Research article

Microbiological contaminants in cosmetics – isolation and characterization

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Abstract: *Cosmetic industries are not obliged to produce sterile cosmetics. Nevertheless, they are liable to assure safety of the product to the potential consumer. The purpose of the study was isolation and identification of microorganisms with the ability to survive and develop in cosmetics. Five cosmetics applied for facial skin and one cosmetic for body care were tested for the presence of contaminating microbiota. Eight microbial strains were isolated from three cosmetics, from which seven were derived from cosmetic applied on the facial skin. One strain was isolated from body care cosmetic. The recovered microbial strains were characterized and identified to the species level as Pseudomonas aeruginosa, Serratia liquefaciens and Candida parapsilosis. The isolates were opportunistic pathogens and may cause skin irritation and infections, especially via wounded epithelium in immunocompromised consumers. Moreover, due to application area, they pose a health risk to the consumer due to easy access to the eye area as well as nasal and oral cavities through usage of cosmetic preparation.*

Keywords: *cosmetics, microbiological contamination, Pseudomonas, Serratia, Candida.*

Introduction

According to the Council Directive (76/768/EEC), established in 1976, a cosmetic product is any substance or preparation, which can be applied onto different parts of the human body (e.g. nails, face, hair, teeth). Its role is to keep body in a good condition, change its appearance as well as remove body odours via perfuming, cleansing or protection [1]. Depending on the application area, cosmetics may be categorized as cosmetics for skin, hair-scalp and oral care as well as fragrances [2]. Cosmetic ingredients govern wide range of products ranging from oily materials, surface active agents, polymers, ultraviolet absorbents to fragrances and vitamins [2, 3]. Companies producing cosmetics are obliged to ensure safety of the product sold, nevertheless, cosmetics are not liable to be sterile [1]. Their microbiological load is strictly controlled at various

manufacture stages and during shelf-life. Cosmetics might contain microbes, due to impurity of raw material and might be contaminated during usage [4, 5]. Microbial spoilage can not only alter physical properties of the product such as colour, taste, odour and viscosity, but also deactivate crucial constituents depriving cosmetic of its features [6, 7]. Microbiological contaminants may produce endotoxins and metabolites causing irritation and allergic reaction of the skin [7]. They can be also pathogens causing hazard to the human health [8].

Microorganisms can survive in environment that fulfil their physical and chemical requirements for proliferation and further development. Most important physical requirements include suitable temperature and pH of the environment. Considering chemical ones, microorganisms require presence of moisture, available and easily metabolized nutrients as well as oxygen [5]. Almost all cosmetics fulfil all of the hereinbefore described requirements for microbial growth. They are rich in a free water, having pH close to neutral. Consumers keep them at home, at room temperature, which is an optimum for proliferation. Most of the cosmetics are found in the bathroom, where the temperature and humidity are high [4]. Depending on the type of closure, different amount of oxygen may access the cosmetic preparation. Composition of cosmetics varies from product to product. The specificity of cosmetic application requires that its ingredients are nourishing and easily assimilated. Hence, such components as proteins, minerals, vitamins and glycerine are easily metabolized source of nitrogen, carbon, hydrogen as well as micro- and macro-elements, necessary for microbial development [4, 5].

The SCCP (Scientific Committee on Consumer Products) in "Notes of Guidance for the testing of Cosmetic Ingredients and their Safety Evaluation" stated that even cosmetics applied by children under three years old, around the eye area and on mucous membrane may be contaminated by saprophytic microorganism up to 100 CFU (Colony Forming Unit) in 0.5 g/mL of the cosmetic preparation [8]. Anyway, such microorganisms as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus brasiliensis* were restricted by EU Pharmacopoeia as most commonly found contaminants posing microbial spoilage in cosmetics and risk to the consumer health. They may not be found in the given volume of cosmetic sample [9]. In order to protect cosmetic product from microbial spoilage to which cosmetic is subjected during use, preservatives are added. Despite their function, addition of preservatives belongs to the one of the most controversial issues concerning cosmetic industry [10].

According to the RAPEX (the Rapid Alert System for non-food consumer products), 173 cosmetics were recalled from the market, from year 2005 to 2008. Among them, 24 were highly contaminated with undesired microorganisms, especially *Pseudomonas aeruginosa* [11]. As stated in RAPEX Reports, 86 cosmetics were notified in 2012 as health risk to the human, from which 8 cosmetics were categorized as a microbiological hazard. In 2013, 13 cosmetics

(calculated basing on weekly reports) were notified as either contaminated with microbial pathogens or having too high microbiological load [12]. The objective of the study was to isolate and identify microorganisms recovered from cosmetics during their use as well as to assess their potential health hazard to the consumer.

Experimental

Materials

Six commercial cosmetic products, abbreviated from A to F were randomly delivered by consumers after approximately 12 months of usage. These included cosmetic applied on facial skin (No A – Anti-Wrinkle Cream, B – Anti-Eye Wrinkle Cream, C – Moisturizing Cream, E – Anti-Redness Cream and F – Moisturizing Cream) and used for body care (No. D – Moisturizing Cream after Shaving and Depilation). Agar slants of nutrient broth supplied with glucose as carbon source (peptone 5.0 g/L, yeast extract 2.0 g/L, meat extract 2.0 g/L, sodium chloride 4.0 g/L, glucose 10.0 g/L, agar 20.0 g/L, distilled water up to 1 L) were used for isolates activation before identification tests.

Methods

Isolation of microorganisms

Depending on the type of cosmetic closure, a plate method with samples seeding or printing were applied to recover microorganisms. A sterile Dilution liquid (peptone K 5.0 g/L, NaCl 8.5 g/L, distilled water up to 1 L, pH 7.2±0.2) was used to dilute cream sample. Recovery of microorganisms from cosmetics was performed on Tryptic Soy Agar with Neutralizers (TSA, Merck KGaA, Darmstadt, Germany), from which single strains were isolated and cultivated on a Plate Count Agar (PCA, Merck KGaA, Darmstadt, Germany) medium. All samples were incubated at 30^{o}C for seven days. 24-hour activated cultures were used for all further analyses and identification.

Identification of isolates

Identification of recovered strains included macroscopic and microscopic characteristics as well as biochemical profiling. Isolated microorganisms were analyzed microscopically. API® tests (bioMerieux, Warsaw, Poland) were chosen and performed for their biochemical profiling. API® 20E set included the following biochemical tests for active enzymes or fermentation/ oxidation reactions: 1 – β-galactosidase, 2 – arginine dihydrolase, 3 – lysine decarboxylase, 4 – ornithine decarboxylase, 5 – citrate utilization, 6 – hydrogen sulphide production, 7 – urease, 8 – tryptophan deaminase, 9 – indole production, 10 – acetoin production, 11 – gelatinase, 12 – fermentation/oxidation of glucose, 13 – fermentation/oxidation of mannitol 14 – fermentation/oxidation of inositol, 15 – fermentation/oxidation of sorbitol, 16 – fermentation/oxidation of rhamnose, 17 – fermentation/oxidation of sucrose, 18 – fermentation/ oxidation of melibiose, 19 – fermentation/oxidation of amygdalin, 20 – fermentation/ oxidation of arabinose. API® 20C AUX set consisted

of biochemical tests for assimilation of the following compounds: 1 – D-glucose, 2 – glycerol, 3 – calcium 2-ketogluconate, 4 – L-arabinose, 5 – D-xylose, 6 – adonitol, 7 – xylitol, 8 – D-galactose, 9 – inositol, 10 – D-sorbitol, 11 – methyl-αD-glucopyranoside, 12 – N-acetyl-glucosamine, 13 – D-cellobiose, 14 – D-lactose, 15 – D-maltose, 16 – D-sucrose, 17 – D-trehalose, 18 – melezitose, 19 – D-raffinose. Recorded results were compared with API® database of reference strains, basing on which the isolated microbial strains were identified to the species level.

Results and Discussion

Contaminants in cosmetics

Microorganisms were isolated from two cosmetics among six investigated (a face care cosmetic – seven isolates and a body care cosmetic – one isolate). Considering morphological features of the recovered strains, seven ones (apart from strain F VII) were either straight rods or curved coccobacilli. All bacteria were motile and did not form special arrangements (Table 1). They were Gram-negative, and gave a positive result in catalase test. Considering strain F VII, the no motile budding cells were yeast species (Table 1).

API® tests are fast and accurate method to perform biochemical profiles of isolated microorganisms and basing on the obtained results to identify them (Table 1, Figure 1, 2, 3). The obtained results for all isolates, except from strain D I, isolated from Moisturizing Cream after Shaving and Depilation, may be considered as accurate, their percentage of identity with official reference strain was higher than 75% (Table 1).

The growth characteristics of isolates confirmed the result of the test. However, the accuracy of identification of strain F III isolated from facial cream was not so high. Compatibility to *Serratia liquefaciens* should be taken into account. The cause of the obtained result may be a wide diversity of environmental species. It should be noted that *Serratia liquefaciens* altogether with *Pseudomonas aeruginosa* were isolated from the contaminated contact lenses of the patient suffering from red eye, which indicates a probability of their coexistence in the skin [13].

In case of identity results for microbial strain D I, the API® database indicated its compatibility to *Pseudomonas aeruginosa* and *Providencia stuartii* with identity equal to 28.0% and 22.9%, respectively. The growth characteristics of the recovered strain may differ the general characteristic features of the *Providencia* and *Pseudomonas* genera due to the wide diversity of species isolated from natural environments, therefore, the obtained result may be considered as reliable.

Despite the fact that seven distinct strains were investigated, they belonged to two species *Pseudomonas aeruginosa* (four strains) and *Serratia liquefaciens* (three strains). Nevertheless, they differ in their biochemical profiles within species (Figure 1, 2), which is also reflected in their identity comparing to the reference strains. It may be resulted from the biochemical diversity of both

species due to wide range of their primary environmental habitats from which they were transferred to the cosmetic environment.

Identification of strain F VII, isolated from Moisturizing Cream, as *Candida parapsilosis* belonging to the yeast species is considered as reliable and accurate one due to very high identity percentage (96.1%) with the official reference strain from API® database (Table 1, Figure 3).

Table 1. Morphological characteristics and biochemical identification of microorganisms isolated from cosmetics

Strain	Morphological features				Biochemical profiling
	size (µm)	shape	motility	pigment formation	identification (identity*)
Cosmetic D					
D I	0.3×1.0÷1.3	straight rods	no motile	no pigment	*Pseudomonas aeruginosa* (28.0%)
Cosmetic F					
F I	0.3×0.6÷1.3	straight rods	motile	green	*Pseudomonas aeruginosa* (98.2%)
F II	0.3×1.0÷1.6	straight rods	motile	green	*Pseudomonas aeruginosa* (76.4%)
F III	0.3×0.3÷1.0	curved coccobacilli	motile	yellowish	*Serratia liquefaciens*
F IV	0.3×0.6÷1.0	curved coccobacilli	motile	yellowish	*Serratia liquefaciens* (98.1%)
F V	0.3×0.6÷1.6	straight rods	motile	green	*Pseudomonas aeruginosa* (76.4%)
F VI	0.3×0.3÷1.0	curved coccobacilli	motile	white	*Serratia liquefaciens* (98.1%)
F VII	1.3÷1.7×3.0÷5.0	elongated ovoid	nonmotile	no pigment	*Candida parapsilosis* (96.1%)

* – comparing to API® database of reference strains

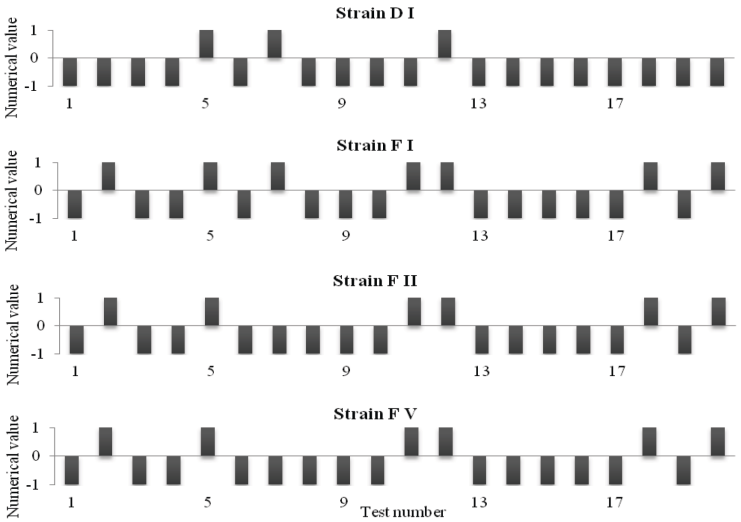

Figure 1. Biochemical profiles of *Pseudomonas aeruginosa* (strains D I, F I, F II and F V) according to API®20E test; "1" – positive, "-1" – negative result

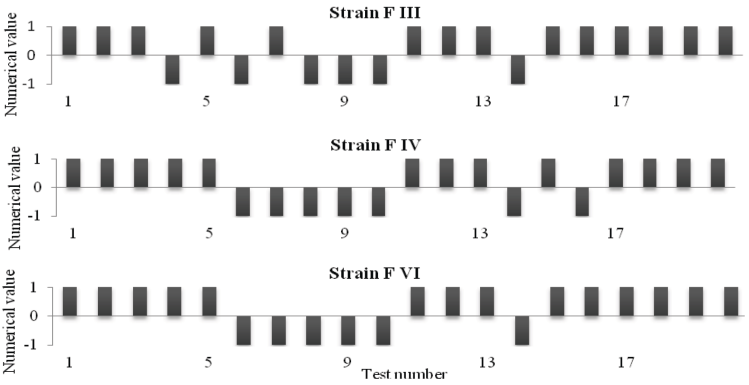

Figure 2. Biochemical profiles of *Serratia liquefaciens* (strains F III, F IV and F VII) according to API®20E test; "1" - positive, "-1" – negative result

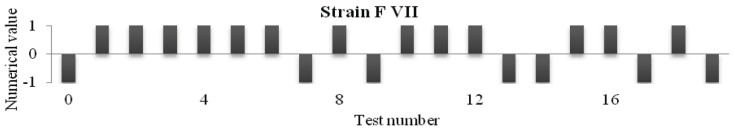

Figure 3. Biochemical profiles of *Candida parapsilosis* (strain F VIII) according to API®20C test; "1" – positive, "-1" – negative result.

Potential hazard of recovered isolates to the consumer's health

Four different microbial species were isolated from the cosmetic environment. Three of them, *Pseudomonas aeruginosa*, *Serratia liquefaciens* and *Candida parapsilosis* were found in the same cosmetic product applied on the facial skin. According to literature, *Pseudomonas aeruginosa* is found in water and soil, some strains were also isolated from skin, animals and plants. It was also isolated from the drainage system and medical equipment [11, 14]. Similarly, *Serratia liquefaciens* was recovered from water, soil, animals, insects and plants [13]. *Candida parapsilosis* is one of the most commonly isolated fungi from the subungual space of hand as well as was also found in other nonhuman environments such as soil, domestic animals, insects [15]. All of the microbial contaminants express similar features: they can survive in harsh conditions, are able to metabolize complex substrates and are resistant to various antimicrobial agents. Since they are ubiquitous in nature, can be easily transported to the cosmetic environment within human hand. *Pseudomonas aeruginosa* belongs to the one of the most commonly isolated bacterium from cosmetic products, whereas *Serratia liquefaciens* was found in antibacterial soap and hand lotion [11, 13]. According to the literature data, *Candida parapsilosis* was recovered from cream [16]. All of the recovered microorganisms are opportunistic pathogens, which basing on the application area may pose a health risk to the consumer due to easy access to the eye area as well as nasal and oral cavities through cosmetic preparation. Abscess formation due to infection was reported for each microorganism. Moreover, eye infections were noted by *Serratia liquefaciens* and *Pseudomonas aeruginosa*, which as well as *Candida parapsilosis* was responsible for fingernails and toenails infections [13, 15, 17]. The immune competent individual is less susceptible to infections with the isolated microorganisms. In contrary, they were reported to cause numerous nosocomial infections of immunocompromised patients, including bloodstream, urinary or fungal peritonitis (in case of *Candida parapsilosis*) infections, which can be lethal [13, 15, 17]. The health risk to potential consumer varies within its own immune system, nevertheless, these microorganisms may cause an infection even in healthy person [18] due to inconsistent skin structure and wounded epithelium as a result of harsh environmental conditions. None of the recovered microbial contaminants should be found in the cosmetic environment.

Conclusions

The performed study confirms that microbiological contamination cosmetic product is a current issue. The steps of microscopic and macroscopic observation of features of recovered microorganisms altogether with biochemical profiling are essential to appropriately identify the microbial strains to the species level. The recovered microorganisms belong to *Pseudomonas aeruginosa*, *Serratia liquefaciens* and Candida *parapsilosis*. They are opportunistic pathogens widely

distributed in nature, which may cause skin irritation and infections, especially via wounded epithelium. Due to application area, they can be a serious threat and the cause of infections of other parts of the body including eye and nails.

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