METABOLIC ENGINEERING FOR RESVERATROL PRODUCTION IN *Escherichia coli*

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Introduction. Resveratrol is a stilbene that has a reported pharmacological potential as an antioxidant and for the treatment of inflamation, cardiovascular diseases and cancer (1). Improvement of resveratrol production in *E. coli* strains has been an issue of research for the last ten years and there have been several efforts to improve the malonyl-CoA pool required for resveratrol synthesis since it is the main bottleneck (2).
It has been demonstrated that *E. coli* strains that lack the phosphotransferase system but have their glucose uptake capacity restored (PTS^-Glc^+) are capable of simultaneous consumption of sugars in a mixture due to an avoided catabolite repression (3) which would be advantageous as this would increase productivity in a culture that need both substrates.
The objective of this work is to evaluate whether supplementing glucose and/or acetate as the carbon source in minimal medium can increase malonyl-CoA levels in the *E. coli* strain VH33 (PTS^-Glc^+) modified for resveratrol synthesis.

Methods. Overnight cultures of *E. coli* strains W3110 and VH33 (PTS^-) in LB were used as preinoculums and then subcultured at an initial OD of 0.1 in shake flask containing 50 mL of M9 medium with Glucose 1g/L + Acetate 1g/L at 37℃ for 12 hours as an adaptation stage. Cells where then subcultured at an initial OD of 0.1 in a shake flask with M9 medium and the corresponding substrate: Glucose 4g/L, Acetate 4g/L or Glucose 2g/L +Acetate 2g/L, at 37℃ for 24 hours. Samples were collected every 2.5 hours for biomass measurement and substrate consumption determination by HPLC.

Results. The substrate uptake profile in the W3110 strain shows that only when glucose has been consumed, acetate uptake can begin. This is avoided in strain VH33 in which both substrates are consumed simultaneously.

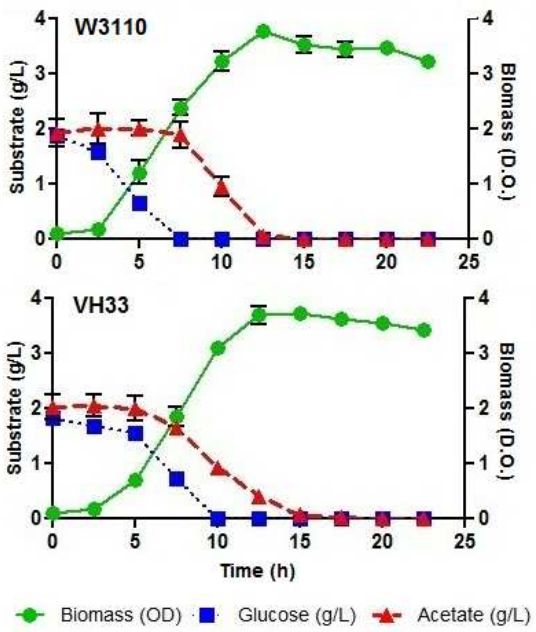

Fig.1 Growth and substrate untake profiles of strains W3110 and VH33 in M9 medium supplemented with glucose 2 g/L and acetate 2 g/L.

The genes 4CL from *Streptomyces coelicolor* and STS from *Arachis hypogaea* necessary for resveratrol biotransformation were successfully cloned in the pTrc99A vector.

Conclusions. The substrate uptake profile of VH33 makes it a suitable strain for increasing the malonyl-CoA pool in an acetate and glucose supplemented medium. The evaluation of resveratrol production using this strain is being currently carried out.

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