Human tetrahydrocannabinol metabolites

An inexpensive way to produce novel drug candidates

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The human metabolites of tetrahydrocannabinol are responsible for a part of the effects of cannabis use and thus are interesting medicinal candidates. We showed that production of 11-hydroxy tetrahydrocannabinol with recombinant whole cells expressing human cytochrome P450 is possible.

The phytocannabinoids produced by *Cannabis sativa*, with the most prominent example of tetrahydrocannabinol (THC), exert a number of medically interesting effects on humans and can be used to treat nausea and vomiting during cancer therapy, eating disorders, spasms, pain, and tremor.

THC in the human body is readily converted to its metabolites 11-hydroxy THC and 11-*nor*-9-carboxy-THC by cytochromes P450. These metabolites play an important role in the effects of THC use. Being able to produce them in large scale might provide new approaches to old medicinal effects and offer a way to use the beneficial impact of THC without the psychoactive effects.

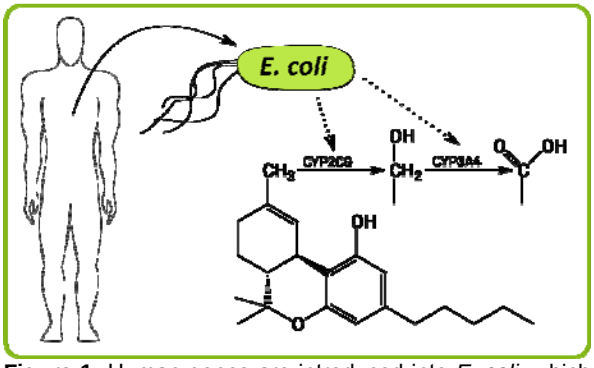

Figure 1: Human genes are introduced into *E. coli*, which is then used as biocatalyst to produce human metabolites of tetrahydrocannabinol.

Escherichia coli DH5α was used to express a modified human Cytochrome P450 2C9. Expression was optimized by fine-tuning the oxygen concentration in the medium, which shows a significant influence on the amount of correctly folded enzyme. The highest enzyme concentration of 1100 nM was achieved under microaerophilic conditions.

Cytochromes P450 require a supply of the redox cofactor NADPH to function. Although *in vitro* conversion of THC with recombinantly expressed P450 is possible using a NADPH regenerating enzyme system, this alternative is very costly. Using just glucose as energy source, non-growing cells without a nitrogen source were able to convert THC efficiently and produce 11-hydroxyl tetrahydrocannabinol up to a concentration of about 55 mg/L with a turnover rate of 1.7×10^{-2} s^{-1} within 24 h.

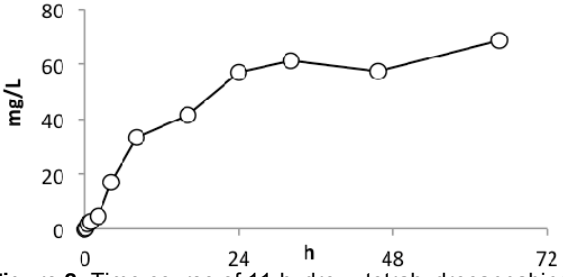

Figure 2: Time course of 11-hydroxy tetrahydrocannabinol concentration. Incubation of *E. coli* DH5α pCW2C9 expressing 1030 µM Cytochrome P450 2C9 in potassium phosphate buffer with 10 g/L Glucose and 314 mg/L tetrahydrocannabinol.

We could show that conversion of tetrahydrocannabinol using whole cells expressing recombinant human cytochromes is a promising approach to synthesize metabolites. This might open the opportunity to use these metabolites as novel drugs and allow a cannabinoid-treatment with less side effects.

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