

Article

Impact of Selected Cosmetic Ingredients on Common Microorganisms of Healthy Human Skin

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Received: 5 July 2019; Accepted: 20 July 2019; Published: 1 August 2019



Abstract: Human skin is a complex ecosystem and is host to a large number of microorganisms. When the bacterial ecosystem is balanced and differentiated, skin remains healthy. However, the use of cosmetics can change this balance and promote the appearance of skin diseases. The skin's microorganisms can utilize some cosmetic components, which either promote their growth, or produce metabolites that influence the skin environment. In this study, we tested the ability of the *Malassezia* species and some bacterial strains to assimilate substances frequently used in dermal formulations. The growth capability of microorganisms was determined and their lipase activity was analyzed. The growth of all *Malassezia* spp. in the presence of free acids, free acid esters, and fatty alcohols with a fatty chain length above 12 carbon atoms was observed. No growth was observed in the presence of fatty alcohol ethers, secondary fatty alcohols, paraffin- and silicon-based substances, polymers, polyethylene glycols, quaternary ammonium salts, hydroxy fatty acid esters, or fatty acids and fatty acid esters with a fatty chain length shorter than 12 carbon atoms. The hydrolysis of esters by *Malassezia* lipases was detected using High Performance Thin Layer Chromatography (HPTLC). The production of free fatty acids as well as fatty alcohols was observed. The growth promotion or inhibition of bacterial strains was only found in the presence of a few ingredients. Based on these results, formulations containing microbiome inert ingredients were developed.

Keywords: *Malassezia* spp.; *Staphylococcus* spp.; *Corynebacterium* spp.; lipases; cosmetics

1. Introduction

The skin provides a diversity of habitats for bacteria, yeasts, and mold, with different microbiota associated with different regions of the skin. Based on traditional cultivating methods, the predominant bacteria are *Propionibacterium acnes* and *Staphylococcus* spp. in oily sites, and *Corynebacterium* spp. and *Staphylococcus* spp. in moist sites. Fungal diversity is dominated by the genus *Malassezia* [1]. Several of these skin microorganisms are involved in dermatological diseases, such as seborrheic dermatitis, atopic dermatitis, and acne vulgaris [2–4].

Malassezia and bacteria such as *Staphylococcus* spp., *Propionibacterium* spp., and *Corynebacteria* spp. secrete multiple lipases with a broad spectrum of activity that hydrolyzes almost all triglycerides in the sebum into fatty acids [5–7]. With the exception of *M. pachydermatis*, all *Malassezia* species known so far require an external lipid source for growth, i.e., they are inevitably lipid dependent [6]. The *Malassezia* genome lacks the genes that encode the cytosolic fatty acid synthase complex (FAS), which explains why these yeasts cannot synthesize de novo C14 or C16 free fatty acids (FFAs) [8]. To overcome this, *Malassezia* utilizes fatty acid sources directly from their host and uses the lipases to obtain FFA. In contrast, most bacteria lack the nutritional advantages offered by the degradation of

sebum triglycerides to their constituent free fatty acids. However, the FFAs produced by bacterial lipases promote cell–cell adhesion and the aggregation of cells promotes cooperative cell nutrition due to effects on the local micro-environment [9]. In vitro studies have shown that purified lipases influenced the functions of different human immune cells, such as the chemotaxis of neutrophils and granulocytes, decreasing the phagocytotic killing of bacteria [10]. In this manner, bacterial colonization can be achieved. On the other hand, it has been shown that environmental conditions, including substrates, can influence the fatty acid composition of some bacterial strains [11].

The application of cosmetics may adversely impact skin if microbial numbers and diversity are changed [12]. However, the interactions of cosmetics with microbiota is not yet fully understood. In relation to fungi and bacterial lipases, cosmetic ingredients based on fatty acid esters are of paramount importance. Mayser et al. reported that some emulsifiers and oils promoted the growth of one or more *Malassezia* species [13]. The same behavior was found by Koyama et al. for other topical vehicles [14]. Growth stimulation and the formation of high amounts of FFAs may be of pathophysiological importance. Additionally, some FFAs produced by the hydrolysis of esters have comedogenic properties and play an important role in the pathogenesis of acne [15]. However, to date no systematic studies have been performed.

In this study, we tested the ability of *Malassezia* species and different bacteria to assimilate ingredients frequently used in dermal formulations. The growth of microorganisms was determined. Additionally, the metabolism of tested substances was analyzed by High Performance Thin Layer Chromatography (HPTLC).

2. Materials and Methods

2.1. Materials

All tested substances are listed in Table 1. The substances were dissolved or suspended according to their solubility in dimethicone (Evonik, Darmstadt, Germany) or in water, both inert to the growth of relevant microorganisms. The used concentrations were 10% for oils, 5% for emulsifiers, and 2% for fatty acids, gelling agents, and thickeners. Fluid substances were additionally used undiluted.

Table 1. Tested cosmetic ingredients.

Ingredient Group	International Nomenclature of Cosmetic Ingredients (INCI)	Producer
Native oils and waxes	<i>Vitis vinifera</i> Grape Seed Oil	Caelo
	<i>Olea europaea</i> Fruit Oil	Caelo
	<i>Persea gratissima</i> Oil	Caelo
	<i>Prunus amygdalus dulcis</i> Oil	Caelo
	<i>Oenothera biennis</i> Oil	Caelo
	<i>Triticum vulgare germ</i> Oil	Caelo
	<i>Brassica campestris oleifera</i> Oil	Caelo
	<i>Arachis hypogaea</i> Oil	Caelo
	<i>Glycine soja</i> Oil	Caelo
	<i>Sesamum indicum</i> Seed Oil	Caelo
	<i>Simmondsia chinensis</i> (Jojoba) Seed Oil	Caelo
	<i>Linum usitatissimum</i> Seed Oil	Caelo
	<i>Ricinus communis</i> (Castor) Seed Oil	Caelo
	Cera alba (Bee wax)	Caelo
	<i>Copernicia cerifera</i> (Carnauba) Wax	Kahlwax
	<i>Butyrospermum parkii</i> (Shea) Butter	BTC Europe GmbH

Table 1. Cont.

Ingredient Group	International Nomenclature of Cosmetic Ingredients (INCI)	Producer
Fatty acid esters	Isopropyl myristate	Caelo
	Isopropyl palmitate	Caelo
	Caprylic/capric triglyceride	Caelo
	Decyl oleate, coco-caprylate/caprato	BASF Personal Care and Nutrition GmbH
	Cetearyl isononanoate	BASF Personal Care and Nutrition GmbH
	Ethyl oleate	Croda
	Oleyl oleate	Fagron
	Decyl oleate	Caelo
	Cetearyl ethylhexanoate	Evonik
	Cetyl palmitate	Fagron
	Glyceryl stearate	Fagron
	PEG-40 stearate	Evonik
	Glyceryl oleate	Evonik
	Tween 80	Caelo
	Span 80	Fluka
	Macrogol 40 glycerolhydroxystearat	Fagron
	PEG-7 glyceryl cocoate	Evonik
	Sucrose stearate	SE Pharma
	Sucrose palmitate	SE Pharma
	PEG-40 hydrogenated castor oil	Evonik
	Methyl glucose isostearate	Evonik
	Polyglyceryl-3 caprate	Evonik
	Polyglyceryl-3 oleate	Evonik
Tween 40	TCI Deutschland GmbH	
Glyceryl stearate citrate	Evonik	
Polyglyceryl-3 dicitrate/stearate	Evonik	
Diisostearoyl polyglyceryl-3 dimer dilinoleate	Evonik	
Polyglyceryl-3 polyricinoleate	IOI OLEO GmbH	
Polyglyceryl-4 isostearate	Evonik	
Lanolin	Caelo	
Fatty acid alcohols	Octyldodecanol	Caelo
	Cetearyl alcohol	Caelo
Fatty alcohol ethers	Ceteth-20	Croda
	Oleth-20	Acros organics
	Laureth 20	Croda
	Steareth-20	Croda
	Cetearyl glucoside	Evonik
Fatty acids	Capric acid	Sigma-Aldrich
	Palmitic acid	Carl Roth
	Oleic acid	Fagron
	Stearic acid	Caelo
Other	Paraffin liquidum	Fagron
	Dimethicone	Evonik
	Squalene	AlfaCesar
	Cyclopentasiloxane	Evonik
	Xanthan	Fagron
	Hydroxyethylcellulose	Fagron
	Cellulose gum (Carmellose natrium)	Fagron
	Carbomer	Caelo
	Acrylamide/sodium acryloyldimethyl taurate copolymer/isohehexadecane and Polysorbate 80	Seppic
	Hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer	Seppic
	Cetyl PEG/PPG-10/1 dimethicone	Evonik
	Poloxamer 407	Fagron
	Acrylates/C10-30 alkyl acrylate crosspolymer	Lubrizol
Palmitamidopropyltrimonium chloride	Evonik	
PEG 300	Caelo	
PEG 550	Sigma-Aldrich	

2.2. Formulations

Using the tested substances, water-in-oil emulsions (W/O) and oil-in-water emulsions (O/W) were produced. The emulsions were based on substances showing no interaction with the tested microorganisms. As positive controls, similar formulations containing *Olea europaea* and *Persea gratissima*

oil (instead of octyldodecanol and caprylic/capric triglyceride) were produced. The composition of both formulations is presented in Table 2.

Table 2. Composition of the tested emulsions.

Oil-in-Water Emulsion	Water-in-Oil Emulsion
Water	Water
Pentylene glycol	Pentylene glycol
Magnesium sulfate	Magnesium sulfate
Glycerin	Glycerin
Sodium lactate	Sodium lactate
Hydroxyethyl acrylate/sodium acryloyl-dimethyl taurate copolymer	Cetyl PEG/PPG-10/1 dimethicone
Acrylates/C10-30 alkyl acrylate crosspolymer	Acrylates/C10-30 alkyl acrylate crosspolymer
Octyldodecanol	Octyldodecanol
Caprylic/capric triglyceride	Caprylic/capric triglyceride

2.3. Microorganisms

All microorganisms tested are listed in Table 3.

Table 3. Strains tested.

Species	Strain No.
<i>Malassezia furfur</i>	CBS 1878
<i>Malassezia furfur</i>	CBS 7019
<i>Malassezia sympodialis</i>	CBS 7222
<i>Malassezia sympodialis</i>	CBS 7977
<i>Malassezia sympodialis</i>	ATCC 42132
<i>Malassezia sympodialis</i>	DSM 6171
<i>Malassezia globosa</i>	CBS 7966
<i>Malassezia globosa</i>	CBS 7705
<i>Malassezia restricta</i>	CBS 7877
<i>Corynebacterium minutissimum</i>	DSM 20651
<i>Staphylococcus epidermidis</i>	DSM 28764
<i>Staphylococcus galinarum</i>	DSM 20610

2.4. *Malassezia* spp. Growth Conditions

Cultures were first grown on modified Dixon agar as described by Gueho et al. [16] at 32 °C. After 8 to 10 days, the cells were harvested. One μL of the *Malassezia* suspension was added together with 100 μL of the substance being tested onto agar selective for pathogenic fungi (Merck, Darmstadt, Germany). Fungal growth was determined at 7 and 14 days.

For determination of metabolic activity, the fungal suspension (approximately 105 CFU/ μL) was incubated with the substance being tested for up to 120 h. After the incubation time, the overlay of the petri dish was completely removed with 2 mL of 2-propanol and centrifuged for 5 min at 14,500 rpm. The supernatant was then used for HPTLC analysis.

2.5. Bacterial Growth Conditions

Corynebacterium minutissimum, *Staphylococcus epidermidis*, and *Staphylococcus galinarum* were grown at 37 °C on Casein-peptone Soymeal-peptone (CASO) agar. After 24 h, the cells were harvested using a 0.9% NaCl solution. One hundred μL of the bacterial suspension was added together with 100 μL of the substance being tested on the appropriate deficient agar. The composition is shown in Table 4. Bacterial growth was determined after 24 h.

Table 4. Composition of deficient agar.

Substance	Concentration
Agar	20 g/L
Lactose	10 g/L
NaCl	5 g/L

2.6. Determination of *Malassezia* spp. and Bacterial Growth

Malassezia species growth was determined visually at 7 and 14 days.

Bacterial growth was determined by plate counting, and if possible, with the OD600 assay. For plate counting, the samples were diluted in a 0.9% NaCl solution to obtain 30 to 300 colonies on a plate. In the OD600 assay, the absorption of the suspension was measured at 600 nm on a UV 1600PC Spectrometer (VWR, Darmstadt, Germany). Previously, a calibration curve was constructed by correlating OD600 values to bacterial counts.

2.7. HPTLC

To determine the metabolic activity of *Malassezia* spp., high performance thin layer chromatography was performed based on previously published research [17]. Ten μ L of each sample was dissolved in 2-propanol and applied to HPTLC Silica 60 plates (Merck, Darmstadt, Germany) with a Linomat V-applicator (Camag, Muttenz, Switzerland) and separated by HPTLC (mobile phase hexane: diethylether: formic acid 80:20:2 *v:v:v*). After development, the plate was dipped in a solution of primulin (Sigma-Aldrich, Taufkirchen, Germany) (stock 0.1% in methyl alcohol; final solution in acetone 1:10 *v/v*). The results were quantified with a Camag TLC Scanner 3 using Camag software (Camag, Muttenz, Switzerland).

3. Results

3.1. *Malassezia* spp. Growth

All tested strains of *Malassezia* species grown on plates of agar selective for pathogenic fungi in the presence of olive oil resulted in growth of white colonies on the plates (Figure 1a). In contrast, no growth was found on the agar plates selective for pathogenic fungi without olive oil (Figure 1b). The ability to use olive oil as an external lipid source was reported by Mayser et al. [17]. Thus, agar selective for pathogenic fungi can be used to study the ability of *Malassezia* species to assimilate cosmetic ingredients.

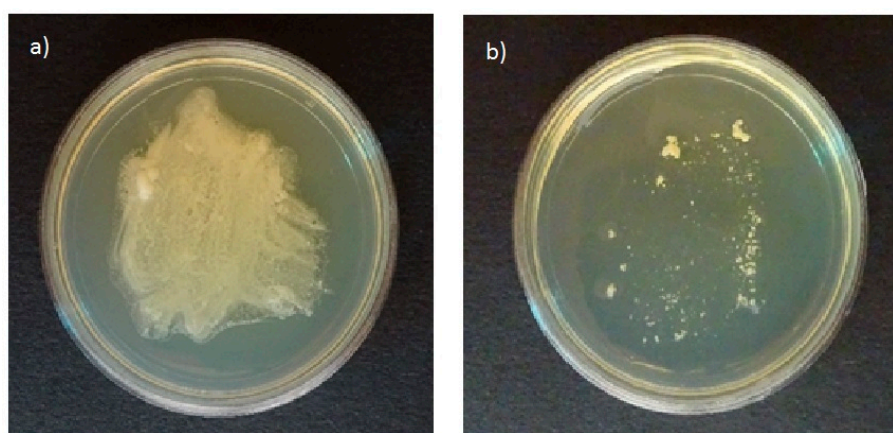


Figure 1. Growth profiles of *Malassezia furfur* on agar plates selective for pathogenic fungi in (a) the presence and (b) the absence of nutritional substances.

The observed growth was strongly dependent on the *Malassezia* species as well as on the tested substance (Table S1 in Supplementary Materials). The strongest growth was observed for the *M. furfur* strains. It was also observed that the growth of *Malassezia* depends on the chemical structure of the tested ingredient. The strongest growth was observed in the presence of natural oils and waxes, primary alcohols, as well in the presence of fatty acid esters with fatty chain lengths above 12 carbon atoms. No growth was observed in the presence of fatty alcohol ethers (e.g., ceteth-20, oleth-20, laureth 20, steareth-20, cetearyl glucoside), secondary fatty alcohols (e.g., octyldodecanol), paraffin- and silicon-based substances, polymers (e.g., hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, cetyl PEG/PPG-10/1 dimethicone, poloxamer 407, acrylates/C10-30 alkyl acrylate crosspolymer), polyethylene glycols, quaternary ammonium salts (e.g., palmitamidopropyltrimonium chloride), hydroxy fatty acid esters, as well as fatty acids and fatty acid esters with fatty chain length shorter than 12C-atoms. However, some exceptions to the above-mentioned rules were observed. In the presence of PEG-40 hydrogenated castor oil and ricinus oil, growth was only observed for the *M. furfur* strain. In the case of esters containing oleic acid (ethyl oleate, decyl oleate, oleyl oleate, and polyglyceryl-3 oleate) and pure oleic acid, growth was observed for *M. furfur* and *M. sympodialis*. No growth was observed for the *M. globosa* and *M. restricta* strains.

No growth of all tested *Malassezia* species was detected in the presence of W/O and O/W emulsions without natural oils. In contrast, a strong growth of all strains was observed in the presence of formulations containing olive and avocado oils.

To determine the metabolic activity of *Malassezia* spp. an HPTLC analysis was performed. *M. furfur* 1878, *M. globosa* 7705, and *M. sympodialis* 7222 were incubated with isopropyl palmitate, oleic acid, oleyl oleate, or caprylic/capric triglyceride for up to 120 h. In the HPTLC chromatogram, the band corresponding to oleic acid occurs at an R_f of 0.27, the oleyl oleate and isopropyl palmitate bands are observed at an R_f of 0.65, and the caprylic/capric triglyceride band at an R_f of 0.42. After incubation with *Malassezia* spp., additional bands were detected. The patterns are presented in Table 5. After incubation with oleic acid an additional band at an R_f of 0.56 was observed. The same band was seen for *M. furfur* when incubated with oleyl oleate. No band corresponding to *M. globosa* at this R_f was observed. After incubation of oleyl oleate with *M. furfur* and *M. globosa*, two additional bands at 0.17 and 0.27 were detected, which correspond to oleic alcohol and oleic acid, respectively. Incubation with isopropyl palmitate resulted in the appearance of an additional band at an R_f of 0.28, corresponding to palmitic acid. After incubation with caprylic/capric triglyceride, an additional band at 0.25 was detected, which corresponds to caprylic and capric acids.

Table 5. Band patterns observed in High Performance Thin Layer Chromatography (HPTLC) chromatograms after incubation of tested substances with *M. furfur* 1878, *M. globosa* 7705, and *M. sympodialis* 7222.

Substance	Band	<i>M. Furfur</i>	<i>M. Globosa</i>	<i>M. Sympodialis</i>
		CBS 1878	CBS 7705	CBS 7222
Oleic acid	1	0.27	0.27	-
	2	0.56	0.56	-
Oleyl oleate	1	0.17	0.17	-
	2	0.27	0.27	-
	3	0.56	-	-
	4	0.65	0.65	-
Caprylic/capric triglyceride	1	0.25	0.25	0.25
	2	0.36	0.36	0.36
Isopropyl palmitate	1	0.28	0.28	0.28
	2	0.65	0.65	0.65

The hydrolysis rate of isopropyl palmitate and its relationship to cell number for *M. sympodialis* was investigated. The results, expressed as the isopropyl palmitate to palmitic acid ratio, are shown in Figure 2. It was observed that with a higher number of cells present, a larger portion of isopropyl palmitate was hydrolyzed, suggesting that the hydrolysis rate of isopropyl palmitate was proportional to cell number. An almost linear decrease of isopropyl palmitate was observed over the incubation time. After 72 h, independent of the initial cell number, about 40% of the initial concentration of isopropyl palmitate remained. However, the amount of palmitic acid produced as a result of hydrolysis was lower than expected. A longer incubation time resulted in a relatively lower concentration of palmitic acid. Accordingly, the isopropyl palmitate to palmitic acid ratio was not constant over time.

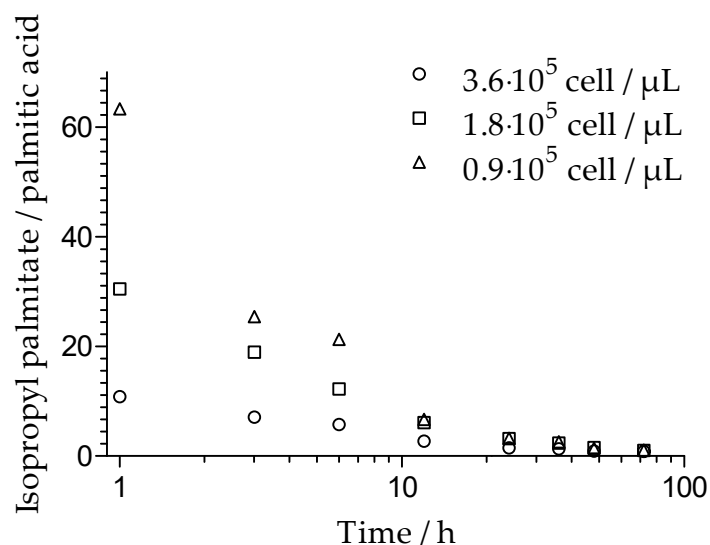


Figure 2. The hydrolysis of isopropyl palmitate over time, expressed as the ratio of isopropyl palmitate to palmitic acid (mg/mg) after incubation with *M. sympodialis* with cell concentrations of 3.6×10^5 cell/ μL , 1.8×10^5 cell/ μL , and 0.9×10^5 cell/ μL .

3.2. Bacterial Growth

A deficient agar for all bacterial species was generated. Agar contains a low amount of nutrients, thus bacterial growth on agar was strongly reduced. The bacteria were incubated with the substances being tested on top of the agar. Peptone was used as a positive control.

No dependence of bacterial growth on general chemical groups of tested substances was found. In most cases, the tested substances had no influence on bacterial growth. However, some exceptions were detected. The most significant interactions were found for *C. minutissimum*. Thus, capric acid inhibits the growth of all tested strains and oleic acid inhibits the growth of *C. minutissimum*. The growth of all strains was observed in the presence of sucrose stearate. The growth of *C. minutissimum* was observed in the presence of ethyl oleate, oleyl oleate, decyl oleate, glycerol oleate, and polyglyceryl-3 oleate. No growth was observed on tested W/O and O/W formulations.

4. Discussion

The human skin is a complex ecosystem with various microenvironmental conditions and hosts many co-existing microorganisms. When the bacterial ecosystem is balanced and varied, the skin remains healthy. However, several environmental factors inclusive of the use of cosmetics can change this balance. Therefore, the influence of cosmetics on microbial diversity and their connection to skin diseases has been explored more extensively in recent years.

Cosmetic products contain many components, which can influence skin microbiome. Some of these, including carbohydrates, proteins, and lipids, can promote microbial growth. On the other hand, they often contain ingredients, e.g., preservatives, which have an inhibitory effect and disturb

the microbiota. It has been shown, that cosmetic ingredients influence the skin microbiome and that microbial diversity can significantly change with the use of cosmetics [18–22]. However, to date no systematic studies have been performed.

In the present study, we tested the influence of many different cosmetic ingredients on microorganism growth. We decided to test the substances at concentrations that are used in cosmetic formulations to better simulate their real influence on skin microbiota. The selection of ingredients was based on the most frequently used cosmetic substances according to the Food and Drug Administration (FDA), Deutscher Arzneimittel Codex (DAC) [23], and other previously published studies [24]. A strong influence of certain groups of ingredients on *Malassezia* spp. growth was found. Natural oils, which mostly consist of fatty acid triglycerides and fatty acid esters with fatty chain lengths above 12C-atoms, presented good growth mediums. It is known that *Malassezia* cannot synthesize fatty acids, and therefore uses skin sebum as a FFA source. Human sebum is a complex mixture of triglycerides, FFAs, wax esters, sterol esters, cholesterol, cholesterol esters, and squalene [25]. These sources are exploited by *Malassezia* by secreting lipases and phospholipases to release FFAs from lipids with fatty acid ester bonds [26]. However, there is no strict requirement for them to be glycerol esters [17]. It was shown that besides natural skin components, various other esters can be used by *Malassezia* as a lipid source. Thus, bacterial growth on natural vegetable oils, emulsifiers, and synthetic oils can also be observed [8,13,14,17]. Our investigations confirm this hypothesis. However, it was apparent that *Malassezia* metabolism was more complex, and that the chemical structure of an ester can strongly influence its nutritional potential. Mayser et al. showed that the alcohol moiety of the ester strongly influences *Malassezia* metabolism [17]. The relationship between growth promotion and hydrolysis rate can be arranged in the following order: ethyl ester > isopropyl ester > decyl ester. Additionally, unsaturated fatty acids were more capable of stimulating growth than saturated fatty acids [17]. By testing various esters, we found additional factors that can play a role in growth promotion. One of these factors is the presence of a hydroxyl group on fatty acid backbones. Thus, growth in the presence of PEG-40 hydrogenated castor oil and ricinus oil was observed only for the *M. furfur* species. In all species, no growth was observed in the presence of macrogol 40 glycerolhydroxystearat. These substances belong to the group of hydroxy fatty acid esters. We tested an additional natural product, lanoline, which is a mixture of hydroxy fatty acid esters (about 30%) with other fatty acid esters (up to 60%) and alcohols. Little or no growth of *Malassezia* spp. on lanoline was observed. Similar observations were made by Mayser et al. for hydroxy fatty acids, such as PEG glycol-35 castor oil and ricinoleic acid [13]. Growth on this medium was only observed for *M. furfur*. Therefore, it was assumed that *M. furfur* is distinct from other *Malassezia* species with regard to the metabolism of ricinoleic acid. We also demonstrated that these results cannot be extrapolated to other hydroxy fatty acid esters. It is likely that other substances belonging to this chemical group cannot promote the growth of *Malassezia*. The slow growth of some *Malassezia* strains on lanoline is probably related to the presence of fatty acid esters other than hydroxy fatty acid esters, which can be metabolized by this species.

Interesting results were obtained using oleic acid as well as esters containing oleic acid. Growth was observed mostly for *M. furfur* and *M. sympodialis* species. No growth was observed for *M. globosa*. However, HPTLC analysis showed an increase in free fatty acids, which could be correlated with the lipase activity of all tested strains. It can be also assumed, that *M. globosa* cannot use oleic acid as a nutrient. Furthermore, the fungistatic properties of oleic acid against *M. globosa* and *M. restricta* strains were reported in a previous study [27]. It is known that *M. globosa* cannot degrade unsaturated species such as oleic acid due to an absence of the enzyme 2,3-enoyl-CoA isomerase. In the same study, it was shown that *M. furfur*, in contrast to *M. globosa*, can use oleic acid as a carbon source [27]. However, in another study no growth of *M. furfur* on oleic acid was observed [28]. The different results may be related to the concentration of the fatty acid used. Mayser used a pure substance, whereas Gordon used concentrations of up to 0.2%. Oleates play an essential role in a number of cellular processes, but at higher concentrations they can have a negative influence on cell survival and cause a lipotoxic effect [29]. In our experiments, apart from hydrolyzed products of fatty acid esters, an

additional HPTLC band at an R_f of 0.56 was detected. This band was identified as an ethyl ester [28]. The formation of fatty acid ethyl esters may be an escape mechanism from excess FFAs and was also observed in other strains of yeast [29]. Interestingly, no detectable formation of fatty acid ethyl esters was found during incubation with isopropyl palmitate, indicating a lower toxic potential of palmitic acid compared to oleic acid.

No growth of *Malassezia* spp. was found on fatty acid esters with fatty chain lengths shorter than 12C-atoms. Medium-chain fatty acids like capric acid (C10:0), caprylic acid (C8:0), or caproic acid (C6:0) are known to have broad antimicrobial activity [30]. The effects of free medium-chain fatty acids on different *Malassezia* spp. were analyzed by Mayser et al. and all strains tested showed growth reduction after exposure to the free fatty acids [31]. In another study, a similar effect was observed for medium-chain triglycerides [32]. Our experiments confirm these data. No growth was found with capric acid, caprylic/capric triglyceride, cetearyl isononanoate, and polyglyceryl-3 caprate. Using decyl oleate and coco-caprylate/caprate, variable growth was observed. Thus, *M. furfur* and *M. sympodialis* showed growth on these media, whereas no growth was observed for *M. globosa* and *M. restricta*. These observations are in accordance with the results obtained for oleic acid esters. We can assume that the hydrolysis of esters with a decyl alcohol moiety is faster than the hydrolysis of esters with a coconut alcohol moiety, because of a shorter chain length. Therefore, a higher concentration of oleic acid exists in the medium compared to caprylic/capric acid. *M. furfur* and *M. sympodialis* use oleic acid as nutrition, whereas *M. globosa* cannot use this acid, as explained above. The amount of caprylic/capric acid medium may be too low to have a toxic effect on the species.

Unexpectedly, the growth of some *Malassezia* spp., especially *M. furfur*, was observed in the presence of a primary fatty alcohol, cetylstearyl alcohol. No growth was observed with a secondary fatty alcohol, octyldodecanol. Until now, the results of the metabolism of fatty alcohols by *Malassezia* were not known. Mayser et al. observed no growth of *Malassezia* spp. on Lanette N and postulated that fatty alcohols cannot be used by this species as nutrition [13]. Lanette N is a mix of ionic surfactant sodium stearyl sulfate and cetylstearyl alcohol. It is possible that the ionic surfactant negatively influences the growth of this species, and therefore no growth was observed. The toxicity of ionic surfactants has been previously reported [33]. Our data suggests that in some cases the metabolism of fatty alcohols by *Malassezia* may be possible.

No growth of most *Malassezia* species was observed in the presence of fatty alcohol ether, paraffin- and silicon-based substances, polymers, polyethylene glycols, and quaternary ammonium salts. Thus, the substances based on these chemical groups cannot be assimilated by *Malassezia* spp. because they either lack fatty acids or the bound fatty acids cannot be cleaved by the *Malassezia* lipases. However, some exceptions are found for *M. furfur*. This species is able to grow in the presence of paraffin liquidum and squalene, but its growth is significantly slower than on other growth media. The mechanism of assimilation is not known, but has been previously studied by other research groups [14].

The results obtained with pure substances were evaluated using complex formulations containing several tested ingredients. The results confirm previous observations. Thus, no growth was observed in the presence of formulations containing inert ingredients. In contrast, strong growth was observed for formulations containing natural oils. We assumed that the results obtained for pure substances can be extrapolated to complex formulations.

The utilization rate of cosmetic ingredients on skin is relevant to their potentially harmful effects. Therefore, the hydrolysis rate, its dependence on cell number, and incubation time were estimated for isopropyl palmitate incubated with *M. sympodialis*. The hydrolysis rate is proportional to cell number, indicating that lipase concentration is cell number dependent. The loss of palmitic acid after a longer incubation time pointed to the assimilation of this substrate by *Malassezia*. This explains the strong growth of *Malassezia* on isopropyl palmitate, as well as on other fatty acid esters.

Most of skin's bacteria are lipid-independent and do not use primarily skin lipids as nutrition. However, some produce lipases, which can hydrolyze skin lipids as well as other fatty acid esters. The hydrolysis of synthetic emulsifiers by *staphylococci* and *corynebacteria* has been reported [34]. The lipolytic

activity of bacteria is strongly dependent on the strain and on the fatty acid nutrition source [35–38]. The fatty acids produced by lipases can give some strains of bacteria benefits by occupying skin and by changing the environment pH. Skin bacteria that produce lipases are *Staphylococcus* spp., *Corynebacterium* spp., and *Propionibacterium* spp., among others. We tested the influence of cosmetic ingredients on the growth of resident skin bacteria. In most cases no influence on growth was found; however, some interactions were observed. Capric acid was found to inhibit the growth of all tested strains and oleic acid inhibited the growth of *C. minutissimum*. The toxic effect of some fatty acids on bacteria is well-documented. Capric acid and lauric acid display a higher bactericidal activity than other saturated fatty acids [39,40]. Fatty acids demonstrate selective bactericidal activity and their influence on bacterial strains is complex [41–43]. Nakatsuji et al. found that lauric acid shows a stronger bactericidal activity against *S. aureus* than that against *S. epidermidis* [44]. Moreover, Hsuan et al. reported that oleic acid preferentially kills *S. aureus* and suggested that gram-positive bacteria are more susceptible to fatty acids than are gram-negative bacteria [45]. Furthermore, it was shown that the antimicrobial activity of fatty acids is strongly dependent on pH and concentration. Thus, less concentrated fatty acids, such as oleic acid, can promote growth, whereas high concentrations have antimicrobial effects [43]. We assumed that the concentrations used in our experiments were above the MIC values (minimum inhibitory concentration) for *C. minutissimum*, causing growth inhibition of this species. In contrast, growth was observed for oleic acid esters: ethyl oleate, oleyl oleate, decyl oleate, glycerol oleate, and polyglyceryl-3 oleate. In this case, oleic acid was bound as an ester and the ester bond must first be cleaved by a lipase to release it. Therefore, the concentration of free oleic acid is considerably lower and can be used by bacteria for growth promotion.

The growth of all bacterial strains observed in the presence of sucrose stearate is attributed to the sucrose moiety, which can be used as an additional carbon source.

In accordance with the results obtained with pure substances, no growth of tested bacterial strains was observed on complex W/O and O/W formulations.

5. Conclusions

Our results show that the use of cosmetic products strongly influences natural skin microbiota. However, this topic is very complex. We tested ingredients that are used in large amounts (above 2%) in formulations in connection with a few microorganisms. On skin, thousands of different species interact together, and therefore a different environment is present compared to that observed in an isolated culture. Therefore, the utilization of cosmetic substances on the skin might be different to the utilization under laboratory conditions. Nevertheless, we could identify groups of substances, which can be problematic in this respect. Specifically, these include free acids, free acid esters, as well fatty alcohols with fatty chain lengths above 12 carbon atoms. In contrast, no interactions were observed in the presence of fatty alcohol ethers, secondary fatty alcohols, paraffin and silicon based substances, polymers, polyethylene glycols, quaternary ammonium salts, hydroxy fatty acid esters, as well fatty acids and fatty acid esters with fatty chain lengths shorter than 12 carbon atoms. The growth influence was observed mostly for *Malassezia* spp., whereas only a few substances showed an influence on the growth of the tested bacteria. This understanding is especially important for people with dermal diseases such as seborrheic dermatitis or acne vulgaris. Although the pathophysiology of these diseases is not yet completely understood, a correlation with the proliferation of the *Malassezia* species was verified [3,4]. Based on our data, suitable skin care products for this group of patients can be designed.

Supplementary Materials: The following materials are available online at <http://www.mdpi.com/2079-9284/6/3/45/s1>, Table S1: Growth of *Malassezia* spp. on cosmetic ingredients.

Author Contributions: Conceptualization, D.D. and T.S.; methodology, D.D.; validation, D.D. and T.S.; formal analysis, D.D.; investigation, S.W. and I.S.; data curation, D.D.; writing—original draft preparation, D.D.; writing—review and editing, T.S., M.M., and F.R.; visualization, D.D.; supervision, T.S., M.M., and F.R.; project administration, D.D., T.S., and M.M.

Funding: This research was funded by Hessen State Ministry of Higher Education, Research, and Arts, grant number 510/16-22.

Acknowledgments: We acknowledge Peter Maysers and Petra Maysers for technical support and helpful discussions.

Conflicts of Interest: The authors declare no conflict of interest.

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