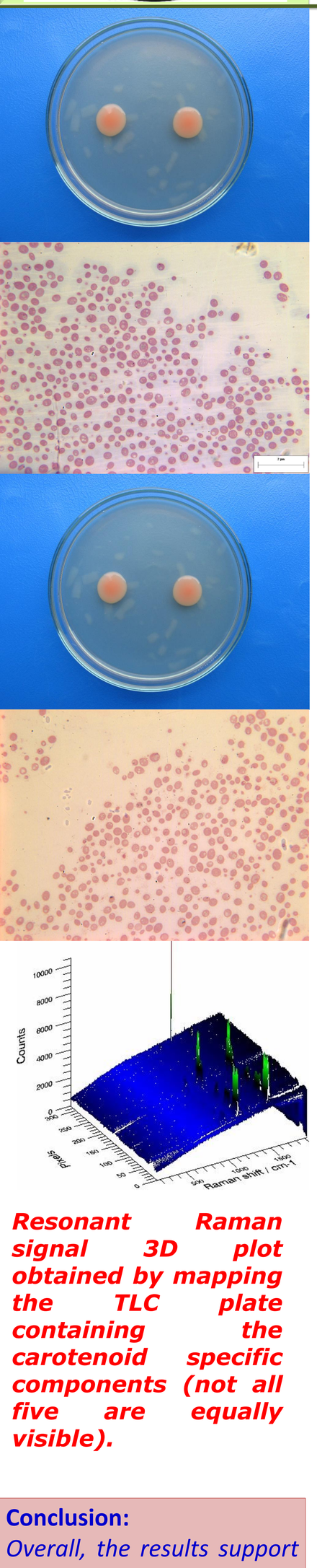
Resonant Raman investigation of the ROS generated stress interference with the carotenogenesis of *R. mucilaginosa*

N.E. Dina[1], A. Colniță[1], C. Mircea[2], A. Moț[3] , M. Parvu[2] , R. Silaghi-Dumitrescu[3]

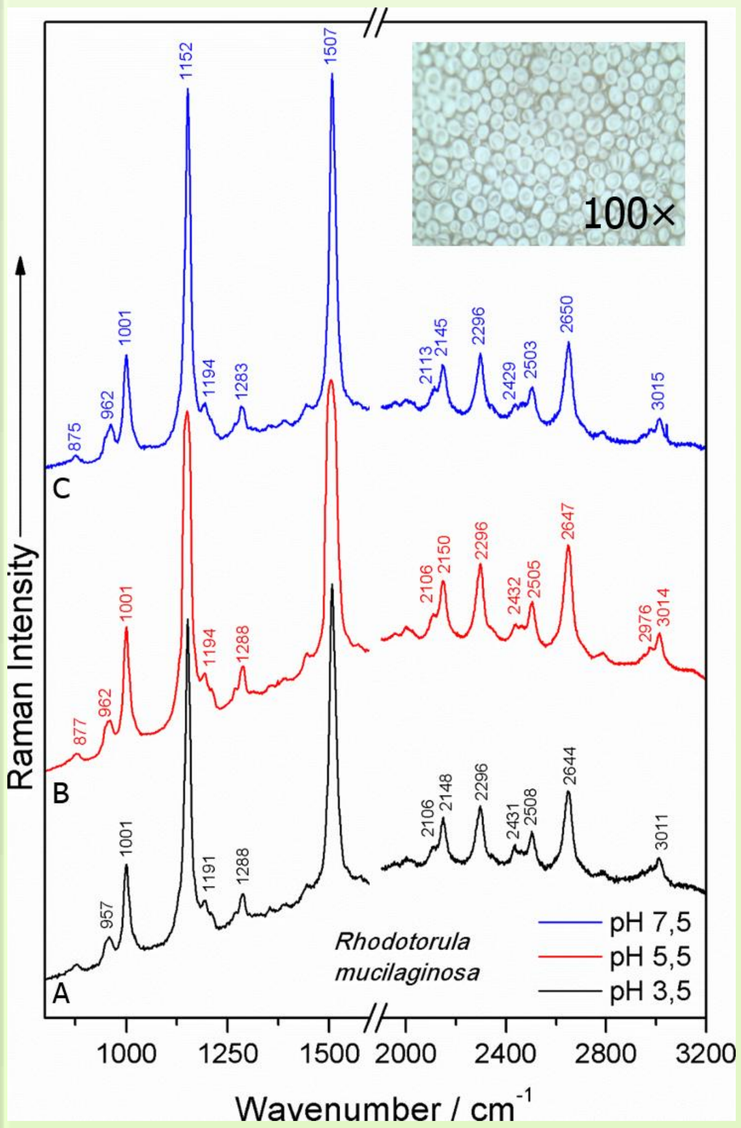
[1]Molecular and Biomolecular Department, National Institute for R&D of Isotopic and Molecular Technologies, 67-103 Donat 400293 Cluj-Napoca, Romania

[2]Faculty of Biology and Geology, Babeş-Bolyai University, 5-7 Clinicilor, 400006 Cluj-Napoca, Romania

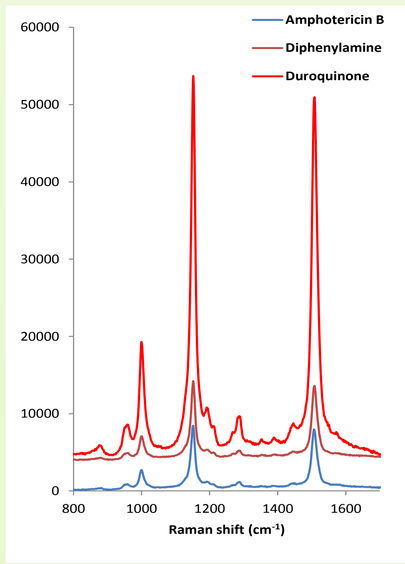
[3] Faculty of Chemistry and Chemical Engineering, 11 Arany Janos 400028 Cluj-Napoca, Babeș-Bolyai University, Romania

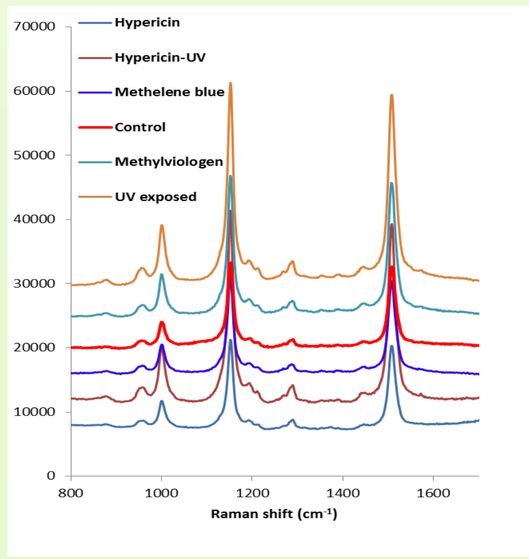


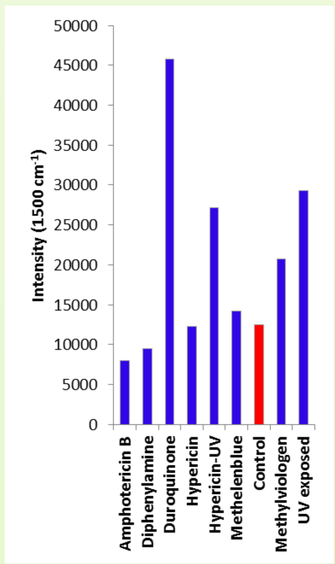
Rhodotorula mucilaginosa (*R. mucilaginosa* synonyms, *R. rubra* and *R. pilimanae*) is a pathogenic pigmented unicellular yeast that might cause human fungemia, especially in immunosuppressed persons. It is known to produce three major carotenoids (i.e. thorularhodin, torulene and β-carotenoids) in order to protect itself from reactive oxygen species (ROS). In order to prevent and control infections, a reliable, highly sensitive and fast method of detection and identification of pathogens is required. In our study, we mainly used Resonant Raman spectroscopy along with High-performance thin layer chromatography, UV-vis absorption spectroscopy and chemometric techniques to monitor and evaluate the three major carotenoids profiles in different conditions (high salt concentrations and different pH values) and in the presence of certain known ROS generating compounds.



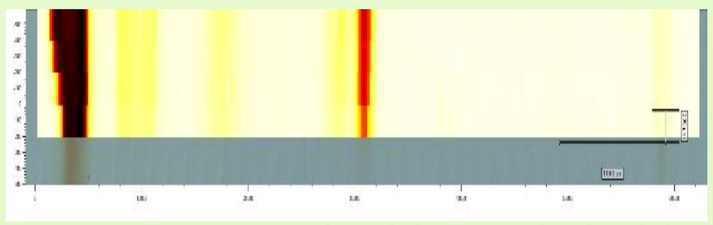
Resonant Raman spectra obtained for microorganisms cultivated in media with pH values of 3.5 (A), 5.5 (B) and 7.5(C) by using the 532 nm laserline. Inset showing 100× microscopic image of R. mucilaginosa in saline buffer, applied on poly-L-lysine adhesive slide.





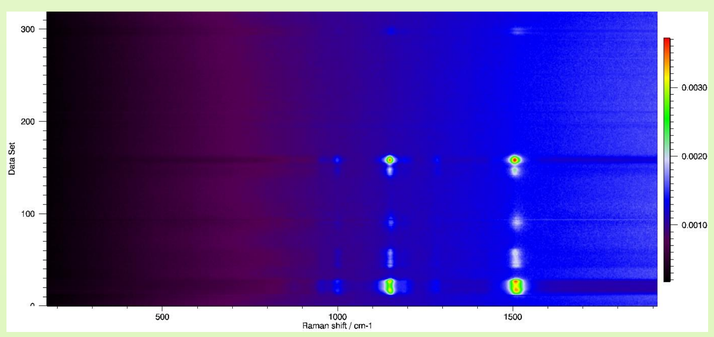


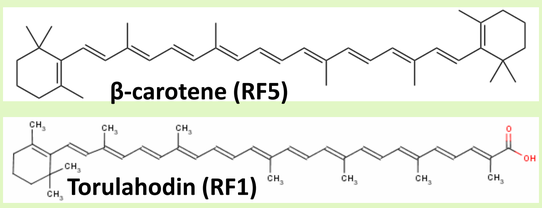
Resonant Raman spectra of the R. mucilaginosa grown in presence of different compounds that control the vitality and the carotenoids content in the cells. Duroquinone (known for superoxide generation) and UV exposure are the strongest stimuli for carotenogenesis while amphotericin (known fungicide) and diphenylamine (inhibitor for carotenogenesis) reduce the carotenoids content (see results plotted in the right chart).



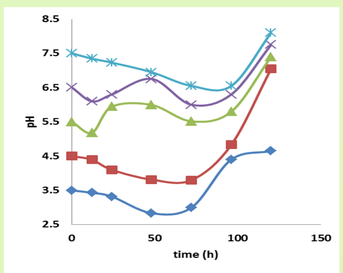
Resonant Raman signal image obtained by mapping the TLC separation plate revealing five carotenoid specific components.

Resonant Raman signal image obtained by mapping the TLC plate containing the carotenoid specific components (not all five are equally visible).

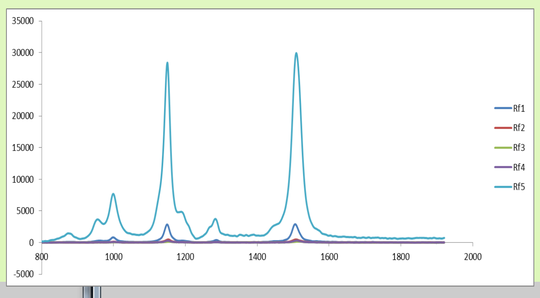




Chemical structure for the two most important components.

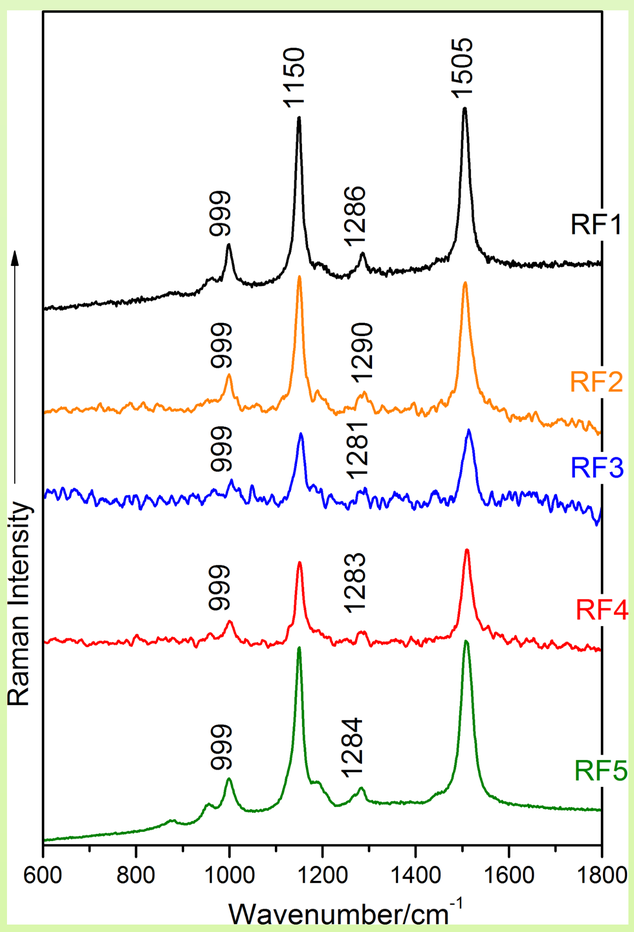


pH variation during R. mucilaginosa growth in time.

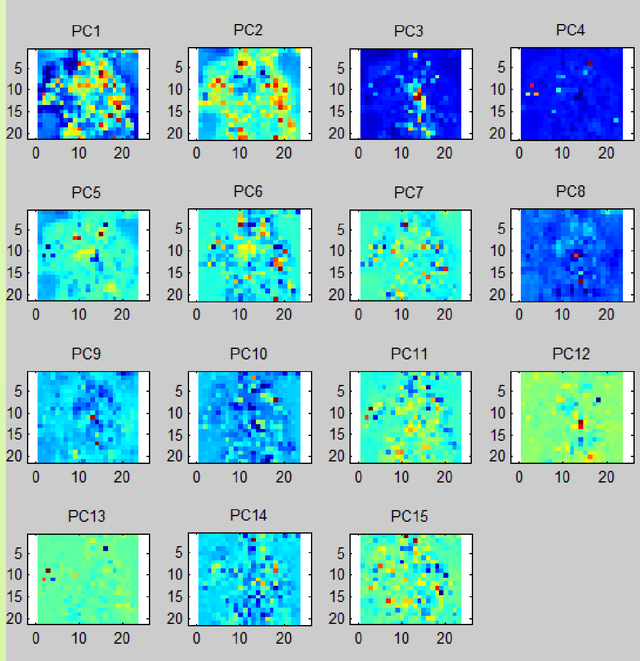


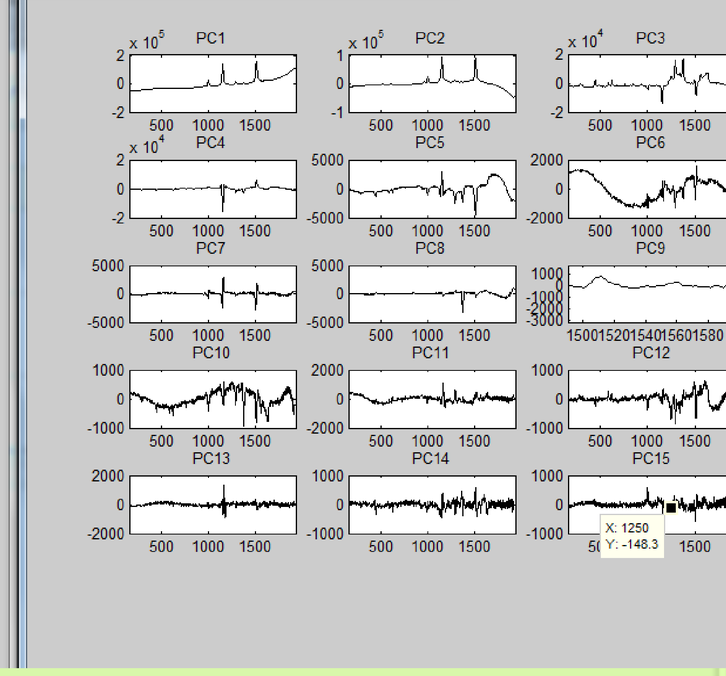
Resonant Raman spectra for carotenoid specific components obtained after HPTLC separation.

Resonant Raman signal 3D plot obtained by mapping the TLC plate containing the carotenoid specific components (not all five are equally visible).



Resonant Raman spectra obtained for each of the five separated carotenoid specific components after C18 column extraction on the TLC plate from R. mucilaginosa: RF1 (torularhodin), RF2-RF4 (torulene-like carotenoids) and RF5 (β-carotene).





PCA analysis correlated to the resonant Raman mapping of R. mucilaginosa cells. PC1, PC2 and PC3 all have a dominant carotenoid specific band at 1507 cm^{-1} and PC4 exhibits a possible signature of carotenoid components at 1523 cm^{-1}, also present in resonant Raman spectrum of the RF3 component separated by TLC.

Conclusion:

Overall, the results support the hypothesis that the carotenogenesis and the carotenoids composition is strongly influenced by the presence of certain ROS species such as oxygen singlet, superoxide generated via different stress pathways.

Acknowledgments:

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