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(54) Title: ENZYMES AND METHODS FOR FERMENTATIVE PRODUCTION OF MONOTERPENE ESTERS

(57) Abstract: The present invention relates to an alcohol acyl transferase which is capable of esterifying a tertiary monoterpene alcohol such that at least 30% by mass of said tertiary monoterpene alcohol is esterified, preferably within 36 h, 24 h, 18 h, 12 h, 6 h, 3 h, 2 h, 1 h, 45 min or 30 min, more preferably in a microbial cell. The invention further relates to a nucleic acid comprising a nucleic acid sequence encoding the alcohol acyl transferase of the invention, or a complementary sequence thereof, and a vector or gene construct comprising the nucleic acid of the invention. Further provided by the present invention is a host cell comprising the vector or gene construct of the invention, and a transgenic non-human organism comprising the nucleic acid of the invention, the vector or gene construct of the invention, or the host cell of the invention. The invention also concerns a method for preparing a monoterpene ester, comprising esterifying a monoterpene alcohol to a monoterpene ester, in the presence of an alcohol acyl transferase of the invention. Specifically, it provides a method for preparing linalyl acetate, comprising esterifying linalool to linalyl acetate, in the presence of an alcohol acyl transferase of the invention. The invention further pertains to the use of the alcohol acyl transferase of the invention, the nucleic acid of the invention, the vector or gene construct of the invention, the host cell of the invention, or the transgenic non-human organism of the invention (i) for heterologous reconstitution of a terpene biosynthetic pathway; (ii) for producing an industrial product, preferably a flavour or fragrance, a biofuel, a fuel composition, a fuel compound, e.g., a blowing agent for diesel fuel compositions, a pesticide, an insect repellent or an antimicrobial; (iii) for producing an aliphatic and/or aromatic monoterpene ester from a monoterpene alcohol, preferably from a tertiary monoterpene alcohol; (iv) for detoxifying a monoterpene alcohol in a microorganism, thereby increasing monoterpene production in said microorganism; (v) in combination with a GPP synthase and/or S- or R- linalool synthase; (vi) for increasing the beneficial effects of acetylation in that the hydrophobic acetate partitions more readily go into an organic phase, as compared to the monoterpene alcohol; (vii) for expressing the alcohol acyl transferase of the invention such that the ratio of monoterpene acetate to monoterpene alcohol is greater than 5:1 or 10:1 or (viii) in a microbial production system for monoterpene esters. The invention also provides a kit comprising the alcohol acyl transferase of the invention, the nucleic acid of the invention, the vector or gene construct of the invention, the host cell of the invention, or the transgenic non-human organism of the invention, and optionally at least one monoterpene alcohol, preferably a tertiary monoterpene alcohol. Finally, the invention relates to a method for the production of a fuel and/or biolubricant compound, wherein the method comprises the steps of: a) Producing one or more monoterpene esters by any one of the methods of the invention; b) optionally, purifying the one or more monoterpene esters produced in step a); and c) converting part or all of the one or more monoterpene esters of step a), or the optionally purified one or more monoterpene esters of step b), to one or more fuel and / or biolubricant compound, preferably selected from the group consisting of: tetrahydrolinalool; 2,6-dimethyloctane (DMO); saturated C20 hydrocarbon dimers; saturated C30 hydrocarbon trimers; hydrogenated methylcyclopentadiene dimers; saturated high density multi-cyclic hydrocarbon compounds suitable for missile propulsion; and hydrogenated C40+ oligomers suitable to produce biolubricant additives; d) optionally combining the one or more fuel or biolubricant compound with additional compounds suitable for a fuel and / or biolubricant; wherein the fuel and / or biolubricant composition has in sum between and including 0.01 % (w/w) to 99.99 % (w/w), of the fuel or biolubricant compound produced from the one or more monoterpene esters obtainable by one of the methods of the invention.

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