

RHAMNOLIPIDS FOR SCALP HEALTH

FIELD OF THE INVENTION

[0001] The present technology, in general, relates to the use of rhamnolipids, for improving and or facilitating the maintenance of a healthy scalp. More particularly, the present technology relates to compositions, and methods, for the treatment and/or prevention of the dandruff cycle by applying compositions comprising a mixture of particular rhamnolipids to a human scalp. In certain embodiments, application of the compositions described herein, in accordance with the methods and regimens described herein, inhibit the growth, colonization, and/or reduces the viability of skin condition causing microorganisms such as the lipophilic yeast *Malassezia*.

BACKGROUND OF THE INVENTION

[0002] The human scalp is characterized by a typically high density of terminal hair growth with numerous sebaceous glands and microorganisms. This environment contributes to the establishment of a specific microenvironment that has implications for the development of skin pathologic conditions, including, for example, seborrheic dermatitis and dandruff, psoriasis, atopic dermatitis, alopecia, and scalp aging.

[0003] Seborrheic dermatitis is a common chronic inflammatory skin disorder that affects the scalp and is characterized by erythema and oily scales (scaly rash). The non-inflammatory version of the disorder affects approximately 50% of the

world's population (with symptoms of a dry and flakey scalp) and is commonly known as dandruff.

[0004] The dandruff cycle starts with an imbalance of intrinsic factors, including: host immunity; skin microbiome; sebaceous gland activity; and/or epidermal barrier damage. One or more of these conditions can create favorable conditions leading to infection by *Malassezia*. *Malassezia* species are lipophilic yeast which account for more than 80% of the fungal population found on the human skin.

[0005] *Malassezia furfur* is the major species responsible for causing dandruff, and associated symptoms and challenges. Other species include *M. dermatis*, *M. equi*, *M. globosa*, *M. japonica*, *M. obtusa*, *M. ovalis* (also known as *Pityrosporum ovale*), *M. pachydermatis*, *M. restricta*, *M. slooffiae*, *M. sympodialis*, or combinations thereof. Once infection is established, seborrheic dermatitis sets in leading to a damaged epidermal barrier, increased sebum secretion, further *Malassezia* colonization (and metabolite secretion), which ultimately results in an amplified inflammatory response. This results in prolonged, sometimes chronic decrease of scalp health and aesthetics. Other conditions such as psoriasis, atopic dermatitis, and accelerated scalp aging have also been associated with damaged skin, oxidative stress, microbial infection, microbiome imbalance, and immunologic response mechanisms.

[0006] Current market solutions involve the use of harsh, and non-select activity chemistries such as coal tar, zinc pyrithione, selenium sulfide, salicylic acid or narrow range antifungal agents which have decreased activity over time. One limitation of these traditional chemistries is that, while such solutions have shown

effectiveness to counter fungal infection and/or descale patches of the scalp by exfoliation for aesthetic purposes, such chemistries fail in the delivery of a holistic, sustainable, natural solution to the dandruff cycle.

[0007] There is therefore an ongoing need for new compositions, and new methods and treatment regimens, that facilitate the maintenance of a healthy scalp. More particularly, there is a need for compositions, and methods, for the treatment and/or prevention of the dandruff cycle, and specifically for inhibiting or preventing the growth, colonization, and/or reducing the viability of the lipophilic yeast *Malassezia*.

[0008] There has also been a recent trend to formulate products with ingredients that are based on renewable raw materials. Such ingredients are considered “green” or “natural”, since they are derived from renewable and/or sustainable sources. As a result, they are more environmentally friendly than ingredients derived from fossil fuels or other non-renewable sources. An ingredient having a high Biorenewable Carbon Index (BCI), such as greater than 80, indicates that the ingredient contains carbons that are derived primarily from plant, animal or marine-based sources.

[0009] Rhamnolipids are interface-active glycolipids produced by various bacterial species and are an example of a “green” ingredient, since they can be prepared by means of fermentation based on renewable raw materials. It would be desirable to provide compositions, and methods of use, that include active ingredients derived from renewable sources, such as rhamnolipids, that can be used to facilitate the maintenance of a healthy scalp. Providing such compositions,

comprising naturally derived rhamnolipids, would satisfy sustainability goals of ensuring sustainable consumption through the use of bio-based antibacterial materials.

[0010] Applicants have determined that particular compositions of rhamnolipid can meet the above objectives while also advancing UN Sustainability Goals (“SDG”). The rhamnolipid compositions of the present technology contribute to better health and well-being by delivering equal or better efficacy in the maintenance of a healthy scalp (e.g., in the prevention and/or treatment of dandruff). The rhamnolipid compositions described herein are advantageously bio-based, renewably sourced actives obtained from a bacterial fermentation process that generates biodegradable waste products that are less impactful on the environment. These benefits further SDG #3 (Good Health and Well-being) and SDG #12 (Responsible Consumption and Production).

SUMMARY OF THE INVENTION

[0011] One aspect of the present technology is directed to compositions and methods for facilitating the maintenance of a healthy scalp. More particularly, the technology described herein relates to compositions, and methods of use, for the treatment and/or prevention of the dandruff cycle. In certain embodiments, application of the compositions described herein, in accordance with the methods and regimens described herein, inhibit the growth, colonization, and/or reduce the viability of skin condition causing microorganisms such as the lipophilic yeast *Malassezia*.

[0012] One further aspect of the present technology regards a natural, holistic alternative to the currently available harsh and non-select chemistries for the prevention and treatment of dandruff. More particularly, the technology described herein regards the use of novel Rhamnolipids compositions to facilitate the maintenance of a healthy scalp microbiome, including inhibiting (or otherwise controlling) the growth, colonization, and/or reducing the viability of the lipophilic yeast *Malassezia*, and progression through the dandruff cycle.

[0013] In one aspect of the present technology, there are provided dandruff treatment compositions comprising: (a) a mixture of rhamnolipid salts; (b) at least one surfactant selected from the group consisting of anionic surfactants, amphoteric surfactants, nonionic surfactants, cationic surfactants, and combinations thereof; and (c) a dermatologically acceptable carrier, wherein, when applied to mammalian skin, the composition exhibits an anti-fungal effect (i.e., inhibits, prevents growth and/or otherwise reduces viability) against *Malassezia* species in the absence of other anti-fungal actives.

[0014] In another aspect, there are provided dandruff treatment compositions comprising: (a) a mixture of Zinc-rhamnolipid salts in an amount of about 0.01% to about 30% by weight, based on the total weight of the composition; and (b) a dermatologically acceptable carrier in an amount to total 100% by weight. When applied to mammalian skin (e.g., to a mammalian scalp), the composition exhibits an anti-fungal effect against *Malassezia* species in the absence of other anti-fungal actives.

[0015] In a still further aspect of the present technology, there are provided dandruff treatment compositions comprising: (a) a mixture of rhamnolipid salts in an amount of about 0.01% to about 30% by weight, based on the total weight of the composition; (b) about 1% to about 5%, about 1% to about 10%, about 1% to about 15%, about 1% to about 20%, about 1% to about 30%, or about 1% to about 40% by weight of the composition of at least one anionic surfactant; (c) about 1% to about 5%, about 1% to about 10%, about 1% to about 15%, about 1% to about 20%, about 1% to about 30%, or about 1% to about 40% by weight of the composition of at least one amphoteric surfactant; and (d) a dermatologically acceptable carrier in an amount to total 100%. When applied to mammalian skin (e.g., to a mammalian scalp), the composition exhibits an anti-fungal effect against *Malassezia* species in the absence of other anti-fungal actives.

[0016] Furthermore, the mixture of rhamnolipid salts comprises mono-rhamnolipids and di-rhamnolipids, for example, in a weight ratio of 10:90 to 90:10, 40:60 to 60:40, or 40:60 to 48:52 mono-rhamnolipids:di-rhamnolipids. The mixture of rhamnolipid salts further comprises, for example, an amount of Rha-C10-C10 mono-rhamnolipid salt of about 5% to about 50%, or about 5% to about 38% by weight, based on the total weight of the mixture of rhamnolipid salts, and an amount of RhaRha-C10-C10 di-rhamnolipid salt of about 30% to about 80%, or about 34% to about 80% by weight, based on the total weight of the mixture of rhamnolipid salts.

[0017] Still further, the mixture of rhamnolipid salts can be present, in the dandruff treatment compositions described herein, in an amount of about 0.01% to

about 30% by weight, about 0.01% to about 20% by weight, about 0.01% to about 10% by weight, about 0.01% to about 5% by weight, about 0.05% to about 30% by weight, about 0.05% to about 20% by weight, about 0.05% to about 10% by weight, about 0.05% to about 5% by weight, about 0.1% to about 30% by weight, about 0.1% to about 20% by weight, about 0.1% to about 10% by weight, about 0.1% to about 5% by weight, about 0.3% to about 20% by weight, about 0.3% to about 10% by weight, about 0.3% to about 5% by weight, about 0.3% to about 3%, about 0.3% to about 2%, or about 0.3% to about 1%, all based on the total weight of the composition.

[0018] A still further aspect of the present technology includes one or more methods for controlling, treating, or preventing dandruff in a subject afflicted with or susceptible to dandruff. These methods comprise, for example, applying to the hair of the subject an effective amount of any one of more of the dandruff treatment compositions described herein.

[0019] Another aspect of the present disclosure includes one or more methods for treating an excess of a *Malassezia* species on mammalian skin, including on a mammalian scalp. These methods include, for example, applying to the mammalian skin an effective amount of any one of more of the dandruff treatment compositions described herein.

[0020] In other aspects, the compositions, and methods disclosed herein positively impact fundamental host factors, including microbiome balance. Likewise, the compositions and methods disclosed herein facilitate the

maintenance of a healthy epidermal layer, as well as facilitate healthy immunological responses, such as facilitating scalp related wound healing.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

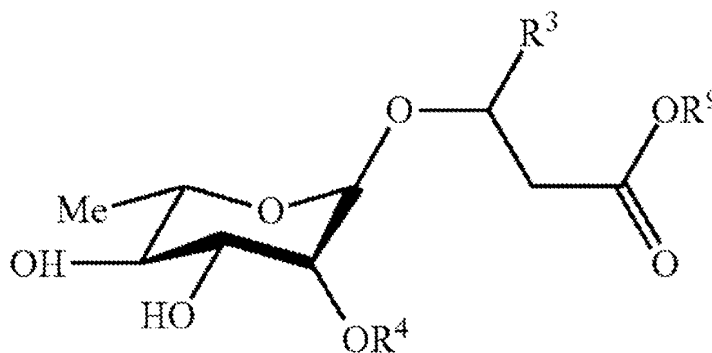
[0021] While the present technology will be described in connection with one or more preferred embodiments, it will be understood by those skilled in the art that the technology is not limited to only those particular embodiments. To the contrary, the presently described technology includes all alternatives, modifications, and equivalents as may be included within the spirit and scope of the appended claims.

[0022] Generally, the present disclosure is directed to compositions and methods for improving and or facilitating the maintenance of a healthy scalp. The methods disclosed herein comprise applying a composition to a mammalian scalp, wherein the composition comprises at least one rhamnolipid, and wherein application of the composition to the mammalian scalp inhibits or mitigates the growth, colonization, and/or viability of a *Malassezia* species, and the progression through the dandruff cycle.

[0023] As defined herein, a "rhamnolipid" is a glycolipid that has a lipid portion that includes one or more, typically linear, saturated or unsaturated B-hydroxy-carboxylic acid moieties and a saccharide portion of one or two units of rhamnose. The term Zn-rhamnolipid or zinc rhamnolipid is a salt of the rhamnolipids described herein, wherein the salt is zinc.

[0024] The saccharide portion and the lipid portion are linked via a B-glycosidic bond between the 1-OH group of a rhamnose moiety of the saccharide portion and the 3-OH group of a B-hydroxy-carboxylic acid of the lipid portion. Thus, the carboxylic acid of one carboxylic acid moiety defines the end of the rhamnolipid. Where more than one rhamnose-moiety is included in a rhamnolipid, each of the rhamnose moieties not linked to the lipid portion is linked to another rhamnose moiety via a 1,4B-glycosidic bond. In embodiments where two or more B-hydroxy-carboxylic acids are present in a rhamnolipid, the B-hydroxy-carboxylic acid moieties are selected independently from each other. B-hydroxy carboxylic acid moieties may in some embodiments be identical. In some embodiments, they are different from each other.

[0025] The present technology generally relates to compositions for the maintenance of a healthy scalp, and to compositions that comprises a particular mixture of rhamnolipids in their salt form. The rhamnolipids may have the following structure (I):

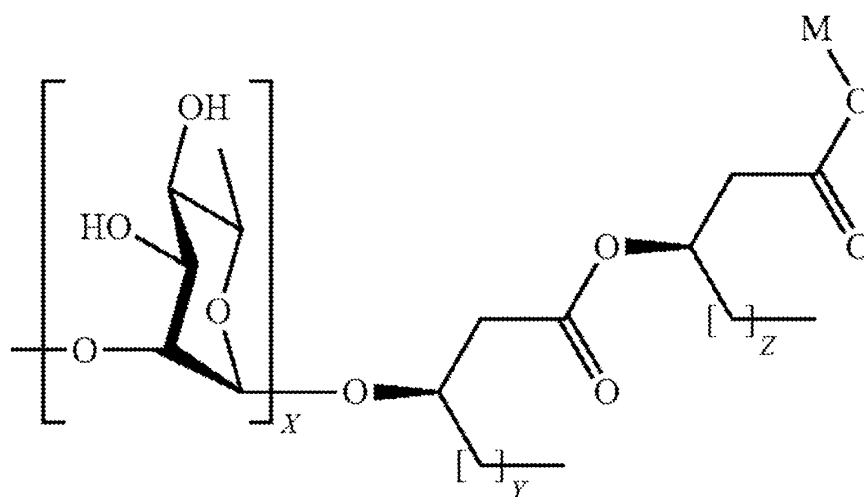


In this formula, R^9 is a hydrogen atom (H) or an aliphatic group that has a main chain of one to about 46, such as one to about 42, one to about 40, one to about 38, one to about 36, one to about 34, one to about 30, one to about 28, including e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms and one to about three, including two, oxygen atoms. In some embodiments, the main chain of the respective aliphatic group carries a terminal carboxylic acid group and/or an internal ester group. As an illustrative example in this regard, R^9 may be of the formula $-\text{CH}(R^5)---\text{CH}_2-\text{COOR}^6$. In these illustrative moieties, R^5 may be an aliphatic moiety with a main chain that has a length from 1 to about 19, such as from 1 to about 17, from 1 to about 15, from 1 to about 13, about 2 to about 13, about 3 to about 13 or about 4 to about 13, including e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 carbon atoms. R^4 in formula (I) is a hydrogen atom (H), or a rhamnopyranosyl moiety. R^6 is a hydrogen atom.

[0026] The term "aliphatic" means, unless otherwise stated, a straight or branched hydrocarbon chain, which may be saturated or mono- or poly-unsaturated and include heteroatoms. The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Herein, an unsaturated aliphatic group contains one or more double bonds (alkenyl moieties). The branches of the hydrocarbon chain may include linear chains as well as non-aromatic cyclic elements. The hydrocarbon chain may, unless otherwise stated, be of any length, and contain any number of branches. Typically, the hydrocarbon (main) chain includes 1 to about 5, to about 10, to about 15 or to about 20 carbon atoms. Examples of alkenyl moieties are straight-chain or branched hydrocarbon moieties

that contain one or more double bonds. Alkenyl moieties generally contain about two to about twenty carbon atoms and one or more, for instance two, double bonds, such as about two to about ten carbon atoms, and one double bond. Examples of alkyl groups are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, the n isomers of these radicals, isopropyl, isobutyl, isopentyl, sec-butyl, tert-butyl, neopentyl, 3,3-dimethylbutyl. Both the main chain as well as the branches may furthermore contain heteroatoms as for instance N, O, S, Se or Si or a carbon atom may be replaced by one of these heteroatoms. An aliphatic moiety may be substituted or unsubstituted with one or more functional groups. Substituents may be any functional group, as for example, but not limited to, amino, amido, carbonyl, carboxyl, hydroxyl, nitro, thio and sulfonyl.

[0027] In a more particular embodiment, the rhamnolipid salts in said structure have the structure (II):



wherein x is 1 or 2, y is 4, 6 or 8, z is 4, 6, or 8, and M is H, or a metal, such as alkali metals Li, Na, or K, alkali earth metals Mg or Ca, or transition metals Mn, Fe,

Cu, or Zn. In the cases of the alkali earth and transition metals, multiple rhamnolipid salt moieties may associate with each metal.

[0028] The mixture of rhamnolipids preferably comprises mono (where $x=1$) and di (where $x=2$) rhamnolipids where y and z are 6 and M is H, Zn, or Na. In some embodiments, the mixture of rhamnolipids is a mixture of zinc-rhamnolipid salts. The mono-rhamnolipid may be referred to as Rha-C10-C10, with a formula of $C_{26}H_{48}O_9$. The IUPAC Name is 3-[3-[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxydecanoyloxy]decanoic acid. The di-rhamnolipid may be referred to as RhaRha-C10-C10, with a formula of $C_{32}H_{58}O_{13}$. The IUPAC name is 3-[3-[4, 5-dihydroxy-6-methyl-3-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxyoxan-2-yl]oxydecanoyloxy]decanoic acid. In general, the mixture of rhamnolipids disclosed herein comprises various types of mono-rhamnolipids and di-rhamnolipids and the mixture specifically encompasses all possible combinations of mono-rhamnolipids and di-rhamnolipids as disclosed herein. Further, unless otherwise stated, an amount of an individual mono-rhamnolipid or di-rhamnolipid as disclosed herein means that the respective mono-rhamnolipid or di-rhamnolipid can be present in the mixture of rhamnolipids in the indicated amount, and the mixture of rhamnolipids disclosed herein specifically includes all possible combinations of amounts of mono-rhamnolipids and di-rhamnolipids as disclosed herein. Generally preferred mixtures of rhamnolipids are SEP-RM rhamnolipid compositions as described below.

[0029] Rha-C10-C10 may be present in the mixture in an amount of about 5% to about 50%, about 5% to about 38%, alternatively about 10% to about 38%,

alternatively about 15% to about 37.5%, alternatively about 20% to about 37.5%, alternatively about 20% to about 50%, alternatively about 25% to about 45%, alternatively about 25% to about 37.5%, alternatively about 30% to about 40%, alternatively about 29% to about 40%, alternatively about 35% to about 38% by weight based on the total weight of rhamnolipids. RhaRha-C10-C10 may be present in the mixture in an amount of about 20% to about 50% by weight, alternatively about 30% to about 80%; alternatively about 34% to about 80%, alternatively about 35% to about 75%, alternatively about 35% to about 65%, alternatively about 35% to about 60%, alternatively about 35% to about 55%, alternatively about 35% to about 50%, alternatively about 35% to about 45%, alternatively about 30% to about 40%, alternatively about 35% to about 38%, alternatively about 36% to about 40% by weight, based on the total weight of rhamnolipids.

[0030] In addition to Rha-C10-C10 and RhaRha-C10-C10, the mixture of rhamnolipids may comprise RhaRha-C10-C12 in an amount of about 5% to about 15%, alternatively about 10% to about 15%, alternatively about 8% to about 14%, alternatively about 9% to about 13%, alternatively about 10% to about 12% by weight based on the total weight of rhamnolipids, and Rha-C10-C12 in an amount of about 0.2% to about 6% by weight, alternatively about 1% to about 5%, alternatively about 2% to about 5%, alternatively about 3% to about 5%, alternatively about 4% to about 5% by weight based on the total weight of rhamnolipids. The mixture of rhamnolipids may also comprise RhaRha-C10-C12:1 in an amount of about 0.2% to about 5% by weight, alternatively about 1% to about

4% by weight, alternatively about 3% to about 5%, based on the total weight of rhamnolipids, an amount of RhaRha-C8-C10 in the range of about 0.2% to about 5% by weight, alternatively about 1% to about 5%, alternatively about 1% to about 4%, alternatively about 2% to about 5%, alternatively about 3% to about 4% by weight, based on the total weight of rhamnolipids, and an amount of Rha-C8-C10 in the range of about 0.2% to about 5% by weight, alternatively about 1% to about 5%, alternatively about 1% to about 4% by weight, alternatively about 2% to about 5%, alternatively about 3% to about 4% by weight, based on the total weight of rhamnolipids. The mixture of rhamnolipids may also comprise RhaRha-C12-12 in an amount of about 0.1% to about 0.5% by weight, alternatively about 0.2% to about 0.4% by weight, alternatively about 0.2% to about 0.3% by weight, based on the total weight of rhamnolipids.

[0031] The mixture of rhamnolipids may comprise a mixture of mono-rhamnolipids and di-rhamnolipids. The mono-rhamnolipids may be present in an amount of about 10% to about 90% by weight, alternatively about 10% to about 80%, alternatively about 10% to about 70%, alternatively about 10% to about 60%, alternatively about 10% to about 50%, alternatively about 15% to about 50%, alternatively about 20% to about 60%, alternatively about 20% to about 50%, alternatively about 30% to about 50%, alternatively about 40% to about 50%, alternatively about 45% to about 50%, alternatively about 44% to about 50% by weight, alternatively about 45% to about 48%, alternatively about 42% to about 48% by weight, based on the total weight of rhamnolipids in the mixture. The di-rhamnolipids may be present in an amount of about 10% to about 90% by weight,

alternatively about 20% to about 70%, alternatively about 30% to about 60%, alternatively about 40% to about 60%, alternatively about 45% to about 55%, alternatively about 50% to about 85%, alternatively about 50% to about 80%, alternatively about 50% to about 70%, alternatively about 50% to about 60%, alternatively about 52% to about 58% by weight, based on the total weight of rhamnolipids. The weight ratio of mono-rhamnolipids:di-rhamnolipids can be from about 10:90 to about 90:10, alternatively about 15:85 to about 85:15, alternatively about 20:80 to about 80:20, alternatively about 25:75 to about 75:25, alternatively about 30:70 to about 70:30, alternatively about 35:65 to about 65:35, alternatively about 40:60 to about 60:40, alternatively about 40:60 to about 45:55, alternatively 45:55 to 55:45, alternatively 47:53 to 53:47, alternatively about 40:60 to about 50:50, alternatively about 40:60 to about 48:52.

[0032] The terms “active”, “% active”, and “% active weight” refer to the amount of the active ingredient without regard to the amount of water or other solvent that may be present with the ingredient.

[0033] As used herein, “effective amount” refers to an amount of an active ingredient or composition that, when administered to mammalian skin (e.g. a mammalian scalp), is capable of accelerating or otherwise facilitating the prevention and or treatment of dandruff. This includes inhibiting or mitigating the growth, colonization, and/or reducing the viability of a *Malassezia* species, and the progression through the dandruff cycle.

[0034] The mono-rhamnolipid may comprise one or more mono-rhamnolipid-mono-lipidic congeners, including for example: Rha-C8-:2; Rha-C8; Rha-C10; Rha-

C12:2; Rha-C12; Rha-C14:2; or combinations thereof. The mono-rhamnolipid may also comprise one or more mono-rhamnolipid-di-lipidic congeners, including for example: Rha-C8-C8; Rha-C8-C10:1; Rha-C10:1-C8; Rha-C8-C10; Rha-C10-C8; Rha-C10-C10:1; Rha-C10-C10; Rha-C8-C12; Rha-C12-C8; Rha-C10-C12:1; Rha-C12:1-C10; Rha-C10-12; Rha-C12-C10; Rha-C10-C14:1; Rha-C12-C12:1; Rha-C10-C14; Rha-C12-C12; Rha-C12-C14; Rha-C14-C14; Rha-C14-C16; Rha-C16-C16; Rha-C10-C10-CH₃; Decenoyl-Rha-C10-C10; or combinations thereof.

[0035] The di-rhamnolipid may comprise one or more di-rhamnolipid-mono-lipidic congeners, including for example: Rha-Rha-C8; Rha-Rha-C10; Rha-Rha-C12:1; Rha-Rha-C12; Rha-Rha-C14; or combinations thereof. The di-rhamnolipid may also comprise one or more di-rhamnolipid-di-lipidic congeners, including for example: Rha-Rha-C8-C8; Rha-Rha-C8-C10; Rha-Rha-C10-C8; Rha-Rha-C10-C10:1; Rha-Rha-C10-C10; Rha-Rha-C8-C12:1; Rha-Rha-C12:1-C8; Rha-Rha-C10-C12:1; Rha-Rha-C12:1-C10; Rha-Rha-C10-C12; Rha-Rha-C12-C10; Rha-Rha-C10-C14:1; Rha-Rha-C12-C12:1; Rha-Rha-C12:1-C12; Rha-Rha-C12-C12; Rha-Rha-C12-C14; Rha-Rha-C14-C12; Rha-Rha-C14-C14; Rha-Rha-C14-C16; Rha-Rha-C16-C14; Rha-Rha-C16-C16; Rha-Rha-C14-C14-C14; Rha-Rha-C10-C10-CH₃; Decenoyl-Rha-Rha-C10-C10; or combinations thereof.

[0036] The rhamnolipids may be produced from a rhamnolipid-producing microorganism that has the capacity to synthesize/produce rhamnolipids under suitable conditions. Such microorganisms include, but are not limited to, bacteria, particularly bacteria of the phyla Pseudomonadota, Actinobacteria, Firmicutes, and Proteobacteria. The rhamnolipids are naturally derived and therefore have a BCI

of 100. In a particular embodiment, the rhamnolipid-producing microorganism for producing the rhamnolipids is *Pseudomonas aeruginosa*. Methods of culturing the rhamnolipid-producing bacteria and the production of rhamnolipids from fermentation are known in the art from, for example U.S. Patent No 11,142,782 and U.S. Patent No. 10,144,943, incorporated herein by reference in their entirety. Methods of purifying the rhamnolipids are also known in the art from, for example, U.S. Patent No 9,884,883 and U.S. Patent No. 10,829,507, incorporated herein by reference in their entirety.

[0037] In addition to the rhamnolipid salts, the dandruff treatment compositions of the present technology may comprise at least one surfactant selected from the group consisting of anionic surfactants, amphoteric surfactants, nonionic surfactants, cationic surfactants, and combinations thereof. In some embodiments, the at least one surfactant is a combination of an anionic surfactant and an amphoteric surfactant. Surfactants that can be used in the dandruff treatment compositions include sulfonated alkyl benzenes, sulfonated methyl esters, sulfonated alpha olefins, paraffin sulfonates, alkyl sulfates, alkyl alkoxy sulfates, alkyl alkoxy carbonates, alkyl sulfonates, alkyl alkoxy sulfonates, acyl lactylates, alkyl isethionates, alkyl sarcosinates, alkyl betaines, alkyl amidopropyl betaines, alkyl sultaines, alkyl amine oxides, and quaternary ammonium compounds. Particular surfactants that can be used in the dandruff treatment compositions include Sodium Laureth Sulfate, Sodium Lauryl Sulfate, Cocamidopropyl Betaine, Sodium C14-16 Olefin Sulfonate, Ammonium Laureth Sulfate, Ammonium Lauryl Sulfate, Sodium Methyl 2-Sulfolaurate, Disodium 2-Sulfolaurate, Cetyl Betaine,

Cocamidopropyl Hydroxysultaine, Sodium Lauroyl Sarcosinate, Glycerol Caprylate/Caprate, behenamidopropyl dimethylamine, brassicamidopropyl dimethylamine, behentrimonium methosulfate, behentrimonium chloride, cetrimonium chloride, stearamidopropyl dimethylamine, salts thereof, and combinations thereof.

[0038] The dandruff treatment compositions can further comprise one or more optional ingredients, such as additives and co-formulants. Examples of additives include stabilizing agents (e.g. trihydroxystearin, ethylene glycol distearate polymers, and mixtures thereof), rheological modifiers (e.g., sodium chloride and sodium xylene sulfonate), emollients, skin conditioning agents, emulsifier/suspending agents, fragrances, pigments, dyes, herbal extracts, vitamins, builders, enzymes, pH adjusters, preservatives (e.g. one or more compound selected from butylene glycol, pentylene glycol, hexylene glycol, 1,2-hexandiol, caprylyl glycol, decylene glycol and glyceryl caprylate), a blowing agent having, for example, one or more volatile materials (e.g., isobutane, propane, and 1,3,3,3-Tetrafluoropropene), and antibacterial agents. Particular examples of additives include, but are not limited to, polyglycerol alkyl esters, glyceryl esters, glycol esters, benzoic acid esters, vitamins, such as Vitamin A, Vitamin E, or pantothenic acid, or combinations of any of the foregoing. In some embodiments, the one or more additives can comprise monoglycerides, diglycerides, or a combination thereof.

[0039] Optional co-formulant ingredients can include a material for improving hair shine and/or reducing hair damage. For example, the dandruff treatment

compositions disclosed herein can be formulated with silicones, siloxanes, natural oils, mineral oils, natural or synthetic waxes, or compounds that remove calcium fatty carboxylate salts from hair, including a calcium chelant (e.g., diethylenetriaminepentaacetic acid (DTPA), etidronic acid, and mixtures thereof).

[0040] In addition, the dandruff treatment compositions disclosed herein can be formulated together with a high melting point fatty compound, including, for example, a fatty alcohol such as cetyl alcohol, stearyl alcohol, behenyl alcohol or brassica alcohol. In still other embodiments, the dandruff treatment compositions disclosed herein can be formulated together with a 2-pyridinol-N-oxide material, including unsubstituted 2-pyridinol-N-oxide, substituted 2-pyridinol-N-oxide and their salts and tautomeric structures. For example, the dandruff treatment compositions disclosed herein can be formulated to together with 1-hydroxy-4-methyl-6-(2,4,4-trimethylpentyl)-2-pyridone monoethanolamine salt.

[0041] The dandruff treatment compositions disclosed herein can also be formulated together with a gel network. Gel networks include lamellar structures (e.g., collection of fine sheets and/or vesicles). Gel networks generally include a lamellar solid crystalline phase, having at least one fatty acid, and at least one secondary component (e.g., at least one secondary surfactant or an additional fatty amphiphile, and water or other suitable solvent).

[0042] In some embodiments, the compositions disclosed herein can also be formulated together with one or more additional anti-fungal or anti-dandruff ingredients. Such ingredients include zinc pyrithione, sulfur, selenium sulfide, coal tar, and soluble anti-dandruff actives, such as one or more compounds selected

from the following groups: azoles (e.g., clotrimazole, ketoconazole, itraconazole, econazole, and luliconazole); hydroxy pyridines (e.g., piroctone olamine, ciclopirox, riloxy, and MEA-Hydroxyoctyloxypyridinone); kerolytic agents (e.g., salicylic acid and other hydroxy acids); strobilurins (e.g., azoxystrobin and metal chelators such as 1,10-phenanthroline, and hinokitiol).

[0043] The dandruff treatment compositions of the present technology also include at least one carrier suitable for personal or dermatological care to bring the total percentage of the composition to 100% by weight. Water is a suitable carrier, and can be deionized water, hard water, soft water, distilled water, tap water or combinations thereof. Water can be used alone as the carrier, or in combination with other carriers suitable for personal care, such as for example, alcohols such as ethanol, isopropanol, or benzyl alcohol; glycols such as propylene glycol, or polyethylene glycol, or combinations thereof.

[0044] The dandruff treatment compositions described herein can have a determined pH range. For example, the dandruff treatment compositions described herein can have a pH in the range of 4.5 to 8.0, 5.0 to 7.5, 5.5 to 7.5, or 6.0 to 7.5.

[0045] The dandruff treatment compositions described herein can be formulated as a solution, aqueous emulsion, gel, paste, or cream.

[0046] In one aspect, the present technology provides an anti-dandruff composition, and methods of use, wherein the composition comprises a mixture of rhamnolipid salts in an amount of 0.01% to 95% by weight, alternatively 0.01% to 30% by weight, alternatively 0.01% to 20% by weight, alternatively 0.01% to 10% by weight, based on the total weight of the composition, wherein the mixture of

rhamnolipid salts comprises mono-rhamnolipids and di-rhamnolipids in a weight ratio of 10:90 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 15:85 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 20:80 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 40:60 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 80:20 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 70:30 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 60:40 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 50:50 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 45:55 mono-rhamnolipids:di-rhamnolipids, alternatively 40:60 to 60:40 mono-rhamnolipids:di-rhamnolipids, alternatively 40:60 to 50:50 mono-rhamnolipids:di-rhamnolipids, alternatively 40:60 to 45:55 mono-rhamnolipids:di-rhamnolipids.

[0047] In another aspect, the present technology provides an anti-dandruff composition, and methods of use, wherein the composition comprises a mixture of rhamnolipid salts in an amount of about 0.1% to about 99% by weight, based on the total weight of the composition (alternatively about 0.05% to about 99% by weight, based on the total weight of the composition), wherein the mixture of rhamnolipid salts comprises mono-rhamnolipids and di-rhamnolipids in a weight ratio of about 10:90 to about 47:53, alternatively about 20:80 to about 47:53, alternatively about 30:70 to about 47:53, alternatively about 40:60 to about 47:53, alternatively about 40:60 to about 45:55, alternatively from 43:57 to 45:55, an amount of Rha-C10-C10 mono-rhamnolipid salt of about 5% to about 50% by weight, alternatively about 5% to about 38% by weight, based on the total weight of

the rhamnolipids in the mixture of rhamnolipids, and an amount of RhaRha-C10-C10 di-rhamnolipid salt of about 30% to about 80% by weight, alternatively about 34% to about 80% by weight, based on the total weight of the rhamnolipids in the mixture of rhamnolipids.

[0048] A further embodiment of the present technology provides a method for controlling, treating, or preventing dandruff in a subject afflicted with or susceptible to dandruff, or a method for treating an excess of *Malassezia* species on mammalian skin, the method comprising administering to the mammalian skin an effective amount of a composition comprising a mixture of rhamnolipid salts, wherein the mixture of rhamnolipid salts comprises mono-rhamnolipids and di-rhamnolipids in a weight ratio of 10:90 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 15:85 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 20:80 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 40:60 to 90:10 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 80:20 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 70:30 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 60:40 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 50:50 mono-rhamnolipids:di-rhamnolipids, alternatively 30:70 to 45:55 mono-rhamnolipids:di-rhamnolipids, alternatively 40:60 to 60:40 mono-rhamnolipids:di-rhamnolipids, alternatively 40:60 to 50:50 mono-rhamnolipids:di-rhamnolipids, alternatively 40:60 to 45:55 mono-rhamnolipids:di-rhamnolipids. The composition exhibits an anti-fungal effect against *Malassezia* species. In certain aspects, the *Malassezia* species is *M. furfur*. Other *Malassezia* species include, for example, *M. dermatis*,

M. equi, *M. restricta*, *M. globose*, *M. ovalis* (also known as *Pityrosporum ovale*), *M. pachydermatis*, *M. restricta*, *M. sympodialis*, *M. obtusa*, *M. slooffiae*, or combinations thereof.

[0049] In some embodiments, the present technology provides a method for treating and/or preventing dandruff, or an excess of *Malassezia* species on mammalian skin, as described above, in which the mixture of rhamnolipids comprises mono-rhamnolipids and di-rhamnolipids in a weight ratio of 40:60 to 45:55, alternatively 40:60 to 48:52, an amount of Rha-C10-C10 mono-rhamnolipid of about 20% to 40% by weight, and an amount of RhaRha-C10-C10 di-rhamnolipid of about 30% to 50% by weight, based on the total weight of the rhamnolipids in the mixture of rhamnolipids.

[0050] The rhamnolipid compositions disclosed herein are associated with certain biological properties. One such property is the capacity to reduce the growth of *Malassezia furfur*. In some embodiments, the rhamnolipid compositions disclosed herein display a characteristic percent reduction of *Malassezia furfur* colony forming units (CFU) per agar plate (relative to a control lacking rhamnolipid and performed as set forth in Example 2 - Embedded Solid Agar-Based Antifungal Efficacy Method below). For example, the rhamnolipid compositions disclosed herein may display a percent reduction of *Malassezia furfur* biological activity (i.e., growth), determined in accordance with the method set forth in Example 2 of between about 25% to about 99%, between about 50% to about 99%, between about 75% to about 99%, between about 25% to about 75%, between about 25% to about 50%, or between about 25% to about 40%.

[0051] In other embodiments, the rhamnolipid compositions disclosed herein display a characteristic percent reduction of *Malassezia furfur* colony forming units (CFU) per mL (relative to a control lacking rhamnolipid and performed as set forth in Example 3 - 96 Well Plate Liquid Method below). For example, the rhamnolipid compositions disclosed herein display a percent reduction of *Malassezia furfur* biological activity (i.e., growth), determined in accordance with the method set forth in Example 3 of between about 40% to about 70%, between about 40% to about 65%, between about 50% to about 70%, or between about 55% to about 65%, when the initial *Malassezia furfur* burden (i.e., microbial challenge) is about 5E+05 (CFU / mL). The rhamnolipid compositions disclosed herein may also display a percent reduction of *Malassezia furfur* biological activity (i.e., growth), determined in accordance with the method set forth in Example 3 of between about 40% to about 80%, between about 40% to about 75%, between about 50% to about 80%, between about 60% to about 80%, or between about 65% to about 75%, when the initial *Malassezia furfur* burden (i.e., microbial challenge) is about 5E+04 (CFU / mL), and/or between about 50% to about 90%, between about 50% to about 85%, between about 60% to about 90%, between about 70% to about 90%, or between about 70% to about 85%, when the initial *Malassezia furfur* burden (i.e., microbial challenge) is about 5E+03 (CFU / mL). The rhamnolipid compositions disclosed herein may also display a percent reduction of *Malassezia furfur* biological activity (i.e., growth), determined in accordance with the method set forth in Example 3 of between about 50% to about 95%, between about 70% to about 95%, between about 80% to about 95%, or between about 85% to about 95%, when the initial

Malassezia furfur burden (i.e., microbial challenge) is about $6E+02$ (CFU / mL) and/or between about 50% to about 97%, between about 70% to about 97%, between about 80% to about 97%, between about 85% to about 97%, or between about 90% to about 97%, when the initial *Malassezia furfur* burden (i.e., microbial challenge) is about $3E+02$ (CFU / mL).

[0052] In some embodiments, the rhamnolipid compositions disclosed herein, when formulated together with anionic, cationic, amphoteric or nonionic co-formulants, also display certain percent reductions of *Malassezia furfur* biological activity (i.e., growth). For example, the rhamnolipid compositions disclosed herein, formulated together with at least one surfactant selected from the group consisting of anionic surfactants, amphoteric surfactants, nonionic surfactants, cationic surfactants, and combinations thereof, have a characteristic percent reduction of *Malassezia furfur* biological activity (i.e., growth), determined in accordance with the method set forth in Example 4 (Select Formulations / Tested using 96 Well Plate Liquid Method), of between about 70% to about 100%, between about 80% to about 100%, between about 90% to about 100%, or between about 95% to about 100%, when the initial *Malassezia furfur* burden (i.e., microbial challenge) is about $2.5E+05$ (CFU / mL).

ITEMS OF THE INVENTION

[0053] The invention further relates to the following items:

[0054] 1. A dandruff treatment composition comprising a mixture rhamnolipid salts in an amount of about 0.01% to about 30% by active weight, based on total weight of the composition, wherein the mixture of rhamnolipid salts comprises

mono-rhamnolipids and di-rhamnolipids having a mono-rhamnolipids:di-rhamnolipids weight ratio of about 40:60 to about 60:40. The rhamnolipid salts may be zinc-rhamnolipids. The rhamnolipid composition also comprises, based on the total weight of rhamnolipids present in the composition, an amount of C10-C10 mono-rhamnolipid of about 29% to about 40% by weight, an amount of C10-C10 di-rhamnolipid of about 35% to about 50% by weight, an amount of C8-C10 mono-rhamnolipid of about 0.2% to about 5% by weight; an amount of C8-C10 di-rhamnolipid of about 0.2% to about 5% by weight; an amount of C10-C12 mono-rhamnolipid of about 2% to about 5% by weight; and an amount of C10-C12 di-rhamnolipid of about 8% to about 14% by weight. The composition also further comprises at least one surfactant selected from the group consisting of anionic surfactants, amphoteric surfactants, nonionic surfactants, cationic surfactants, and combinations thereof, and at least one dermatologically acceptable carrier in an amount to total 100% by weight of the composition.

[0055] 2. The composition of the previous item wherein the at least one surfactant is one or more of Sodium Laureth Sulfate, Sodium Lauryl Sulfate, Cocamidopropyl Betaine, Sodium C14-16 Olefin Sulfonate, Ammonium Laureth Sulfate, Ammonium Lauryl Sulfate, Sodium Methyl 2-Sulfolaurate, Disodium 2-Sulfolaurate, Cetyl Betaine, Cocamidopropyl Hydroxysultaine, Sodium Lauroyl Sarcosinate, Glyceryl Caprylate/Caprates, behenamidopropyl dimethylamine, brassicamidopropyl dimethylamine, behentrimonium methosulfate, behentrimonium chloride, cetrimonium chloride, or stearamidopropyl dimethylamine, such as a combination of Sodium Laureth Sulfate, Sodium Lauryl

Sulfate, and Cocamidopropyl Betaine, wherein the amount of Sodium Laureth Sulfate is about 1% to about 2% by weight, the amount of Sodium Lauryl Sulfate is about 1% to about 2% by weight, and the amount of Cocamidopropyl Betaine is about 0.5% to about 2% by weight, based on the weight of the composition; or a combination of Sodium Lauryl Sulfate and Cocamidopropyl Betaine, wherein the amount of Sodium Lauryl Sulfate is about 2% to about 4% by weight and the amount of Cocamidopropyl Betaine is about 0.5% to about 2% by weight, based on the weight of the composition; or a combination of Sodium C14-16 Olefin Sulfonate and Cocamidopropyl Betaine, wherein the amount of Sodium C14-16 Olefin Sulfonate is about 2% to about 4% by weight and the amount of Cocamidopropyl Betaine is about 0.5% to about 2% by weight, based on the weight of the composition; or a combination of Ammonium Laureth Sulfate, Ammonium Lauryl Sulfate, and Cocamidopropyl Betaine, wherein the amount of Ammonium Laureth Sulfate is about 1% to about 3% by weight, the amount of Ammonium Lauryl Sulfate is about 1% to about 3% by weight, and the amount of Cocamidopropyl Betaine is about 0.5% to about 2% by weight, based on the weight of the composition; or a combination of Sodium Methyl 2-Sulfolaurate, Disodium 2-Sulfolaurate, Cetyl Betaine, and Cocamidopropyl Hydroxysultaine, wherein the amount of Sodium Methyl 2-Sulfolaurate and Disodium 2-Sulfolaurate together is about 1% to about 2% by weight, the amount of Cetyl Betaine is about 1% to about 2% by weight, and the amount of Cocamidopropyl Hydroxysultaine is about 0.5% to about 3%, or 1% to about 2% by weight, based on the weight of the composition. The one or more surfactants may be Sodium Methyl 2-Sulfolaurate and Disodium 2-Sulfolaurate in a

combined amount of about 1% to about 5% by weight, based on the weight of the composition; or Sodium Lauroyl Sarcosinate in an amount of about 1% to about 5% by weight, based on the weight of the composition; or Glyceryl Carprylate/Caprates in an amount of about 0.1% to about 1.0% by weight, based on the weight of the composition; or Sodium Lauryl Sulfate in an amount of about 2% to about 5% by weight, based on the weight of the composition; or Sodium C14-16 Olefin Sulfonate in an amount of from about 2% to about 5% by weight, based on the weight of the composition.

EXAMPLES

[0056] The presently described technology and its advantages will be better understood by reference to the following examples. These examples are provided to describe specific embodiments of the present technology. By providing these examples, the inventors do not limit the scope and spirit of the present technology.

Example 1 – Preparation of Rhamnolipid Composition

[0057] After fermentation, whole broth containing the rhamnolipids undergoes multiple purification steps. The specific combination of steps depends on the rhamnolipid actives and purity requirements of the target application. Certain rhamnolipid mixtures used herein are purified by a solvent extraction process. As used herein, the acronym SEP-RM refers to Solvent Extraction Purified - Rhamnolipid Mixture (or composition).

[0058] For example, the SEP-RM Rhamnolipid composition used herein is prepared in accordance with at least the following processing steps: (1)

fermentation of appropriate Rhamnolipid producing bacterium; (2) biomass separation; (3) sterilization; (4) clarification (e.g. filtration); (5) acidulation; (6) bleaching; (7) washing; (8) solvent extraction; (9) neutralization and dilution; and (10) final polishing step(s). As discussed below, the solvent extraction process/step is intended to yield a higher purity rhamnolipid mixture/composition for personal care applications, such as those described herein. The additional solvent extraction steps are necessary to achieve, for example, a lighter color and milder odor profile required for the personal care market.

[0059] At the end of fermentation, the whole broth typically contains rhamnolipid along with biomass and other by-products of fermentation. To separate the biomass solids, the broth can be centrifuged. The resulting centrifuged broth is then subjected to sterilization (e.g., high temperature sterilization), after which, the centrifuged sterilized broth is clarified by filtration to remove suspended solids.

[0060] Further purification is achieved by treating the clarified broth with acid, which converts the rhamnolipid to a water-insoluble form that settles to the bottom and separates from bulk aqueous phase. This dense acidulated rhamnolipid is then isolated (and referred to as Acidulated, Concentrated Clarified Broth (ACCB)). ACCB is then treated with bleaching agent, then water washed to remove residual bleaching agent and other water-soluble impurities. At this point, the decolorized washed ACCB is concentrated (e.g., $\geq$ about 45% actives, or between about 35% to about 55% actives) and purer (e.g., $\geq$ about 75% purity, or between about 65% to about 85% purity).

[0061] Solvent Extraction Process - To achieve better purity, color, and odor, the decolorized Acidulated, Concentrated Clarified Broth (ACCB) undergoes a solvent extraction process. This is generally performed by dissolving the decolorized, washed ACCB in organic solvent, preferably ethyl acetate. The rag layer that typically forms is separated from the bulk solution. Activated carbon is then added to the rhamnolipid solution in ethyl acetate for further decolorization and deodorization. The slurry is filtered, and the resulting solution is stripped under vacuum to remove ethyl acetate. The highly concentrated ACCB obtained as residue is then neutralized and diluted (e.g., from between about 20% to about 30% actives, preferably about 25% actives) to give crude SEP-RM. As a final polishing step, crude SEP-RM is then washed with ethyl acetate to extract the antifoam and yield the final product as optically clear SEP-RM at about 25% active and about 85% purity.

[0062] Zinc salt rhamnolipids were prepared by adding an equimolar amount of ZnCl_2 to rhamnolipid actives (such as SEP-RM) in an aqueous solution and mixing at ambient temperature for about 20 minutes. The solution was then centrifuged and separated into layers. The top layer was decanted, and the bottom layer was washed with ~20g DI water & centrifuged. The washing procedure was repeated and the bottom layer was collected and dried in a 50C vacuum oven to obtain the Zn-rhamnolipid salt.

Mono-Rhamnolipid:Di-Rhamnolipid Ratio and Congener Distribution

[0063] The mono-rhamnolipid:di-rhamnolipid ratio, and congener distribution for SEP-RM rhamnolipid compositions of the present disclosure can be determined

using HPLC (High Performance Liquid Chromatography). As understood by the person of ordinary skill in the art, HPLC will separate rhamnolipid congeners by chain length and number of rhamnose moieties (with the more polar congeners eluting first). Fractions are collected and analyzed by mass spec to identify each peak. The congener distribution is reported as area percent (with actives being calculated relative to known standards).

[0064] The HPLC congener distribution of a standard SEP-RM rhamnolipid composition of the present disclosure, is presented below:

SEP-RM (Solvent Extraction Purified – Rhamnolipid Mixture)	
Congener	HPLC Relative Area %
Di (C8C10)	3.21
Mono (C8C10)	3.29
Di (C10C10)	37.10
Mono (C10C10)	36.71
Di(C10:C12:1)	3.82
Di (C10C12)	11.18
Mono (C10C12)	4.41
Di(C12:C12)	0.28
Total Mono-Rhamnolipid wt%	44.41
Total Di-rhamnolipid wt%	55.59

[0065] Mono-rhamnolipids may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in an amount of about 35% to about 55%,

alternatively about 38% to about 52%, alternatively about 40% to about 50%, alternatively about 42% to about 48%, alternatively about 42% to about 46%, alternatively about 43% to about 45%, alternatively about 44% by weight based on the total weight of rhamnolipids in the mixture.

[0066] Di-rhamnolipids may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in an amount of about 45% to about 65%, alternatively about 50% to about 60%, alternatively about 52% to about 58%, alternatively about 53% to about 57%, alternatively about 54% to about 56%, alternatively about 55% by weight based on the total weight of rhamnolipids in the mixture.

[0067] The weight ratio of mono-rhamnolipids:di-rhamnolipids, in SEP-RM rhamnolipid compositions of the present disclosure, can be from about 35:65 to about 65:35, alternatively from about 38:62 to about 62:38, alternatively from about 40:60 to about 60:40, alternatively from about 42:58 to about 58:42, alternatively from about 43:57 to about 57:43, alternatively about 41:59 to about 44:56, alternatively about 42:58 to about 44:56, alternatively about 42:58, alternatively about 43:57, alternatively about 44:56, or alternatively about 50:50.

[0068] The mono-rhamnolipid C8-C10 congener may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in the range of about 1% to about 6% by weight, alternatively about 2% to about 5% by weight, alternatively about 3% to about 4% by weight, alternatively about 3% by weight, or alternatively about 4% by weight, based on the total weight of rhamnolipids present in the composition.

[0069] The mono-rhamnolipid C10-C10 congener may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in the range of about 20% to about 50% by weight, alternatively about 25% to about 45% by weight, alternatively about 30% to about 40% by weight, alternatively about 32% to about 38% by weight, alternatively about 34% to about 38% by weight, alternatively about 35% to about 37% by weight, alternatively about 36% to about 37% by weight, alternatively about 36 % by weight, or alternatively about 37 % by weight, based on the total weight of rhamnolipids present in the composition.

[0070] The mono-rhamnolipid C10-C12 congener may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in the range of about 1% to about 8% by weight, alternatively about 2% to about 6% by weight, alternatively about 3% to about 5% by weight, alternatively about 4% to about 5% by weight, alternatively about 4 % by weight, or alternatively about 5% by weight, based on the total weight of rhamnolipids present in the composition.

[0071] The di-rhamnolipid C8-C10 congener may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in the range of about 1% to about 6% by weight, alternatively about 2% to about 5% by weight, alternatively about 3% to about 4% by weight, alternatively about 3% by weight, or alternatively about 4% by weight, based on the total weight of rhamnolipids present in the composition.

[0072] The di-rhamnolipid C10-C10 congener may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in the range of about 20% to about 50% by weight, alternatively about 25% to about 45% by weight, alternatively

about 30% to about 40% by weight, alternatively about 32% to about 38% by weight, alternatively about 34% to about 38% by weight, alternatively about 35% to about 38% by weight, alternatively about 36% to about 38% by weight, alternatively about 37% by weight, or alternatively about 38% by weight, based on the total weight of rhamnolipids present in the composition.

[0073] The di-rhamnolipid C10-C12.1 congener may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in the range of about 1% to about 7% by weight, alternatively about 2% to about 6% by weight, alternatively about 3% to about 5% by weight, alternatively about 3% to about 4% by weight, alternatively about 3% by weight, or alternatively about 4% by weight, based on the total weight of rhamnolipids present in the composition.

[0074] The di-rhamnolipid C10-C12 congener may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in the range of about 8% to about 14% by weight, alternatively about 9% to about 13% by weight, alternatively about 10% to about 12% by weight, alternatively about 11% to about 12% by weight, alternatively about 11%, or alternatively about 12% by weight, based on the total weight of rhamnolipids present in the composition.

[0075] The di-rhamnolipid C12-C12 congener may be present, in SEP-RM rhamnolipid compositions of the present disclosure, in the range of about 0.1% to about 0.5% by weight, alternatively about 0.2% to about 0.4% by weight, alternatively about 0.2% to about 0.3% by weight, alternatively about 0.25% to about 0.3% by weight, alternatively about 0.2%, alternatively about 0.25%, or

alternatively about 0.25% by weight, based on the total weight of rhamnolipids present in the composition.

[0076] The SEP-RM rhamnolipid composition provided herein can be processed either with, or without a bleaching step (occurring after acidulation, and before the solvent extraction step(s)). For the SEP-RM rhamnolipid compositions of the present disclosure, the mono-rhamnolipid:di-rhamnolipid ratio, and the associated congener distribution, are unaffected by whether a bleaching step is included, or not included, in the purification process.

Example 2 - Embedded Solid Agar-Based Antifungal Efficacy Method

[0077] In order to evaluate the efficacy of Rhamnolipid compositions disclosed herein against *Malassezia furfur*, an embedded solid agar-based method was employed. According to this method, test samples, are diluted into a full-strength nutrient agar-based matrix, and dispensed in Petri dishes at 20mL per dish. The test species *Malassezia furfur* ATCC 14521 (i.e., *M.furfur*) is delivered at different levels to each dish, using fully propagated and adjusted cultures. The nutrient agar-based matrix / *Malassezia furfur* mixture is kept at $45\pm 2^{\circ}\text{C}$, thoroughly mixed, and allowed to solidify by cooling down to $20\text{--}25^{\circ}\text{C}$. The plates are then incubated in $30\pm 2^{\circ}\text{C}$ for up to 7 days until mature growth develops on controls. The absence of growth when compared to controls is characteristic of antifungal activity.

[0078] The nutrient agar-based matrix employed included: Potato Dextrose Agar (PDA) (BD 213400, general media for fungal species); and Modified Dixon Agar (MD) (ATCC Medium 2693, consisting of protein, carbon, oil, and acids preferred by *Malassezia*). Test samples employed included Rhamnolipid compositions,

prepared using solvent extraction as described above (e.g., SEP-RM / Lot A, having 26.9% active, 47.2% Mono-Rhamnolipid (RL) and 52.8% Di-RL; and SEP-RM / Lot B having 25.7% active, 47.6% Mono-RL and 52.4% Di-RL).

Test results are as presented below:

Percent Reduction of <i>M. furfur</i>					
Agar Matrix	Tested Final % Active of Rhamnolipid Alone	pH	Inoculum Burden Colony Forming Units (CFU) per plate		
			1340-1595	134-160	13-16
PDA	2.7%	7.0	93%	99%	91%
	0.3%	6.1	<78%*	30%	25%
MD	2.7%	6.7	<81%*	0%	25%
	0.3%	6.0	<81%*	0%	0%

*<LD = below limit of detection. Based on reduced numbers per maximum recovery detection limit of 300 CFU/plate.

Example 3 - 96 Well Plate Liquid Method

[0079] In order to evaluate the efficacy of Rhamnolipid compositions disclosed herein against *Malassezia furfur*, a 96 well plate liquid culture method was employed. In this method samples are diluted into a reduced-strength nutrient broth-based liquid and dispensed in plate wells up to 300uL at a time. *Malassezia furfur* is delivered, at different levels, to each set of wells using fully propagated and adjusted culture. Resazurin, a blue fluorogenic dye used as a redox indicator in cell viability and proliferation assays, is added to each sample and control wells. The mixture is kept at 20-25°C and thoroughly mixed. The plates are then incubated at 30±2°C for up to 3 days, until mature biological activity is detected from the controls first visually (resazurin changes color when metabolically processed) and then

quantitatively (by use of fluorescent enabled plate reader). Tests were conducted using Modified Dixon (MD) at 0.3x strength, which is a scalp relevant and microbially preferred nutrient source matrix.

[0080] Test samples employed included Rhamnolipid compositions, prepared using solvent extraction as described above (SEP-RM / Lot A, having 26.9% active, 47.2% Mono-Rhamnolipid (RL) and 52.8% Di-RL; and SEP-RM / Lot B having 25.7% active, 47.6% Mono-RL and 52.4% Di-RL). Rhamnolipid was added at 1.0% active to each test mixture.

The test results are presented below:

Test Sample	<i>M. Furfur</i> burden (CFU / mL)	Antifungal Activity (Percent Reduction of Biological Activity measured by FL Units of Resazurin)
1.0% Rhamnolipid	5E+05	63.4%
	5E+04	70.5%
	5E+03	84.9%
	3E+03	83.2%
	1E+03	87.6%
	6E+02	95.2%
	3E+02	96.7%

Example 4 – Select Formulations

[0081] The efficacy of Rhamnolipid compositions disclosed herein, against *Malassezia furfur*, was evaluated in the presence of anionic, amphoteric, or nonionic co-formulants. The tests were performed using the 96 well plate liquid

culture method described above, at an active concentration as described below in the specific test samples. Test samples employed included Rhamnolipid compositions, prepared using solvent extraction as described above (SEP-RM / Lot B, having 47.6% Mono-RL and 52.4% Di-RL). As indicated below, rhamnolipid was added at 1.0% active to each test mixture.

[0082] Rhamnolipids (SEP-RM / Lot B, having 25.7% active, 47.6% Mono-RL and 52.4% Di-RL) were admixed in water with a variety of co-formulants, as outlined in the following tables (wherein % (w/w) numbers are based on the total weight of the composition:

Sample	% (w/w)	Component
1	1.7	Sodium Laureth Sulfate (STEOL CS-230 C)
	1.7	Sodium Lauryl Sulfate (STEPANOL WA EXTRA HA)
	1.0	Cocamidopropyl Betaine (AMPHOSOL HCG-HP)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
2	3.3	Sodium Lauryl Sulfate (STEPANOL WA EXTRA HA)
	1.0	Cocamidopropyl Betaine (AMPHOSOL HCG-HP)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
3	3.3	Sodium C14-16 Olefin Sulfonate (BIO-TERGE AS-40 HP)
	1.0	Cocamidopropyl Betaine (AMPHOSOL HCG-HP)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
4	1.7	Ammonium Laureth Sulfate (STEOL CA-330 C)
	1.7	Sodium Lauryl Sulfate (STEPANOL AMV)
	1.0	Cocamidopropyl Betaine (AMPHOSOL HCG-HP)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
6	1.3	Sodium Methyl 2-Sulfolaurate and Disodium 2-Sulfolaurate (ALPHA-STEP PC-48)
	1.5	Cetyl Betaine (AMPHOSOL CDB-HP)
	1.5	Cocamidopropyl Hydroxysultaine (AMPHOSOL CS-50)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
7	1.3	Sodium Methyl 2-Sulfolaurate and Disodium 2-Sulfolaurate (ALPHA-STEP PC-48)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
8	1.7	Sodium Lauroyl Sarcosinate (MAPROSYL 30-B)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
9	0.2	Glyceryl Carprylate/Caprate (STEPAN-MILD GCC)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
10	3.3	Sodium Lauryl Sulfate (STEPANOL WA EXTRA HA)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
11	3.3	Sodium C14-16 Olefin Sulfonate (BIO-TERGE AS-40 HP)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
12	4.3	Sodium Methyl 2-Sulfolaurate and Disodium 2-Sulfolaurate (ALPHA-STEP PC-48)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
13	4.3	Sodium Lauroyl Sarcosinate (MAPROSYL 30-B)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
14	0.5	Glyceryl Carprylate/Caprates (STEPAN-MILD GCC)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
15	4.3	Sodium Lauryl Sulfate (STEPANOL WA EXTRA HA)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
16	4.0	Sodium C14-16 Olefin Sulfonate (BIO-TERGE AS-40 HP)
	1.0	Rhamnolipid SEP-RM / Lot B

Sample	% (w/w)	Component
20	3.3	Sodium C14-16 Olefin Sulfonate (BIO-TERGE AS-40 HP)
	1.0	Cocamidopropyl Betaine (AMPHOSOL HCG-HP)
	1.0	Rhamnolipid SEP-RM / Lot B

[0083] To evaluate efficacy, *Malassezia furfur* was added to the Rhamnolipid formulations described above, in accordance with the 96 well plate liquid culture method described above. The individual samples were diluted into a reduced-strength nutrient broth-based liquid and dispensed in plate wells up to 300uL at a time. *Malassezia furfur* was delivered, at a level of 2.5E+05 cfu/ml, to each set of wells using fully propagated and adjusted culture. Resazurin, a blue fluorogenic dye used as a redox indicator in cell viability and proliferation assays, is added to each sample and control wells. The mixture is kept at 20-25°C and thoroughly mixed. The plates are then incubated at 30±2°C for up to 3 days, until mature biological activity is detected from the controls first visually (resazurin changes color when metabolically processed) and then quantitatively (by use of fluorescent enabled plate reader). The test was conducted using Modified Dixon (MD) at 0.3x strength, which is a scalp relevant and microbially preferred nutrient source matrix.

[0084] As set forth below, Rhamnolipid as prepared above (SEP-RM / Lot B, having 47.6% Mono-RL and 52.4% Di-RL) demonstrated efficacy (47% inhibition (versus 2.5E +05 cfu/ml *M. furfur* challenge) at 1% (w/w, based on the total weight of the composition). The SEP-RM / Lot B Rhamnolipid performed significantly better than the comparative sample (commercially available rhamnolipid product having 4:96 Mono:Di Rhamnolipid ratio), which demonstrated only 19% inhibition (versus

2.5E+05 cfu/ml *M. furfur* challenge). When co-formulants were added to 1% SEP-RM / Lot B, having 47.6% Mono-RL and 52.4% Di-RL) (i.e., Samples described above) performance increased to the maximum detectable of 100% inhibition (versus 2.5E+05 cfu/ml *M. furfur* challenge).

[0085] The test results are presented below:

Category	Sample	Average	StDev
Rhamnolipid Alone	SEP-RM	46.9%	6.0%
	Comparative Sample	18.9%	0.5%
Rhamnolipid (SEP-RM) Formulated Samples	S1	100.0%	0.2%
	S3	100.0%	0.5%
	S4	99.6%	0.4%
	S6	98.3%	1.2%
	S8	100.0%	0.0%
	S9	96.3%	0.4%
	S10	99.2%	1.1%
	S11	98.0%	4.0%
	S12	100.0%	0.0%
	S13	100.0%	0.0%
	S15	100.0%	0.0%
	S16	100.0%	0.0%
	S20	100.0%	0.0%

[0086] National brand anti-dandruff products were also tested in accordance with the protocol set forth above (this Example, and Example 3). As indicated below, the efficacy of the SEP-RM rhamnolipid formulations (set forth above), matches the performance of three dandruff national brand products (0.3x strength) and Quaternary Ammonium Compounds (1839-220) at 0.3x RTU:

Sample	% Active	Average	StDev
Head & Shoulders (H&S) Deep Scalp Cleanser (Zinc P)	0.333	100.0%	0.0%
	0.033	100.0%	0.0%
	0.003	100.0%	0.0%
H&S Supreme N&S (Zinc P)	0.333	100.0%	0.0%
	0.033	99.0%	1.4%
	0.003	100.0%	0.0%
H&S Clinical Strength (Selenium Sulfite)	0.333	100.0%	0.0%
	0.033	77.8%	0.8%
	0.003	51.0%	6.2%
1839-220 (QAC)	0.100	100.0%	0.0%

Example 5 - Zn-Rhamnolipid Activity

[0087] To evaluate the efficacy of Zn-rhamnolipid compositions disclosed herein against *M. furfur*, the 96 well plate liquid culture method described in Example 3 was employed. The test samples included a mixture of rhamnolipids prepared using solvent extraction as described above and converted to Zn-rhamnolipid salts. The mixture of Zn rhamnolipids was added at 1.0% active to either water or a base shampoo composition comprising 10% active weight of sodium lauryl sulfate, 3% active weight cocamidopropyl betaine and 3% active weight sodium laureth sulfate, and water to total 100% by weight. Comparative samples were prepared from a national brand (H&S) dandruff shampoo product containing 1% zinc pyrithione, and a Zn-rhamnolipid sample prepared from a commercially available rhamnolipid composition comprising about 3% mono-rhamnolipids and 97% di-rhamnolipids converted to Zn-rhamnolipid salts as described above. Water alone, base

composition alone, and zinc pyrithione in water acted as controls. The sample dilutions tested and the test results are shown below.

Average Percent Reduction of Biological Activity (at Specified Disease Burden in CFU/mL)								
Sample	Carrier	Tested Zn%	Tested PPM	3.6 x 10 ⁴	1.4 x 10 ⁵	5.7 x 10 ⁵	2.3 x 10 ⁶	9.1 x 10 ⁶
Base Control	Base	0.000 00%	0.00	94%	97%	97%	98%	100%
		0.000 00%	0.00	16%	11%	6%	10%	8%
		0.000 00%	0.00	25%	23%	0%	0%	0%
Zn-Rhamnolipid (Comparative Sample)	Base	0.068 67%	686.6 7	100%	100%	98%	97%	100%
		0.006 87%	68.67	100%	99%	95%	100%	100%
		0.001 37%	13.73	26%	29%	24%	0%	0%
Water	Water	0.000 00%	0.00	5%	0%	0%	0%	0%
		0.000 00%	0.00	27%	14%	0%	0%	0%
		0.000 00%	0.00	21%	16%	0%	0%	0%
Zn-Rhamnolipid (SEP-RM alone)	Base	0.016 67%	166.6 7	100%	100%	100%	100%	100%
		0.001 67%	16.67	100%	95%	63%	15%	0%
		0.000 33%	3.33	4%	16%	17%	0%	0%
Zn-Rhamnolipid (SEP-RM in base)	Base	0.068 67%	686.6 7	91%	94%	94%	93%	88%
		0.006 87%	68.67	100%	100%	95%	81%	26%
		0.001 37%	13.73	80%	25%	21%	0%	0%
Zn-Rhamnolipid (SEP-RM in water)	Water	0.068 67%	686.6 7	88%	92%	91%	84%	56%
		0.006 87%	68.67	100%	100%	98%	84%	59%
		0.001 37%	13.73	46%	25%	29%	44%	57%

H&S 1% ZPT	N/ A	0.068 67%	686.6 7	99%	100%	100%	100%	100%
		0.006 87%	68.67	100%	100%	96%	91%	63%
		0.001 37%	13.73	100%	100%	99%	89%	59%
ZPT in water	Wa ter	0.068 67%	686.6 7	100%	100%	100%	100%	100%
		0.006 87%	68.67	100%	100%	100%	100%	97%
		0.001 37%	13.73	100%	100%	100%	100%	100%

[0088] Example 6 – Zn-Rhamnolipids Combined With Additional Anti-Fungal

[0089] The 96 well plate liquid culture method described in Example 3 was used to evaluate the efficacy of Zn-rhamnolipids in combination with different anti-fungal actives in either water or the base shampoo composition described in Example 5. The anti-fungal actives tested were salicylic acid and zinc pyrithione. The sample active concentrations tested and the test results are shown below.

Sample	Carrier	Tested Zn%	Tested PPM	Sal. Acid %	Average % Reduction of Biological Activity (at Specified Disease Burden in CFU/mL)				
					3.6 x 10 ⁴	1.4 x 10 ⁵	5.7 x 10 ⁵	2.3 x 10 ⁶	9.1 x 10 ⁶
Sal Acid + Zn-Rhamnolipid (SEP-RM) in water	Water	0.016 67%	166.6 7	0.666 7	100%	100%	100%	100%	100%
		0.0016 7%	16.67	0.066 7	100%	100%	92%	100%	100%
		0.0003 3%	3.33	0.013 3	83%	71%	59%	0%	0%
Sal Acid + Zn-Rhamnolipid (SEP-RM) in base	Base	0.016 67%	166.6 7	0.666 7	100%	100%	100%	100%	100%
		0.0016 7%	16.67	0.066 7	100%	100%	100%	100%	100%
		0.0003 3%	3.33	0.013 3	60%	50%	35%	0%	0%

		0.0003 3%	3.33		98%	81%	86%	19%	0%
		0.0000 7%	0.67		49%	35%	35%	30%	0%
ZPT + Zn-Rhamnolipid (SEP-RM) in base	Base	0.00	166.67		100%	100%	100%	100%	98%
		0.00	16.67		100%	100%	99%	89%	59%
		0.00	3.33		100%	100%	100%	82%	79%
ZPT + Zn-Rhamnolipid (SEP-RM) in water	Water	0.00	33.33		100%	100%	100%	98%	86%
		0.00	3.33		97%	98%	99%	98%	100%
		0.00	0.67		100%	100%	100%	96%	100%
ZPT alone in base	Base	0.016 67%	166.67		100%	100%	100%	100%	100%
		0.0016 7%	16.67		95%	98%	93%	68%	72%
		0.0003 3%	3.33		100%	100%	98%	83%	82%
ZPT in base	Base	0.068 67%	686.67		100%	100%	100%	100%	96%
		0.0068 7%	68.67		100%	100%	100%	100%	100%
		0.0013 7%	13.73		100%	98%	100%	100%	100%
ZPT in water	Water	0.068 67%	686.67		100%	100%	100%	100%	100%
		0.0068 7%	68.67		97%	97%	98%	100%	100%
		0.0013 7%	13.73		100%	100%	100%	100%	100%

[0090] The present technology is now described in such full, clear and concise terms as to enable a person skilled in the art to which it pertains, to practice the same. It is to be understood that the foregoing describes preferred embodiments of the present technology and that modifications may be made therein without departing from the spirit or scope of the present technology as set forth in the appended claims. Further, the examples are provided to not be exhaustive but illustrative of several embodiments that fall within the scope of the claims.

Claims

1. A composition for treating dandruff comprising:

- (a) a mixture of rhamnolipid salts in an amount of about 0.01% to about 30% by weight, based on the total weight of the composition, wherein the mixture of rhamnolipid salts comprises mono-rhamnolipids and di-rhamnolipids in a weight ratio of 10:90 to 90:10 mono-rhamnolipids:di-rhamnolipids based on the total weight of the mixture of rhamnolipid salts, an amount of Rha-C10-C10 mono-rhamnolipid salt of about 5% to about 50% by weight based on the total weight of the mixture of rhamnolipid salts, and an amount of RhaRha-C10-C10 di-rhamnolipid salt of about 30% to about 80% by weight based on the total weight of the mixture of rhamnolipid salts;
- (b) at least one surfactant selected from the group consisting of anionic surfactants, amphoteric surfactants, nonionic surfactants, cationic surfactants, and combinations thereof; and
- (c) a dermatologically acceptable carrier in an amount to total 100% by weight,

wherein the composition reduces *Malassezia* species present on mammalian skin in the absence of other anti-fungal actives.

2. A composition for treating dandruff comprising:

- (a) a mixture of zinc-rhamnolipid salts in an amount of about 0.01% to about 30% by weight, based on the total weight of the composition, wherein the mixture of rhamnolipid salts comprises mono-rhamnolipids and di-rhamnolipids in a weight ratio of 10:90 to 90:10 mono-rhamnolipids:di-rhamnolipids based on the total weight of the mixture of rhamnolipid salts, an amount of Rha-C10-C10 mono-rhamnolipid salt of about 5% to about 50% by weight based on the total weight of the mixture of rhamnolipid salts, and an amount of RhaRha-C10-C10

- di-rhamnolipid salt of about 30% to about 80% by weight based on the total weight of the mixture of rhamnolipid salts; and
- (b) a dermatologically acceptable carrier in an amount to total 100% by weight,
- (c) wherein the composition reduces the *Malassezia* species present on mammalian skin.
3. The composition of claim 2, wherein the composition further comprises at least one surfactant selected from the group consisting of anionic surfactants, amphoteric surfactants, nonionic surfactants, cationic surfactants, and combinations thereof.
 4. The composition of any one of claims 1-3, wherein the amount of rhamnolipid salts is about 0.1% to about 10%, 0.1% to about 5%, about 0.3% to about 5%, about 0.3% to about 3%, about 0.3% to about 2%, or about 0.3% to about 1% by weight of the composition.
 5. The composition of claim 1 or 3-4, wherein the at least one surfactant is present in an amount of about 2% to about 40% by weight of the composition.
 6. The composition of any one of claims 1 or 3-5, wherein the at least one surfactant comprises an anionic surfactant in an amount of about 1% to about 40% by weight of the composition.
 7. The composition of any one of claims 1 or 3-6, wherein the at least one surfactant comprises an amphoteric surfactant in an amount of about 1% to about 40% by weight of the composition, about 1% to about 30% by weight of the composition, about 1% to about 20% by weight of the composition, or about 1% to about 10% by weight of the composition.
 8. The composition of any one of claims 1-7, wherein the mixture of rhamnolipid salts comprises mono-rhamnolipids and di-rhamnolipids in a weight ratio of 40:60 to 60:40 mono-rhamnolipids:di-rhamnolipids, 45:55 to 55:45 mono-rhamnolipids:di-rhamnolipids, or 47:53 to 53:47 mono-rhamnolipids:di-rhamnolipids based on the total weight of the mixture of rhamnolipid salts.

9. The composition of any one of claims 1-8, wherein the mixture of rhamnolipid salts comprises from about 20% to about 60% by weight, from about 30% to about 50% by weight, from about 40% to about 50% by weight, or from about 45% to about 48% by weight, of mono-rhamnolipids based on the total weight of the mixture of rhamnolipid salts.
10. The composition of any one of claims 1-9, wherein the mixture of rhamnolipid salts comprises from about 20% to about 70% by weight, from about 30% to about 60% by weight, from about 40% to about 60% by weight, from about 45% to about 55% by weight, or from about 50% to about 55% by weight, of di-rhamnolipids based on the total weight of the mixture of rhamnolipid salts.
11. The composition of any one of claims 1-10, wherein the mixture of rhamnolipid salts comprises an amount of Rha-C10-C10 mono-rhamnolipid salt of about 20% to about 50% by weight, about 25% to about 45% by weight, about 30% to about 40% by weight, or about 35% to about 38% by weight, based on the total weight of the mixture of rhamnolipid salts.
12. The composition of any one of claims 1-11, wherein the mixture of rhamnolipid salts comprises an amount of RhaRha-C10-C10 di-rhamnolipid salt of about 20% to about 50% by weight, about 25% to about 45% by weight, about 30% to about 40% by weight, or about 35% to about 38% by weight, based on the total weight of the mixture of rhamnolipid salts.
13. The composition of any one of claims 1-12, wherein the mixture of rhamnolipid salts comprises an amount of Rha-C8-C10 mono-rhamnolipid salt of about 1% to about 10% by weight, about 1% to about 5% by weight, about 2% to about 5% by weight, or about 3% to about 4% by weight, based on the total weight of the mixture of rhamnolipid salts.
14. The composition of any one of claims 1-13, wherein the mixture of rhamnolipid salts comprises an amount of RhaRha-C8-C10 di-rhamnolipid salt of about 1% to about 10% by weight, about 1% to about 5% by weight, about 2% to about 5% by

weight, or about 3% to about 4% by weight, based on the total weight of the mixture of rhamnolipid salts.

15. The composition of any one of claims 1-14, wherein the mixture of rhamnolipid salts comprises an amount of Rha-C10-C12 mono-rhamnolipid salt of about 1% to about 10% by weight, about 1% to about 5% by weight, about 2% to about 5% by weight, about 3% to about 5% by weight, or about 4% to about 5% by weight, based on the total weight of the mixture of rhamnolipid salts.
16. The composition of any one of claims 1-15, wherein the mixture of rhamnolipid salts comprises an amount of RhaRha-C10-C12 di-rhamnolipid salt of about 1% to about 20% by weight, about 5% to about 15% by weight, about 10% to about 15% by weight, or about 10% to about 12% by weight, based on the total weight of the mixture of rhamnolipid salts.
17. The composition of any one of claims 1-16, wherein the composition comprises one or more additives.
18. The composition of claim 17, wherein the one or more additives comprise one or more of pH adjustment agents, skin conditioners, antioxidants, preservatives, fragrances, pigments, and dyes.
19. The composition of claim 17, wherein the one or more additives comprises monoglycerides, diglycerides, or a combination thereof.
20. The composition of any one of claims 1 or 3-19, wherein the at least one surfactant is selected from the group consisting of sulfonated alkyl benzenes, sulfonated methyl esters, sulfonated alpha olefins, paraffin sulfonates, alkyl sulfates, alkyl alkoxy sulfates, alkyl alkoxy carbonates, alkyl sulfonates, alkyl alkoxy sulfonates, acyl lactylates, alkyl isethionates, alkyl sarcosinates, alkyl betaines, alkyl amidopropyl betaines, alkyl sultaines, alkyl amine oxides, quaternary ammonium compounds, salts thereof, and combinations thereof.
21. The composition of any one of claims 1-20, wherein the dermatologically acceptable carrier comprises water.

22. The composition of any one of claims 1-21, wherein the dermatologically acceptable carrier comprises one or more alcohols, glycols, or solvents.
23. The composition of any one of claims 1-22, wherein the composition further comprises at least one anti-fungal active selected from the group consisting of zinc pyrithione, sulfur, selenium sulfide, salicylic acid, coal tar, ketoconazole, and piroctone olamine.
24. The composition of any one of claims 1-23, wherein the *Malassezia* species is *M. furfur*, *M. dermatis*, *M. equi*, *M. globosa*, *M. japonica*, *M. obtusa*, *M. ovalis* (also known as *Pityrosporum ovale*), *M. pachydermatis*, *M. restricta*, *M. slooffiae*, *M. sympodialis*, or combinations thereof.
25. The composition of any one of claims 1-24, wherein the composition has a pH in the range of 4.5 to 8.0.
26. The composition of any one of claims 1-25, wherein the composition is a solution, a gel, a paste, or a cream.
27. A method of controlling, treating, or preventing dandruff in a subject afflicted with or susceptible to dandruff comprising applying to the scalp and/or hair of the subject an effective amount of the dandruff treatment composition of any one of the preceding claims.
28. A method of controlling, treating, or preventing an excess of *Malassezia* species on mammalian skin comprising applying to the mammalian skin an effective amount of the dandruff treatment composition of any one of the preceding claims.
29. The method of claim 28, wherein the *Malassezia* species is *M. furfur*, *M. dermatis*, *M. equi*, *M. globosa*, *M. japonica*, *M. obtusa*, *M. ovalis* (also known as *Pityrosporum ovale*), *M. pachydermatis*, *M. restricta*, *M. slooffiae*, *M. sympodialis*, or combinations thereof.