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# mint condition

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**It is very likely that mint – and its close cousin menthol – is one of the most popular flavours or sensations known worldwide. Is there any population left on Earth that hasn't sucked a mint sweet or chewed on mint gum? Mint is drunk in beverages, and brushed onto teeth. Added to sauces, and put into chocolates. Smeared onto chests and added to paper handkerchiefs. Why is it that mint and menthol are found, one way or another, almost everywhere on this planet? Transport would be an obvious answer. But there is more to it than that. Besides the numerous health benefits, mint – and menthol – have a quality that is readily appreciated by many: freshness. This sensation is the legacy of two kindred proteins – P450 cytochromes – found in mint plants.**

Mentha

by Fir0002/Flagstaffotos



The virtues of mint plants have been appreciated for millennia, and like the great majority of medicinal herbs, the mint plant is named after a Greek mythological character: the nymph Minthe. Persephone was jealous of Pluto's love for Minthe, so she promptly transformed her into a plant. Unfortunately, Pluto was not able to restore Minthe to her former state but assured her that she would not be forgotten since her fragrance would be distinctive and pleasant. Especially when she was trod upon… Minthe became a Mediterranean weed whose benefits were widely acknowledged. Dried mint leaves have been found in Egyptian tombs. The Romans used it extensively and introduced the plant to Great Britain on one of their visits. The British then introduced it to many parts of the world as they colonized different parts of it.

The two most popular mint plants are spearmint (*Mentha spicata*) and peppermint (*Mentha piperata*). Known for literally thousands of years, their essential oils are used to treat numerous ailments, such as headaches, indigestion, diarrhea, motion sickness, colds, gallstones and infections to name a few. What is it that does us so much good? The answer is menthol and carvone. Spearmint hosts the enzyme limonene-6-hydroxylase which is involved in the production of carvone – the chemical entity which gives the well-known spearmint flavour. The peppermint plant, on the other hand, hosts limonene-3-hydroxylase, the enzyme involved in the production of menthol. Both limonene hydroxylases belong to the large P450 cytochrome family whose members all have a central role in producing thousands of natural plant products amongst which the hundreds of oxygenated monoterpenes – to which belong carvone and menthol – that are the source of the aromas and flavours so particular to specific essential oils.

Carvone and menthol are end products following the hydroxylation – by the spearmint and peppermint hydroxylases respectively – of one same chemical entity: limonene. Limonene-6-hydroxylase hydroxylates limonene on C6 thus producing trans-carveol which is subsequently modified to become carvone. Limonene-3-hydroxylase, however, hydroxylates limonene

on its C3 thus producing trans-isopiperitenol which – five steps later – is modified to become menthol. The two enzymes are very similar, and their substrate binding sites very restrictive – a discovery which came as a surprise to scientists. Indeed while, as a rule, in the P450 cytochrome family any change of activity usually requires a certain number of mutations, only one mutation is needed to modify the limonene hydroxylases' binding activity.

This particular mutation converts a phenyl-alanine into an isoleucine in the sequence of the spearmint hydroxylase. Originally a limonene-6-hydroxylase, this phenylalanine to isoleucine mutation causes the spearmint enzyme to become a limonene-3-hydroxylase! The spearmint enzyme is thus capable of synthesizing menthol like its cousin, the peppermint hydroxylase! Such a mutation points to the fact that these particular amino acids are not only essential but are most probably involved in the orientation of the substrate limonene within the binding pocket so that it is hydroxylated either at position C3, or position C6.

Single mutations which are capable of changing so drastically a protein's function are of great interest in the world of research. Not only do they point to very specific minute regions in a protein's sequence, but they provide valuable information for the understanding of instances such as substrate binding, substrate orientation, pocket binding structure, enzyme function and metabolic pathways. Needless to say, they are of high biotechnological interest. In the case of the limonene-3- and limonene-6- hydroxylases for instance, the study implies that their substrate binding pockets must be small and pretty tight, and one mutation is capable of influencing substrate orientation in a very subtle way. Naturally, such studies are of great importance within the world of commerce for the yield of peppermint oil, for example, by way of the genetic engineering of *E.coli* or yeast. Nothing many of us would complain about; it is so rare to be able to enjoy something with the knowledge that it is also good for you.

## Cross-references to UniProt

Cytochrome P450 71D15, *Mentha piperita* (Peppermint) : Q9XHE6
Cytochrome P450 71D18, *Mentha spicata* (Spearmint) : Q9XHE8

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