ABOUT ESSENTIAL OILS BIOTECHNOLOGY ON THE BASE OF MICROBIAL SYNTHESIS

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One of the high-priority aims is an expansion of the world assortment of natural essential oils produced by industry – which is currently counted to be around 180 names widely used in pharmaceutical, perfumery, cosmetic, confectionary, soap, and alcohol beverage productions. The revelation of perspective producers is an actual goal in modern biotechnology of aromatic products, in addition to including the scent of fresh rose flowers. The comparative analysis of cultures of microorganisms, which are referred to different taxonomic positions, shows that the quantity of synthesized volatile aromatic substances reaches 180 mg/l cultural liquid during the first two days of growth on fermentative medium. These bio-objects possess the highest growth speed that offers them an advantage and simultaneously increases product outcome yield on useful equipment units. The basic components of essential oils are geraniol and β-phenylethanol. Nerol, citronellol, neral, and geranial were also observed among these basic components.

Keywords: biotechnology, essential oil, microbial synthesis

Essential oils are fragrant, volatile substances, which different plants parts, essentially flowers, leaves, fruits, roots, may contain. They are lightly distillated with a vapor from raw plant material. The essential oils have a wide range of cosmetic and medical actions due to the presence of up to 500 complex organic compounds with different chemical structure in their composition. The properties of essential oils are apparent by their complex pharmacological, biochemical and clinical effects due to their action on three levels: molecular, psychoemotional, and on the level of nervous system – because every smell has several chemical substances. The mechanisms of essential oils are made up of local, reflex and total resoptive actions. Molecules of aromatic compounds, when interacting with olfactory receptors, evoke emotional response. In turn, the emotional reaction can provide mental and physiological changes in the organism. The essential oils cause stimulatory or sedative effects on the central nervous system, apparent immunomodulatory action, or regulate oxidative processes in the organism. The efficiency of essential oils is compared against a wide range of bacteria, fungi, viruses. The increases of the causative agent's sensitivity to antibacterial medicines are marked by their complex administration with essential oils [1].

The essential oils are highly propagated in the vegetable world, from fungi and algae till flowering plants. On the Earth, around 3000 plants exist that can be a source of essential oil. But nowadays the limited amount of higher plants is used in industry. The quality of essential oil depends considerably on ecological factors such as location, where volatile-oil-bearing plants are cultivated. In addition, plantation cultivation is characterized with seasonal prevalence. Biotechnological production is deprived from mentioned disadvantages. But the biotechnology of essential oils production in culture of isolated cells and tissues is not competitive in comparison with the biotechnology based on the microbial synthesis.

Bacteria, yeasts, actinomycetes, fungi, algae – which are able to synthesize essential oils and aroma substances de novo and also bioconvert them from less valuable components (fatty acids, alcohols, alkanes, etc.) – are of particular interest as a non-traditional source of these substances in connection with a rapid development of modern industrial biotechnology. In nature there are around 100,000 known species of microorganisms, but just only several hundreds of them that synthesize products or provide reactions that are useful for mankind are studied [2].

The study of new sources of fragrant substances has begun in completed earlier investigations. The estimation was carried out by analysis of accumulation level and composition of essential oil, speed of culture growth and other properties which are important for biotechnological production [3-9]. In addition, it is worth paying attention to the investigation of mechanisms of aroma forming substances synthesis, the increasing clarification of metabolic means on influencing this process [10, 11].

The comparative analysis of cultures of blue-green, green, and red algae, referred to as: *Calothrix, Cylindrospermum, Anabaena, Nostoc, Spirulina, Chlorella, Cyanidum* families, showed that the quantity of synthesized fragrant substances is around 3mg/ml cultural liquid of *Nostoc sp*. (Table). But the use of *Chlorella vulgaris, Spirulina platensis*, and other microalgae biomass is perspective for production of spirituous extractions that resemble oakmoss resinoid – which enters into the composition of perfumes as odour fixator, and also as a self-contained pigment aromatic source.

Microorganisms that are essential oils producers

Micro-organism	Taxonomic position (kingdom, division, class, family)	Basic component composition of aromatic product	Reference
Bacillus sp.	*Bacteria, Firmicutes, Bacilli, Bacillaceae*	β-phenylethanol	[9]
Thiocapsa sp.	*Bacteria, Proteobacteria, Gammaproteobacteria, Chromatiaceae*	β-phenylethanol	[9]
Ectothio-rhodospira sp.	*Bacteria, Proteobacteria, Gammaproteobacteria, Ectothiorhodospiraceae*	β-phenylethanol	[9]
Spirulina platensis	*Bacteria, Cyanobacteria, Chroobacteria, Phormidiaceae or Plantae, Cyanophycota, Cyanophyceae, Oscillatoriaceae*	monoterpenoid alcohols, paraffin	[4]
Nostoc punktiformei	*Bacteria, Cyanobacteria, Hormogoneae, Nostocaceae or Plantae,Cyanophyta, Hormogoniophyceae, Nostocaceae*	monoterpenoid alcohols, paraffin	[4]
Chlorella vulgaris	*Plantae, Chlorophyta, Chlorophyceae, Chlorellaceae*	monoterpenoid alcohols, paraffin	[4]
Scenedes-mus acutus	*Plantae, Chlorophyta, Chlorophyceae, Scenedesmaceae*	monoterpenoid alcohols	[4]
Saccharo-myces sp.	*Fungi, Ascomycota, Hemiascomycetes, Saccharomycetaceae*	β-phenylethanol	[9]
Endomy-copsis sp.	*Fungi, Ascomycota, Hemiascomycetes, Endomycetaceae*	β-phenylethanol	[9]
Eremothe-cium ashbyi	*Fungi, Ascomycota, Hemiascomycetes, Eremotheciaceae*	geraniol, β-phenylethanol	[7]
Eremothe-cium gossypii	*Fungi, Ascomycota, Hemiascomycetes, Eremotheciaceae*	geraniol, β-phenylethanol	[7]
Aspergillus foetidus	*Fungi, Ascomycota, Eurotiomycetes, Trichocomaceae*	lactones, terpene and aromatic alcohols, aldehydes, ketones	[7]
Penicillium canescens	*Fungi, Ascomycota, Eurotiomycetes, Trichocomaceae*	lactones, terpene and aromatic alcohols, aldehydes, ketones	[7]
Trichoder-ma viride	*Fungi, Ascomycota, Sordariomycetes, Hypocreaceae*	lactones, terpene and aromatic alcohols, aldehydes, ketones	[7]
Ceratocystis paradoxa	*Fungi, Ascomycota Sordariomycetes, Ophiostomataceae*	lactones, terpene and aromatic alcohols, aldehydes, ketones	[7]
Ceratocystis pilifera	*Fungi, Ascomycota Sordariomycetes, Ophiostomataceae*	lactones, terpene and aromatic alcohols, aldehydes, ketones	[7]

The strains which produce aromatic substances in quantity of around 95 mg/l are revealed among studied representatives of bacteria (genera *Thiocapsa, Ectothiorhodospira, Bacillus*) and yeasts (genera *Saccharomyces, Endomycopsis*). As a rule β-phenylethanol is contained in aromatic products of these cultures. In comparison with other groups of microorganisms, they possess the highest growth speed that offers the advantage of higher end product outcome yield on useful equipment units [5].

The study of fungi cultures, such as mycelial fungi, deserves particular attention in its ability to accumulate biologically active metabolites which are important for industrial use. The aimed search of perspective objects for aromatic products in biotechnology in the range of genera *Trichoderma, Ceratocystis, Aspergillus, Eremothecium* gives the possibility to characterize differences between species, strains by level of biosynthetical activity and essential oil composition. During analysis of twenty-one cultures of genus *Trichoderma* a high variability is revealed by these characteristics. The essential oil synthesis *E.ashbyi* reaches 180 mg/l cultural liquid during first two days of growth in fermentative medium. This can be comparable with that of the essential oil content in 500-600 g of rose flowers [6-9]. The basic components of essential oil are geraniol and β-phenylethanol, while nerol, citronellol, neral, gernial were later identified. Geraniol, β-phenylethanol and other essential oil components affect spasmolyticly, antiinflammantory and antiseptically [12]. The essential oil synthesized by *E.ashbyi* closely approaches the functions of the essential oil of fresh rose flowers and has a scent. The component composition of essential oil of *E.gossypii* is similar, but the monoterpene alcohols ratio is closer to their content in Bulgarian rose oil.

This natural product, whose price on the world market reaches eighty dollars for one gram, is in extreme demand as more than half of world perfume brands are produced on the base of rose oil. It is also used in medicine and pharmaceutics. The oil is used as a corrigant of pharmaceutical products for their taste and smell improvement. The rose oil and the rose water are widely applied in perfumery, cosmetic, confectionary, soap, etc. industries. The rose oil possesses moderately antibacterial (bacteriostatic) effects, because β-phenylethanol inhibits macromolecules synthesis, but is not toxic for all microorganisms and strains at the same degree [13]. The rose oil regulates the adrenal work, possesses antipyretic, is anti-inflammatory, is anti-edematous, is choleretic, hepatoprotective action, is used in the treatment of stomatitis, parodontosis, cutaneous and other diseases.

The rose oil accounts for 0,025 % of composition on average, so for production of 1 kg oil the manual collection and processing of around 4 tonnes of petals is necessary. The rose water remains after oil distillation while oil accounts for 0,02 % of its composition. The main supplier of rose water in the world market is Iran, but the oil is not produced there. In the world, rose oil of good quality and volume – which is nowadays around 600 kg/year – is produced just only in four countries: Taif, Saudi Arabia; Kazanlyk, Bulgaria; Istanbul, Turkey; and the Tashkent region, Uzbekistan [14]. Until 1992, the rose oil production by hydrodistillation method in USSR republics (Ukraine, Moldova, etc.) was around four tonnes per year. But since then, it has sharply reduced due to the economical crisis in CIS countries [15]. For instance, in 2005, in Crimea, only 600 kg rose essential oil (extract) were produced. That is less than maximal levels reached in this region by a factor of two [16].

Thus, the revelation of perspective producers of essential oil – including rose odour direction – is an urgent problem in biotechnology of aromatic products, which are very close to endogenous compounds of human body by their content and actions. In addition, many aroma forming substances are able to engage in spontaneous interactions – stacking – yet are conditioned by geometrical limits. Such nanostructures have a high potential for the application in different fields of bionanotechnology (nanobiotechnology) [17]. One of the high-priority aims is an expansion of world assortment of natural essential oils produced by industry – that is counted to be around 180 names – at the expense of the introduction of new taxonomically different microorganisms-overproducers.

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