Study of *Aspergillus niger* resistance to low concentration preservatives in media with essential oils

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Filamentous fungi are well-known contaminants and destructors of various substrates. The search of anti-fungal treatment methods to preserve materials and food are actual. One of approaches is based on plant's essential oils that mediate the interaction of plants with the environment and could combat fungi by providing cell protection. The main compounds of such action are terpenes and terpenoids, in some cases nitrogen- and sulfur-containing chemicals, coumarins and homologues of phenylpropanoids [1]. For example, essential oils of *Litsea cubeba* (Lauraceae) and its component citral are capable to exhibit anti-fungal activity against *Alternaria alternata*, *Aspergillus niger*, *Fusarium verticilloides* and *Neocosmospora solani*, through damage of their cell wall and cell membrane to various degrees, cytoplasm leakage and by inhibiting DNA, RNA, protein and peptidoglycan biosynthesis [2]. Studies have shown a dose-dependent inhibitory effect of essential oils that produced stronger suppression of fungal and bacterial growth along a concentration gradient. The level and character of inhibition depends on the specific compound and fungus. Some agents exhibit linear inhibition effect, while others may reach a plateau stage where further concentration increase does not significantly enhance inhibition [3]. Besides, fungi can develop resistance or tolerance to the antimicrobial agent over the time, and then to require application of the agent higher concentrations as to achieve the same level of inhibition. Resistance mechanisms can involve changes in cell membrane permeability, efflux pumps, or the evolvement of the specific resistance genes [1].

The aim of the research was to analyze inhibitory action of compound mixtures that consisted from weakened preservatives and essential oils on fungi, especially on *A. niger*. It was hypothesized that a cell wall structure of *A. niger* can contribute to the fungus resistance to a treatment based on combination of weakened preservatives when combined with essential oils.

Two different agar media were used in the study: potato dextrose agar and Sabouraud agar. There were prepared sampled Petri dishes plates with different concentrations of a cosmetic product that had combination of essential oils and other substances (water, urea, *Helianthus annuus* seed oil, stearic acid, glycerol stearate, PEG-100 stearate, propylene glycol, glycerol, panthenol, *Ricinus communis* seed oil, carbomer, triethanolamine, *Olea europaea* fruit oil, *Persea americana* oil, *Macadamia ternifolia* seed oil, tocopherol acetate, *Vitellaria paradoxa* oil, *Ribes nigrum* seed, *Salvia rosmarinus* leaf oil, *Citrus limon* seed oil, ethylhexylglycerin, phenoxyethanol) which had been added to the agar media by 10 ml. Added suspension contained also 9 ml of CPLP (TAT Broth Base, for proliferation and regeneration of fungi and bac-

teria from a variety of highly viscous or gelatinous materials). Concentrations of a cosmetic product for tests were prepared as 1 %, 2 %, 5 % and 10 %; besides, there were tested different dilutions of the product as 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}). Control plates with agar media were without any product with essential oils. All studied Petri dishes plates were inoculated by standardized test strain of *A. niger* under controlled laboratory conditions and incubated in a thermostat at a temperature of 25°C for five days, with collected intermediate result on the second day, 48 h.

The tests with cultivation of *A. niger* on the media with different concentrations of essential oils had demonstrated that the lower was the content of the studied product with essential oils in the cultivation medium, the lower level of *A. niger* was observed (Table 1).

Table 1. The tests results of *Aspergillus niger* cultivation on the media with different concentrations of essential oils

Potato-dextrose agar				Sabouraud agar	
Concentration	Number of colonies	Dilution	Number of colonies	Dilution	Number of colonies
1 %	162	10^{-4}	36	10^{-4}	15
2 %	216	10^{-3}	51	10^{-3}	40
5 %	452	10^{-2}	596	10^{-2}	628
10 %	< 1000	10^{-1}	< 1000	10^{-1}	< 1000
Control	0	Control	0	Control	0

The fungus *A. niger*, like other fungi, has a cell wall primarily composed of chitin, a tough and rigid polysaccharide. Chitin provides structural integrity and protection to fungal cells. The presence of chitin makes fungal cell walls more resistant to chemical agents, including some preservatives and that could be the explanation of observations received during the experiment. In general, cell wall of *A. niger* consists chiefly of neutral carbohydrates (73–83 %) and hexosamine (9–13 %), with smaller amounts of lipids (2–7 %), proteins (0.5–2.5 %) and phosphorus (less than 0.1 %). Analysis had shown the presence of six sugars: glucose, galactose, mannose, arabinose, glucosamine and galactosamine, all in the D-configuration, except that a small amount of L-galactose may be present. Sixteen common amino acids are also present [4]. At the same time, knowledge of filamentous fungi, as well as of *A. niger*, cell wall structure, mechanisms regulating cell wall biogenesis, and cell wall stress responses, is still limited in comparison with that of *Saccharomyces cerevisiae*. Cell wall structure of *Aspergillus* species has an alkali-soluble and alkali-insoluble fractions. The first one composed mainly of linear-chain α-1,3-glucan that performs functions related to pathogenic expression as well as other functions of protecting cells from certain types of cell wall stress and helping in promotion of normal growth and regulation of conidiation. The alkali-insoluble fraction is composed mainly of β-1,3-glucan, chitin, and galactomannan [5]. Chitin is a β-1,4-linked homopolymer of N-acetylglucosamine which synthesis is important for hyphal development. Chitin synthases are integral plasma membrane enzymes that catalyze N-acetylglucosamine polymerization. All

known seven classes of chitin synthases are present in *Aspergillus*, though classes III, V, VI, and VII are specific to filamentous fungi and certain dimorphic yeasts only. Enzymes of III, V, VI classes play crucial roles in hyphal tip growth and maintenance of cell wall integrity [5], and could be responsible for higher levels of *Aspergillus* fungi survival under the essential oil stress conditions.

Resistance of *A. niger* to environmental factors is also supported by specific structure of spores that are produced as a part of the life cycle. The spores have much thicker and more resistant cell walls in comparison to actively growing fungal hyphae. The presence of spores in a culture may contribute to resistance as they can act as a reservoir of viable cells that can withstand preservative treatments.

Further investigations of essential oils influence on *A. niger* spore germination and metabolites are necessary. Some preservatives may affect nutrient uptake or utilization differently in fungi compared to other microorganisms, as *A. niger* was the only survival organism between different tested fungi and bacteria in preliminary experiments.

References

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