Localization of strawberry (*F. ananassa*) and *M. extorquens* genes of biosynthesis of strawberry flavour by *in situ* hybridization

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INTRODUCTION

Strawberry flavour is one of the most popular fruit flavours worldwide with numerous applications in food industry. Therefore, the biosynthetic origin of the most important strawberry flavour components such as 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one (DMHF) (Zabetakis and Holden, 1997), is a challenging research area.

Moreover some precursors of DMHF are biosynthesised by the endophyte *Methylobacterium extorquens*. In particular alcohol dehydrogenase (ADH) enzymes of *Methylobacterium extorquens* are highly involved in the biogenesis of DMHF's precursors. Therefore the expression of the bacterial methanol dehydrogenase and the plant DMHF biosynthesis gene were localized in the tissues of raw and ripe strawberries by *in situ* hybridization

METHODS

Expression of the bacterial methanol dehydrogenase subunit 1 (CP001510; Mxa) and the plant DMHF biosynthesis gene (GB No. AY158836; FaQR) were determined in the strawberry tissue (raw and ripe) by *in situ* hybridization.

Probes specific for the aforementioned sequences were developed, and these as well as bacteria-specific probes (*Eubacterial* E11 and *Methylobacterium-specific* MB; Pirttilä *et al.* 2000) were labelled with digoxigenin. Specifically, the probe sequences of the methanol dehydrogenase (Mxa) and *Fragaria x ananassa* quinone oxireductase (FaQR) genes were amplified from the genomic DNA of *Methylobacterium extorquens* and strawberry leaves by PCR. The resulting PCR products were cloned into pGEM T-easy vector and labelled with digoxigenin-UTP by *in vitro* transcription with SP6 and T7 RNA polymerase (DIG RNA Labelling Kit (SP6/T7), ROCHE).

The strawberry samples were collected from ripe and raw fruits. Surface sterilized fruit samples were fixed in fixing solution at 4°C overnight, dehydrated, cleared through an ethanol-*t*-butanol series and embedded in paraffin. The paraffin sections were attached on silane-coated slides by baking at 55°C overnight. The hybridization was performed at 42°C overnight using hybridization solution containing 0.5 ng/ml of each probe.

RESULTS-CONCLUSIONS

- The signal of bacterial ADHs gene and plant DMHF biosynthesis gene were detected at the same locations in the plant tissue as the signal of MB and E11.
- The signal of bacterial ADHs gene and plant DMHF biosynthesis gene were mainly detected in the intercellular spaces of receptable vascular tissue and intracellularly in the tissues of achenes.
- The ripe plant cells exhibited mainly unspecific signal, as the samples treated with controls (sense probe, no probe) were stained as well.

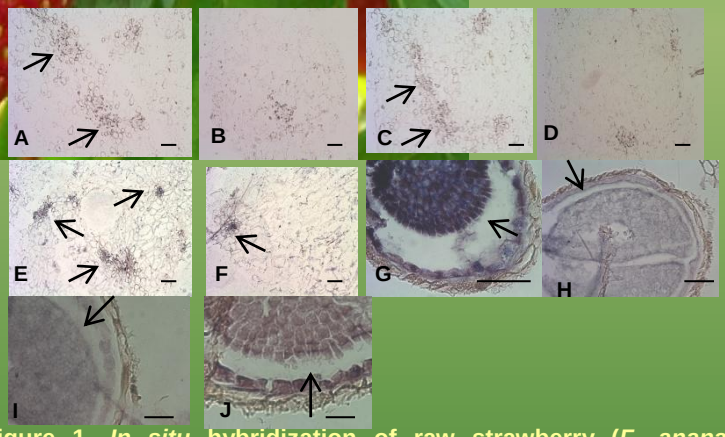

Figure 1. *In situ* hybridization of raw strawberry (*F. ananassa)* vascular and achene tissues with probes labelled with digoxigenin. (A) vascular strawberry tissues, hybridized with the antisense MxaT7 probe, (B) vascular strawberry tissues, hybridized with the negative control of sense MxaSP6 probe, (C) vascular strawberry tissues, hybridized with the antisense FaQRT7 probe, (D) vascular strawberry tissues hybridized, with the negative control of sense FaQRSP6 probe, (E) vascular strawberry tissues, hybridized with the *Eubacterial* probe E11, (F) vascular strawberry tissues, hybridized with the *Methylobacterium-specific* probe MB, (G), (H), (I) and (J) achene tissue, hybridized with the *Methylobacterium-specific* probe MB, the *Eubacterial* probe E11, the antisense MxaT7 probe and the antisense FaQRT7 probe, respectively. Scale bar = 20 μm.

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This project was a COST STSM (Ref. N.: 10547) funded by the Cost Action FA1103.