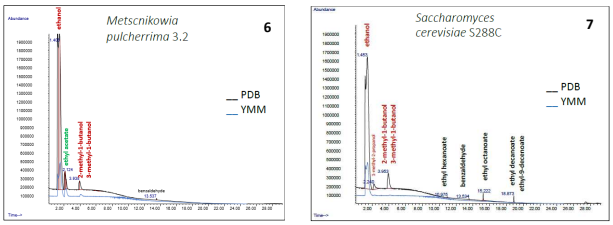
Method

We cultured eight yeast strains: 1) *Saccharomyces cerevisiae* S288C, 2) *Candida* sp. 3.3, 3) *Saccharomycopsis vini* 1.2.3, 4) *Hanseniaspora uvarum* 2.2, 5) *Hanseniaspora uvarum* 3.4, 6) *Hanseniaspora uvarum* 1.2.1, 7) *Issatchenkia terricola* 2.1, 8) *Metschnikowia pulcherrima* 3.2 on two different growth media: YMM-Yeast Minimal Medium and PDB-Potato Dextrose Broth. The volatile profile emitted by each living yeast culture was characterised using direct headspace analysis and gas chromatography-mass spectrometry (DHS-GC-MS).

Results

Results showed that PDB was a better medium than YMM, as the chromatograms resulting from the GC-MS analysis showed a higher number and intensity of volatile compounds when the yeast strains were cultured in the nutrient-rich medium PDB. Figures 6 and 7 are showing clearly the performance difference of the yeast cultures in the two media. *M. pulcherrima* (6) has a very high emission of ethanol, which seems just a minimal peak in the YMM. *S. cerevisiae* has a whole set of esters visible in PDB (black line), but not in YMM (blue line). As a consequence, YMM is not a suitable medium for the growth of our yeast strains and PDB has been selected to go on with the analyses. Peaks corresponding to the volatiles have been labelled in red (alcohols), green (acetates) and black (esters). The experiment has been done on all the 8 strains selected.



3. 8 mL yeast culture sampled into glass vials ready to be frozen at -80 °C and then utilized for GC-MS analyses. **4. and 5.** Equipment utilized in the UniBz laboratories for the analyses of volatile compounds with DHS-GC-MS: GC-MS (7890A-5975C, Agilent Technologies, Santa Clara, USA) with incubator/shaker and multipurpose autosampler (Gerstel, Mülheim an der Ruhr, Germany).



6. and 7. Chromatograms after DHS-GC-MS: comparison between the growth media: PDB-Potato Dextrose Broth (black line) and YMM-Yeast Minimal Medium (blue line).

Experiment 2 – Characterisation of volatiles and differences among yeast strains

Method

The aim of this experiment was to identify any difference in the volatile profiles of the selected 8 strains cultured in PDB. We analysed the chromatograms after the DHS-GC-MS analysis and characterised the detected volatiles using Chemstation software (Agilent). Afterwards we performed a statistical analysis (ANOVA) to determine the significance of our data. R software was used for the statistics.

Results

The outcome of this experiment revealed that there are indeed differences among the yeast strains that we selected and analysed: no one was identical to another. We characterised 16 VOCs in total. Pictured in 8, 9, and 10 are the chromatograms of the strains emitting most of the volatiles identified. Results showed that *Saccharomyces cerevisiae* S288C release the highest rate of alcohols and ethyl esters, while the strains *Candida* sp. 3.3 and *Hanseniaspora uvarum* 1.21 release much more acetates and almost no alcohols. The strain *Saccharomycopsis vini* 1.23 instead (fig. 8), showed a high emission of one acetate and some alcohols, but no esters. *H. uvarum* strains do not seem to produce much, but the acetates emitted could be of some significance in terms of attraction towards *Drosophila suzukii*. The most promising yeast instead, seems to be *S. vini* 1.23, as it is the only strain releasing a monoterpene. In a parallel work, it is considered to be an attractive yeast to *D. suzukii* (see poster Urban Spitaler, 2018).

Conclusion and Future Expectations

Our work demonstrates the possibility of using acceptance of some yeast strains to be carried on for further analysis towards *Drosophila suzukii.* It is likely that the pest insect might respond differently to these strains, since they are fruit-associated, and probably they will be attracted or repelled by the detected volatiles. More trials are currently under assessment for the volatiles' characterisation, and electroantennography experiments will be performed on *D. suzukii* to test the yeast volatiles. Hopefully, we will utilize the outcomes of the progressing experiments as valuable data for field trials.

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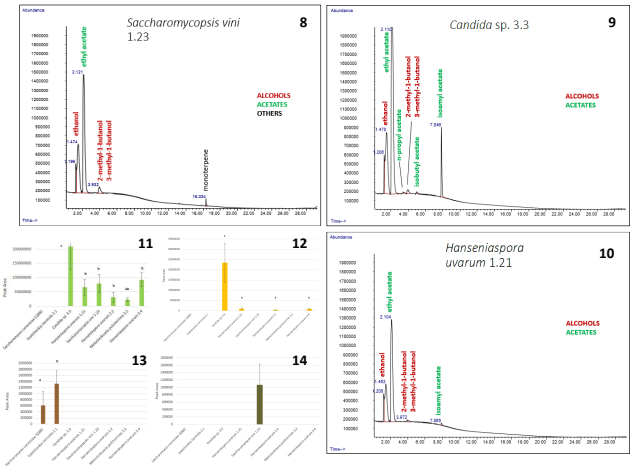

Typical chromatograms of 3 yeast cultures: **8.** *Saccharomycopsis vini* 1.23, **9.** *Candida* sp. 3.3, **10.** *Hanseniaspora uvarum* 1.21. Alcohols are written in red, acetates in green and other compounds in black colour. **11. 12. 13. 14.** Histograms representing the statistical differences after ANOVA ($p < 0.001$) and post-hoc Tukey HSD, among the yeast strains. The most characteristic compounds detected are showed: ethyl acetate (11), isoamyl acetate (12), ethyl octanoate (13), and the monoterpene (14).