



## **Producing plant essential oil compounds through industrial biotechnology**

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Plant essential oils play an important role in today's economy, for flavouring, fragrance, cosmetics and healthcare applications. Most of these oils can be extracted from ample waste materials such as citrus peels and pine trunks, or from dedicated plant cultivations, such as mint, thyme and patchouli. In some cases, the source of the oils is a more rare species, and may pose cultivation challenges. Examples of those include the Asian sandalwood tree (*Santalum album*) and the candeia tree (*Eremanthus erythropappus*), both of which represent trees which need more than 15 years before they can be harvested, and face extinction due to illegal cutting in the wild. In such cases, their main essential ingredients, which are often sesquiterpenes, can also be produced by microbial fermentation. Microbial production of sesquiterpenes is often mediated by terpene synthases derived from plants. These terpene synthases may behave with very different efficiencies when expressed in microbes, and for this reason it is important to be able to choose the most efficient terpene synthase for a specific product. In this work we aim to develop methods to identify the most efficient sesquiterpene synthases for specific products.

In a traditional approach, two very closely related genes were isolated from *Cinnamomum camphora*. One of these genes acts as a monoterpene synthase, when expressed in *Nicotiana*, while the other gene mediates production of santalenes, which represent important ingredients of sandalwood oil. A library of hybrids between the monoterpene- and sesquiterpene synthases leads to identification of enzymes with altered product specificity.

In a computational approach, the rapidly growing databases for plant genomes is interrogated for terpene synthases, and machine learning is applied to identify candidate synthases for a specific sesquiterpene, or a category of sesquiterpenes. This algorithm has been trained using a novel database of characterized sesquiterpene synthases ([www.bioinformatics.nl/sesquiterpene/synthasedb](http://www.bioinformatics.nl/sesquiterpene/synthasedb)). Indeed, using the developed tool, a number of novel bisabolyl cation synthases from *Citrus bergamia* 'Femminello' were identified successfully from a cDNA sequence library.

Thus novel approaches have been developed for generating superior microbial production platforms, to replace vulnerable plant resources for the flavour and fragrance industry.

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