Title Characterisation of two alcohol acyltransferases from kiwifruit (*Actinidia* spp.) reveals distinct substrate preferences

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Abstract

Volatile esters are key compounds of kiwifruit flavour and are formed by alcohol acyltransferases that belong to the BAHD acyltransferase superfamily. Quantitative RT-PCR was used to screen kiwifruit-derived expressed sequence tags with proposed acyltransferase function in order to select ripening-specific sequences and test their involvement in alcohol acylation. The screening criterion was for at least 10-fold increased transcript accumulation in ripe compared with unripe kiwifruit and in response to ethylene. Recombinant expression in yeast revealed alcohol acyltransferase activity for *Actinidia*-derived *AT1*, *AT16* and the phylogenetically distinct *AT9*, using various alcohol and acyl-CoA substrates. Functional characterisation of AT16 and AT9 demonstrated striking differences in their substrate preferences and apparent catalytic efficiencies ($V'_{Max}K_M^{-1}$) . Thus revealing benzoyl-CoA:alcohol*O*-acyltransferase activity for AT16 and acetyl-CoA:alcohol*O*-acyltransferase activity for AT9. Both kiwifruit-derived enzymes displayed higher reaction rates with butanol compared with ethanol, even though ethanol is the main alcohol in ripe fruit. Since ethyl acetate and ethyl benzoate are major esters in ripe kiwifruit, we suggest that fruit characteristic volatile profiles result from a combination of substrate availability and specificity of individual alcohol acyltransferases.