food&health

mibelle GROUP biochemistry

miRNA Quantification in Cultured Apple Fruit Cells and Fresh Apple Fruit Tissue

S-1015 & S-1057
Test institute: Mibelle AG Biochemistry, 5033 Buchs, Switzerland (in-house study)
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INTRODUCTION

The aim of this study was to compare the content of microRNAs (miRNAs) in cultured apple fruit cells (Biomass used for PhytoCellTec™ Md Nu production) and fresh fruits from Uttwiler Spätlauber apples (*Malus domestica*). In addition, miRNA levels of the cultured cells from the start of the culture (day 1, respectively day 5) and the typical harvest time point (day 12 respectively day 14) were compared.

miRNAs are small single-stranded non-coding RNA molecules found in many cell types of various organisms including plants and humans. miRNAs function in the post-transcriptional regulation of gene expression by suppressing protein biosynthesis and play crucial roles in the regulation of stem cell functions.

STUDY DESIGN

Test samples

Cultured apple cells (*Malus domestica,* Uttwiler Spätlauber)
Fresh apples (*Malus domestica,* Uttwiler Spätlauber)

Cell cultivation and harvesting

Cultured cells received from callus tissue of *Malus domestica* were harvested at day 1 and day 14 (or, in the second study, day 5 and day 12) of culture. Apples were cut into small pieces. All samples were frozen at -80 °C and pulverized using liquid nitrogen and a mortar.

Isolation and quantification of miRNA

A Plant miRNA Purification Kit (Norgen Biotek Corp., Canada) was used to isolate and purify small RNAs (<200 nucleotides) including miRNA from the powder. Quantification of the samples was performed by UV spectroscopy (UV-1800 UV-Vis Spectrophotometer; Shimadzu Scientific Instruments Inc., USA), measuring the absorbance of each sample at 260 and 280 nm. Resulting concentrations were normalized to the dry weight of each sample. Data for the first study resulted from two independent experiments and technical replicates (n=4 in total). Data for the second study resulted from three technical replicates.

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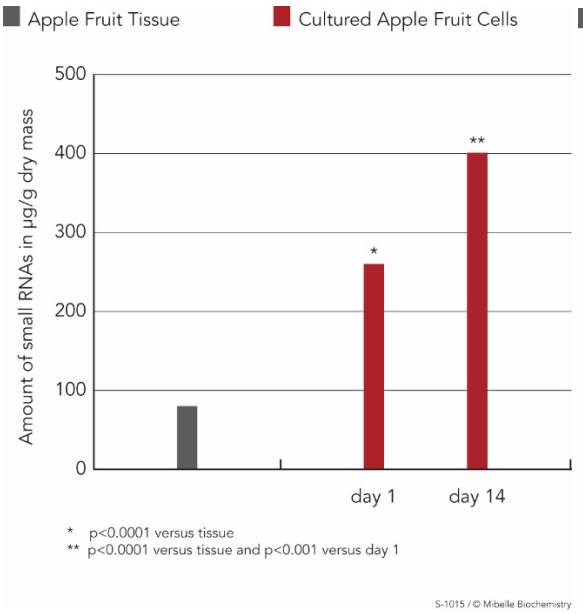
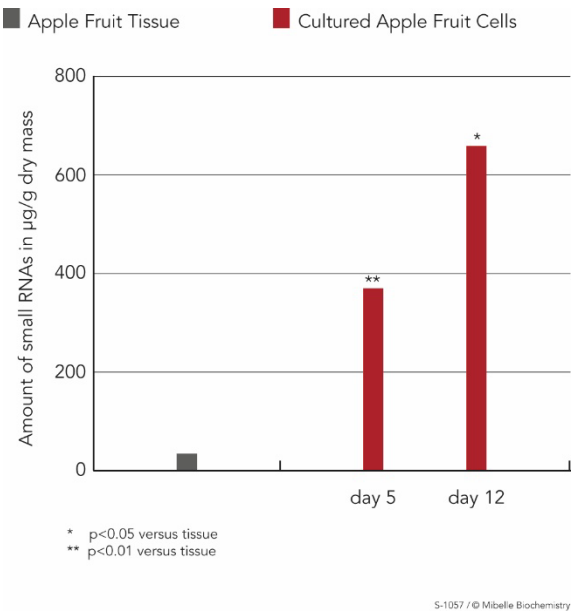
RESULTS

Expression of miRNAs in cultured apple cells was significantly higher by 231 % than in the tissue of the corresponding apples. In addition, the amount of miRNA was significantly increased in cultured cells after 14 days of culture (time point when the cells are used for the production of PhytoCellTec™ Md Nu) by 56 % compared to the start of the culture. Increased miRNA expression after 14 days of cell culture was reproducible and showed a batch-to-batch consistency.

In the second study, again the quantity of miRNA in cultured apple cells was significantly higher (more than 10x) than in the tissue of the corresponding apples. In addition, the amount of miRNA was increased in cultured cells after 12 days of culture by 78 % compared to the cells in the exponential growth phase. Increased miRNA quantities of cultured cells at the later time point compared to the apple cells in the exponential growth phase was reproducible.





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