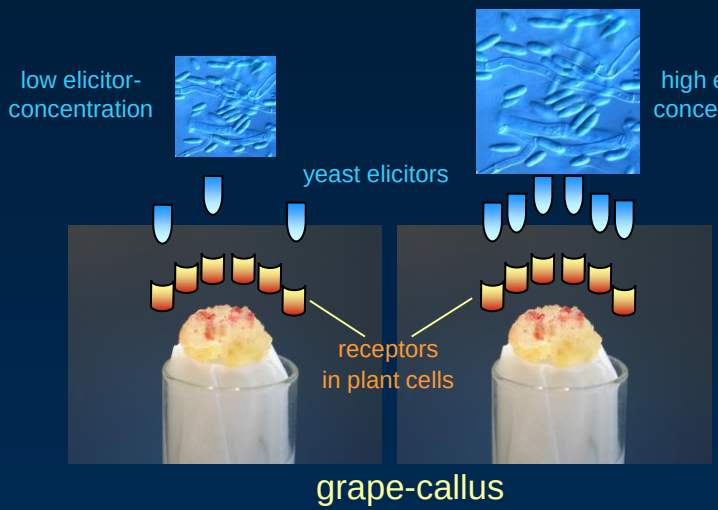
# Induction of resveratrol-derivatives in *Vitis vinifera* callus cultures by the epiphytic yeast *Aureobasidium pullulans*

*Dr. Susanne Rühmann, Prof. Dr. Dieter Treutter*
Institute of Fruit Science TU München Center of Life Sciences, Weihenstephan,
Alte Akademie 16, D-85350 Freising, Germany, Tel. ++49-8161-713129; Fax ++49-8161-715385;
susanne.ruehmann@wzw.tum.de

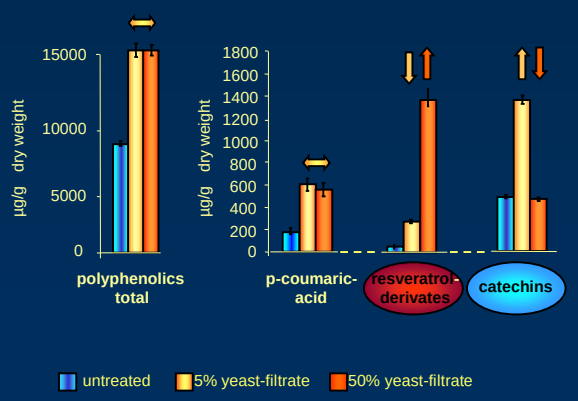
*Aureobasidium pullulans*, a non-pathogenic, epiphytic yeast, shows postharvest antagonistic effects aginst *Botrytis cinerea* and *Penicillium expansum* on apple (Lima et al. 2003) and on grapes (Schena et al. 1999). On the other hand phenolic compounds are in many cases implicated in plant defence mechanisms (Treutter 2005; Feucht et al.1994). In grape, the most prominent compounds acting against gray mould and mildew are resveratrol and it's derivatives like piceid and viniferine (Jeandet et al. 2002).
The present study aims to investigate the influence of the epiphytic yeast *Aureobasidium pullulans* on the secondary metabolism of callus cultures of *Vitis vinifera* in order to elucidate its potential for activating the accumulation of defensive compounds.

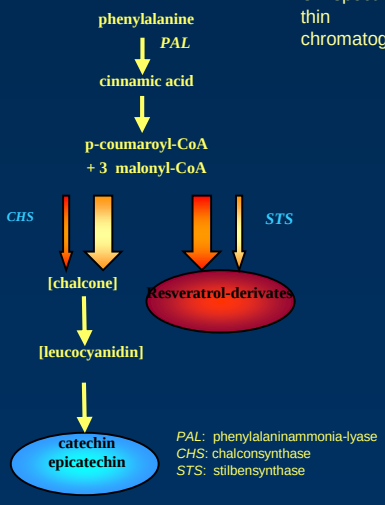
As experimental system, callus from *Vitis vinifera* 'Regent' was cultivated on modified MS-medium (Murashige and Skoog).




*Aureobasidium pullulans* was cultivated in liquid medium on a shaker (100 U/min) for 48 h and subsequently autoclaved to mortify the yeast cells. After centrifugation of the yeast suspension the supernatant was added to the culture medium of grapevine callus with final concentrations of 50% and 5% yeast-suspension.

Samples were harvested after 5, 10, 24 and 48 h. The phenylpropanoids were investigated by HPLC and identified by UV-spectroscopy and thin layer chromatography.




The predominant stilbenes in grape callus cultures were trans- and cis-piceid. Resveratrol as aglycone occurred constitutively only in trace amounts. Treatment with the yeast suspension induced an accumulation of the aglycone resveratrol after 10 hours. Therafter, resveratrol was metabolised to oligomeric viniferins. A low elicitor concentration results in a pronounced accumulation of catechins concurrently with a low accumulation of resveratrol-derivates. A high concentration of yeast suspension led to an increased accumulation of stilbenes at the cost of catechins. The yeast elicitors seem to influence the synthesis of enzymes like chalcone synthase (CHS) and stilbene synthase (STS) which coincides with an increased gene expression of the STS-gene and enzymatic activity of STS while the gene expression of the CHS-gene in the investigated callus treatment decreased (Pfeiffer et al. 2006). Parallel to the increased resveratrol-content in the induced callus culture the course of disease after an inoculation with *Botrytis cinerea* was delayed. In conclusion, extracts from *Aureobasidium pullulans* induced the biosynthesis of resistance related compounds by controlling the expression of the corresponding genes.

*Feucht, W., Treutter, D., Christ, E., 1994: Accumulation of flavanols in yellowing beech leaves from forest decline sites. Tree Physiology 14, 403-412*

*Jeandet, P., Douillet-Breuil, A.C., Bessis, R., Debord, S., Sbaghi, M., Adrian, M., 2002: Phytoalexins from the Vitaceae: Biosynthesis, Phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. Journal of Agricultural and Food Chemistry 50, 2731-2741*

*Lima, G., De Curtis, F., Castoria, R., De Cicco, V., 2003: Integrated control of apple postharvest pathogens and survival of biocontrol. European Journal of Plant Pathology 109, 341-349*

*Pfeiffer, J., Rühmann, S., Fischer, T.C., Treutter, D., Forkmann, G. 2006: Induction of Phenylpropanoid Resistance Factors in Grapevine. JIEP. 2006 Winnipeg*

*Schena, L., Ippolito, A., Zahavi, T., Cohen, L., Nigro, F., Droby, S., 1999: Genetic diversity and biocontrol activity of Aureobasidium pullulans isolates against postharvest rots. Postharvest Biology and Technology 17, 189-199*

*Treutter, D., 2005: Significance of Flavonoids in Plant Resistance and Enhancement of Their Biosynthesis. Plant Biology 7: 581-591*

**Induction of resveratrol-derivates in *Vitis* vinifera callus cultures by the epiphytic yeast *Aureobasidium pullulans***

Rühmann Susanne, Dieter

Institute of Fruit Science TU München Center of Life Sciences, Weihenstephan, Alte Akademie 16, D-85350 Freising, Germanny, Tel. ++49-8161-713129; Fax ++49-8161-715385; s.ruehmann@wzw.tum.de

Gray mould and mildew are the most important pathogenic fungi in grape cultivation. For developping an environmentaly friendly pest management a profound knowledge on plant resistant mechanisms is required. Recent approaches investigated the possible use of non-pathogenic micro-organisms like yeasts for controlling pathogenic fungi. *Aureobasidium pullulans*, a non-pathogenic, epiphytic yeast, shows postharvest antagonistic effects aginst *Botrytis cinerea* and *Penicillium expansum* on apple (Lima et al. 2003) and on grapes (Schena et al. 1999). On the other hand phenolic compounds are in many cases implicated in plant defence mechanisms (Treutter 2005; Feucht et al.1994). In grape, the most prominent compounds acting against gray mould and mildew are resveratrol and it's derivatives like piceid and viniferine (Jeandet et al. 2002).

The present study aims to investigate the influence of the epiphytic yeast *Aureobasidium pullulans* on the secondary metabolism of callus cultures of *Vitis vinifera* in order to elucidate its potential for activating the accumulation of defensive compounds.

As experimental system, callus from *Vitis vinifera* "Regent" was cultivated on modified MS-medium (Murashige and Skoog). *Aureobasidium pullulans* was cultivated in liquid medium on a shaker (100 U/min) for 48 h and subsequently autoclaved to mortify the yeast cells. After centrifugation of the yeast suspension the supernatant was added to the culture medium of grapevine callus with final concentrations of 50% and 5% yeast-suspension. Samples were harvested after 5, 10, 24 and 48 h. The phenylpropanoids were investigated by HPLC and identified by UV-spectroscopy and thin layer chromatography.

The predominant stilbenes in grape callus cultures were trans- and cis-piceid. Resveratrol as aglycone occured constitutively only in trace amounts. Treatment with the yeast suspension induced an accumulation of the aglycone resveratrol after 10 hours. After that resveratrol was metabolised to oligomeric viniferins. A low elicitor concentration results in a pronounced accumulation of catechins concurrently with a low accumulation of resveratrol-derivates. A high concentration of yeast suspension led to an increased accumulation of stilbenes at the cost of catechins. The yeast elicitors seem to influence the synthesis of enzymes like chalconesynthase (CHS) and stilbenesynthase (STS) which coincides with an increased gene expression of the STS-gene and enzymatic activity of STS while the gene expression of the CHS-gene in the investigated callus treatment decreased (Pfeiffer).

Parallel to the increased resveratrol-content in the induced callus culture the course of disease after an inoculation with *Botrytis cinera* was delayed. In conclusion, extracts from *Aureobasidium pullulans* induced the biosynthesis of resistance related compounds by controlling the expression of the corresponding gene

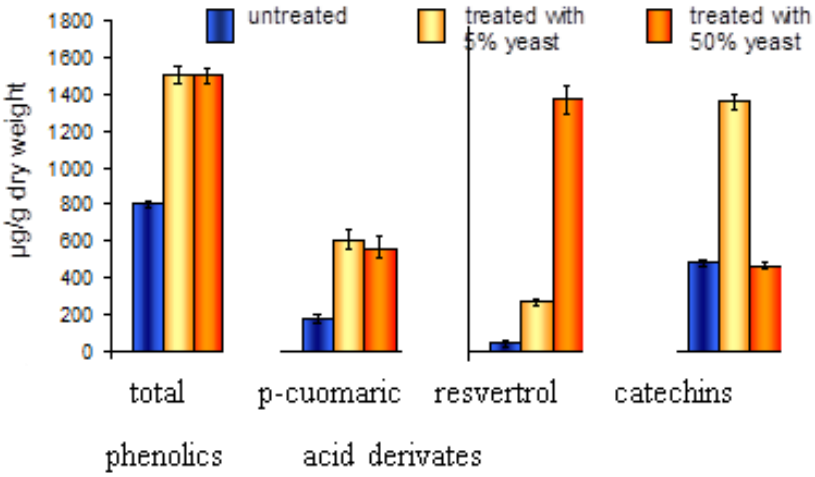



*Figure A: total phenolics, p-couomaric-acid, resveratrol-derivates and catechins of callus from Vitis vinifera „Regent" 48 hours after treatment with 5 and 50% yeast suspension*

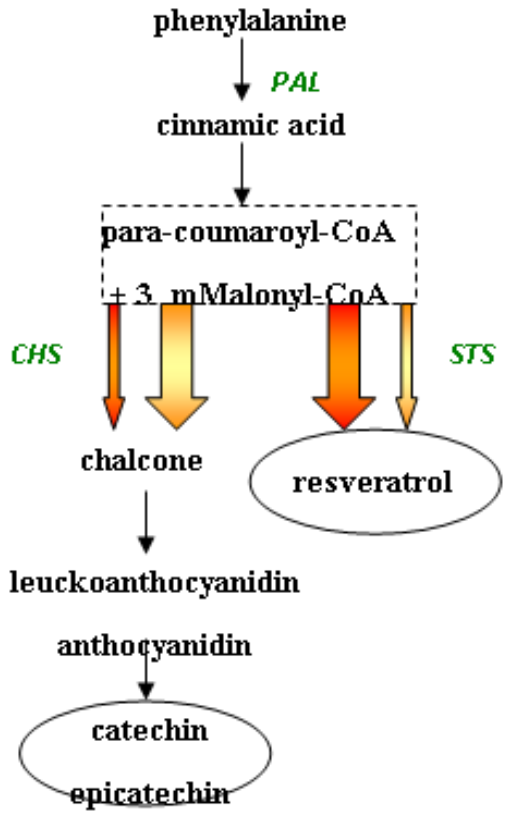



*Figure B: compendium of phenylpropanoid methabolism (HELLER und FORKMANN 1994, JEANDET et al. 2002)*


FEUCHT, W., TREUTTER, D., CHRIST, E., 1994: Accumulation of flavanols in yellowing beech leaves from forest decline sites. *Tree Physiology* 14, 403-412

JEANDET, P., DOUILLET-BREUIL, A.C., BESSIS, R., DEBORD, S., SBAGHI, M., ADRIAN, M., 2002: Phytoalexins from the Vitaceae: Biosynthesis, Phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. Journal of Agricultural and Food Chemistry 50, 2731-2741

LIMA, G., DE CURTIS, F., CASTORIA, R., DE CICCO, V., 2003: Integrated control of apple postharvest pathogens and survival of biocontrol. European Journal of Plant Pathology 109, 341-349

SCHENA, L., IPPOLITO, A., ZAHAVI, T., COHEN, L., NIGRO, F., DROBY, S., 1999: Genetic diversity and biocontrol activity of Aureobasidium pullulans isolates against postharvest rots. Postharvest Biology and Technology 17, 189-199

Treutter, D., 2005: Significance of Flavonoids in Plant Resistance and Enhancement of Their Biosynthesis. Plant Biology 7: 581-591