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"Cosmetic composition containing a compound with stimulatory activity on interleukin-6 production"

The present invention relates to a cosmetic composition containing an active compound which is capable of stimulating *in vivo* interleukin-6 (IL-6) production by human keratinocytes.

Among the wide range of cytokines produced by cells, the specific stimulation of IL-6 synthesis is of particular interest for use in cosmetology. Besides its activating effects on cellular and humoral immunity, IL-6 plays an active part in the mechanisms of epidermis regeneration. *In vitro*, IL-6 stimulates collagen synthesis, as well as keratinocyte and fibroblast proliferation. *In vivo*, in transgenic mice which overexpress IL-6 in the skin, a thickening of the stratum corneum is observed. This increase of the superficial layer of the epidermis occurs without a pro-inflammatory manifestation, and is thought to reflect the improvement in the protection of the skin against local lesions. This set of data, which is derived from the literature, indicates that IL-6 promotes the repair of damage caused to the epidermis and slows the ageing of the skin.

Reference may thus be made to K. Yoshisaki *et al.*, Cytokine, 1990 2, 381-387; R.M. Grossman *et al.*, Proc. Natl. Acad. Sci. USA, 1989, *86*, 6367-6371; Kirbauer *et al.*, J. Immunol., 1989, *142*, 1922-

1928; K. Turken *et al.*, Proc. Natl. Acad. Sci. USA, 1992, 89, 5068-5072; J. Taylor-Papadimetriou *et al.*, Cell. Differ., 1982, *11*, 169-180; L. Aarden *et al.*, Lymphokines, 1985, *10*, 175-182. It is well known that in ageing of the skin, firstly, in the epidermis, the horny layer is thickened but epidermopoiesis is decreased and cellular cohesion at the basal membrane is modified. In order to help skin combat ageing, it is thus important to provide it with an active agent which is capable of stimulating keratinocyte proliferation so as to bring about a thickening of the epidermis. Secondly, in the dermis, the cells which are responsible for constructing the dermal architecture, the fibroblasts, have increasingly reduced activity, the consequence of which is a decrease in collagen, elastin and glycosaminoglycan synthesis. In parallel, the effects of exposure to light lead to an accelerated degradation of existing dermal fibres (actinic ageing). This set of effects leads to a disorganized dermal structure which deprives the skin of its mechanical qualities of elasticity, firmness and tonicity, and thus promotes the appearance of wrinkles.

In order to oppose the effects of ageing, it is thus important to provide the skin with an active agent which will stimulate fibroblasts and help them to reconstruct their environment. In order to be useful, this effect should not be accompanied in parallel by an

increase in the enzymatic activity of fibre degradation, in particular of collagenase. It should not modify the natural functions of the skin either.

It has now been found that the components or extracts which are capable of stimulating IL-6 production by human keratinocytes *in vivo* constitute "active agents" which can be used for preparing cosmetic compositions; more particularly, it has been found that cosmetic compositions containing a compound which is capable of stimulating IL-6 production by human keratinocytes *in vivo* can be used in the reorganization of skin tissue without, however, interfering with its natural functions. The compounds with stimulatory activity on IL-6 through human keratinocytes, also termed hereafter "active agents" [lacuna] are shown to be capable of inducing an action per se on the stimulation of keratinocyte proliferation (autocrine action of IL-6 on keratinocytes which is described in the literature).

Moreover, the compounds with stimulatory activity on IL-6 through human keratinocytes have been shown to be capable of inducing, via their action on the fibroblast, an increase in tonicity of the dermal environment of this cell. These compounds even induce a slight increase in the number of collagen fibres, without increasing collagenase activity.

Thus, a first subject of the present invention relates to a cosmetic composition containing a compound which is capable of stimulating interleukin-6 production by human keratinocytes *in vivo* mixed with a cosmetic excipient.

The compound with stimulatory activity on IL-6 production, or active agent, which is contained in the cosmetic composition can be a nonpeptide compound, a peptide, an extract of cells or of tissues of plant origin, or a product obtained by fermentation of a microorganism, for example of a bacterium or of a fungus and more particularly of a yeast.

The properties of the strain SEBR 2002 will be described below, along with the method for obtaining the extract with stimulatory activity on interleukin-6 production by keratinocytes.

Strain

Discovery

The organism which produces the extracts with stimulatory activity on IL-6 production by human keratinocytes according to this particular aspect of the present invention is a strain of yeast which was isolated from an oilfield water sample taken from an oil drilling site in the Loiret (France), which has been assigned the internal number SEBR 2002. A sample of this microorganism was deposited on 11 February 1997

with the C.N.C.M. of the Pasteur Institute, where it was registered under the reference I 1844.

The biochemical properties of this microorganism have been determined on API 50 CH galleries (specific for sugars), which are sold by BioMérieux, and with the YT biological test (specific for yeasts sold by Biolog Inc. USA). It has thus been determined that this microorganism belongs to the family of *Basidiomycetes*, genus *Rhodotorula*.

It is a polymorphic mesophilic yeast. It develops well at 28°C on a YPG (yeast peptone glucose agar) culture medium; the coloration of the colonies is orangy-pink.

This organism exhibits properties which make it possible to relate it to the species minuta.

This yeast strain *Rhodotorula sp*, which has been deposited with the C.N.C.M. of the Pasteur Institute under the number I 1844, and its producer mutants also constitute a subject of the present invention.

The isolation of this novel strain was carried out by following the conventional method which consists in diluting a small amount of water of the sample to various concentrations, and in spreading a small volume of each dilution onto the surface of a Petri dish containing a nutrient agar medium. After a few days of incubation at 28°C, which enables the

microorganisms to develop, the various colonies are individually sampled and subcultured on nutrient agars in order to obtain more abundant cultures thereof.

After culturing on nutrient agar medium and several successive subculturings which make it possible to obtain an abundant and pure culture of the strain of interest, a conservation batch 0 of the stock strain, and then primary and secondary seed batches, are manufactured.

For this, a yeast suspension is prepared starting from a Petri-dish culture on a nutrient agar medium and using a recovery medium; this medium contains a cryoprotector for ensuring good viability of the microorganism during the conservation by freezing.

The obtained yeast suspension is distributed into cryotubes, which are conserved at -80°C: these tubes constitute batch 0.

Following the same protocol, but starting from a tube of batch 0, a primary seed batch is prepared.

Then, still according to the same protocol, a secondary seed batch is prepared starting from a cryotube of the primary seed batch.

The manufacture of seed batches 0, 1 and 2 insures long-lasting access to the strain and thus to the desired activity.

Method

The method for preparing the extracts with stimulatory activity on IL-6 production by human keratinocytes consists in culturing this novel strain I 1844, or its producer mutants, on a medium and under suitable culture conditions, and in then extracting the active fraction.

The active fraction can be in the biomass or in the supernatant.

Fermentation

The culturing of the strain I 1844 can be carried out by any aerobic culture method. For this purpose, various type of machine are used which are commonly used in the fermentation industry. The following approach can in particular be adopted for carrying out the procedures.

Flask culturing

Using the secondary seed batch, Petri dishes are seeded which, after incubation for two to three days, provide a yeast suspension which is used to seed stirred Erlenmeyer flasks containing a suitable medium. The stirred flask can also be directly seeded with a tube of the seeding batch. The stirred-flask culturing can last from one to three days.

The production of activity is observed by extracting the obtained biomass by filtration or centrifugation with organic solvents.

Right from the first culture step in flasks, it may be advantageous to carry out two successive culture steps: a first step for multiplying the cells and propagating the biomass, and a second for the production. In this latter case, a duration of one to two days is sufficient for the first step.

The activity of stimulation of IL-6 production by human keratinocytes which is contained in the biomass extracts of the cultures is expressed by a stimulation index or factor, termed SI_{IL-6} (SI), which is calculated by forming the ratio of the amounts of IL-6 produced by induction by the extracts to the basal (noninduced) value measured:

$$SI_{IL-6} = \frac{\text{Induced IL-6 production}}{\text{Basal IL-6 production}}$$

A biomass extract is considered to be active when, assayed at 1‰ final concentration with respect to the dry extract, on SVK 14 keratinocytes, it produces an SI between the values 1.2 and 6, preferably from 1.5 to 4.5.

One litre of culture makes it possible to obtain approximately from 10 to 50 ml of extract which has a stimulation index of between 1.5 and 4.5 for a 100-fold dilution (1/100).

The desired activity can be obtained by extracting the biomass of the flask cultures, but, in order to obtain the desired activity in greater amount,

it appears to be advantageous to prepare a fermenter culture and then to extract its biomass.

Fermenter culturing

The fermenter is seeded with a 1- to 2-day-old stirred flask culture; it is preferable for the culture still to be in the exponential phase.

In a fermenter, depending on the culture conditions used, the activity may be observed right from the first hours of culture, but it is advantageous to wait for the stationary growth phase to be reached before proceeding with the extraction. The fermenter culturing of I 1844 makes it possible to better control the culture conditions which are described below, for example the pH and the aeration.

The stimulatory activity through keratinocytes obtained in a fermenter may vary depending on the culture conditions used. One litre of culture makes it possible to obtain, after extraction of the biomass, approximately 50 ml of extract with an SI of between 1.5 and 4.5 for a dilution of 100-fold (1/100).

Culture conditions

The culture medium used in the fermentation method should contain at least one source of carbon which can be assimilated, one source of nitrogen which can be assimilated and mineral elements. As sources of carbon which can be assimilated, use may be made for

example of carbohydrates such as glucose, mannose, maltose, dextrins, glycerol, amino acids and proteins.

As sources of carbon which can be assimilated, use may also be made of acetic, suberic, citric, propionic, succinic and 2-ketoglutaric acids, or animal or plant oils.

The best sources of nitrogen which can be assimilated are to be found among proteins, peptones and amino acids. These sources comprise, for example, casein, lactalbumin and gluten, and the hydrolysates thereof, fish meal, yeast extracts or peptones.

The biomass production can be increased by adding, during culturing, one or the other of these two principal substrates.

Among the mineral elements added to the culture medium to ensure microorganism growth and to optimize the assimilation of the carbon and nitrogen sources by the cells of the microorganism, mention may be made of potassium, sodium, iron, magnesium, calcium or manganese salts, as well as phosphorus compounds such as phosphates and trace elements.

The culture is stirred and aerated for a duration which varies between 10 hours and 60 hours, which makes it possible to obtain advantageous results.

Preferably, the pH of the culture medium is maintained at a slightly acid value and the optimum

incubation temperature is between 23°C and 38°C, the preferred range being 25°C to 33°C.

The culture conditions such as the composition and pH of the medium, the incubation temperature, the stirring speed and the fermentation aeration may vary within broad limits, and are chosen so as to obtain the best possible results.

Extraction

The production of the active extract exhibiting a stimulatory activity on IL-6 production by keratinocytes requires several extraction steps.

A first step consists in separating the biomass from the rest of the must; for this, it is possible to use centrifugation, tangential microfiltration, rotary drum filtration or any other method conventionally used by persons skilled in the art to separate the biomass from a fermentation must. The biomass is then brought into contact for a few hours with a mixture of solvents which makes it possible to extract molecules of hydrophobic nature.

The preferred duration of bringing into contact is overnight, i.e. approximately 15 hours.

The biomass and the loaded organic phase are then separated by frontal filtration, and the biomass is discarded.

The organic phase is then evaporated off under vacuum at a temperature ranging from room temperature to 50°C until a dry extract is obtained.

Preparation of the active extract

The dry extract thus obtained can be taken up in various solvents conventionally used in cosmetology.

The uptake concentration is chosen so as to allow complete dissolution of the extract and so as to be compatible with the subsequent use.

The extract with stimulatory activity on IL-6 production by keratinocytes is introduced, in a proportion of 5 to 10% of dry extract according to the activity, into a 50/50 (v/v) ethanol/water mixture which constitutes the preferred solvent according to the invention. If production of a powder instead of a solution is desired, the dry extract can be simply freeze-dried.

An assay of stimulatory activity on IL-6 production by keratinocytes carried out on an aliquot portion of each extract obtained makes it possible to evaluate its activity and to verify the reproducibility of the method.

In order to remove any trace of residual biomass and to ensure its microbiological stability, the extract is filtered through a membrane with a 0.2 μm exclusion threshold, and distributed aseptically into sterile flasks. The aseptic preparation of these

solutions makes it possible to avoid adding a preservative. Various biochemical assays were carried out and made it possible to demonstrate that the extracts derived from this novel microorganism I 1844 have a very valuable stimulatory activity on IL-6 production by keratinocytes. Their powerful activity was demonstrated in various assays which predict a repair of the various damage caused to the epidermis, and the slowing-down of skin ageing.

The characterization of the stimulant effect of the extract I 1844 on *in vitro* IL-6 synthesis by human keratinocytes was carried out as follows.

Extract I 1844 and products used

Various samples of the extract I 1844 which were derived from various fermenter cultures carried out under similar conditions were used. The samples were conserved at 4°C in a 50/50 (v/v) ethanol/water mixture, at a concentration of 0.1 g/g of dry extract, and then diluted to the final concentration desired at the time of use.

The reference product for inducing IL-6 synthesis was LPS (*E. coli* 055:B5) Sigma, which was conserved at -20°C at a concentration of 10 mg/ml in PBS. PolymyxinB, which is an LPS inhibitor, was supplied by Sigma.

Dilution of the products

The products were diluted in the keratinocyte incubation medium (cf. below). Except where otherwise indicated, the I 1844 extracts prepared as indicated above were evaluated at the final concentration of 1‰ with respect to the dry extract. LPS was assayed at a concentration of 10 µg/ml.

Evaluation model

Cells

The human keratinocyte line used was the SVK 14 line (J. Taylor-Papadimetriou *et al.*, Cell. Differ., 1982, *11*, 169-180) which was maintained in culture in DMEM medium containing 4.5 g/l of glucose, 100 mmol of sodium pyruvate, 50 U/ml of penicillin, 50 µg/ml of streptomycin and 10% of foetal calf serum (FCS). By way of comparison, normal human lymphocytes (PBMNC) which were purified on a Ficoll® gradient, and which originated from healthy volunteers, as well as keratinocytes originating from the A 431 line (ATCC CRL 1555) and normal human keratinocytes derived from breast plastic surgery (Biopredic, Rennes) were cultured in the same medium as that described above, but also containing 10 mg/ml of EGF and 50 µg of bovine hypophysis extract.

Incubation with the products

For IL-6 induction, the cells are seeded in triplets in a final volume of 200 µl at a density of

5×10^4 cells/well in 96-well culture microplates. After incubation for 24 hours at 37°C in an atmosphere containing 95% air and 5% CO_2, the medium is removed and renewed with the same volume of medium with 1% of FCS containing the products at the concentrations indicated. After a further 18 to 24 hours of culture, the culture supernatant of each triplet is removed, pooled and frozen at -20°C until the determination of the cytokine content.

Cytokine assays

IL-6, IL-1β, TNFα and IL-8 were quantified with the aid of ELISA assay kits (R&D, Abington, UK). Each supernatant was evaluated in duplicate according to the manufacturer's indications. The detection limit for these assays is 3 pg/ml. In addition to the immunological assay of the protein by the ELISA technique, the active form of IL-6 was also evaluated by biological assay on B9 murine hybridoma cells (L. Aarden *et al.*, Lymphokines, 1985, 10, 175-182).

Expressing the results

The results are expressed either in absolute value of amount of secreted cytokines (in pg/ml of protein for the ELISA assays or U/ml for the B9 biological assay of IL-6: one unit being defined as being the amount of cytokine which induces 50% of the maximum B9 cell proliferative effect), or in stimulation index SI as defined above which is

calculated as being the ratio between induced cytokine production and basal production:

$$SI_{IL-6} = \frac{\text{Induced IL-6 production}}{\text{Basal IL-6 production}}$$

Results

The extract with stimulatory activity on IL-6 production will hereafter be termed simply "extract I 1844". The number of the extract used for each experiment, as well as its concentration, are indicated.

Effect of the extract I 1844 on keratinocyte IL-6 stimulation

The extract I 1844 stimulates SVK 14-keratinocyte IL-6 synthesis in concentration-dependent manner. Taking the mean amount of basal IL-6 produced + 3 SD (11.8 + [3 × 0.8] = 13.6 pg/ml) to be the positivity threshold, the extract I 1844 significantly stimulates IL-6 synthesis at and above concentrations in the region of 0.12% (SI = 1.23), the EC_{50} is equal to 0.25% and the maximum effect is obtained at the concentration of 2% for which IL-6 synthesis is stimulated by a factor of 2.3.

The study was extended to another human keratinocyte line (A 431 line), as well as to normal human keratinocytes. Unlike what was observed on the SVK 14 line, the extract I 1844 is capable of stimulating IL-6 production by a factor greater than 2

both in another keratinocyte line (A 431) and in a primary culture of normal keratinocytes.

The effect of the extract I 1844 is thus not limited to lines, but can be generalized to normal cells.

Specificity of the effect of the extract I 1844

The specificity of the effect of the extract I 1844 was verified at various levels by demonstrating:

- that the effect was not due to a contamination with bacterial LPS, the capacity of which to stimulate IL-6 production is known, and that cells of lymphoid origin which are also capable of producing IL-6 were insensitive to the extract I 1844.

- that among the cytokines which can be synthesized by keratinocytes, only IL-6 production was stimulated under the influence of the extract I 1844.

The presence of polymyxin, which is an LPS inhibitor, does not modify the inductive effect of the extract I 1844 on SVK 14-keratinocyte IL-6 production, whereas the LPS-induced stimulation is logically inhibited by this compound. This result indicates that the effect observed with the extract I 1844 is not caused by a contamination due to a bacterial endotoxin. In addition, the evaluation carried out by B9 bioassay indicates that the extract I 1844-stimulated IL-6 production is biologically active.

The comparative study on different cellular targets shows that blood lymphocytes (PBMNC), unlike keratinocytes, are insensitive to the stimulatory activity effect of the extract I 1844 on IL-6 production. Conversely, LPS, which is capable of stimulating both cell types, is effective in both cases. This study thus shows that the extract I 1844 stimulates keratinocytes without affecting cells of lymphoid origin.

Finally, the extract I 1844 stimulates IL-6 synthesis without simultaneously increasing the level of IL-1, IL-8 or TNFα. Among the cytokines, IL-8 is of special nature since it is capable, via its chemotactic properties, of locally recruiting neutrophil polynuclear cells. It thus plays an important role in inflammation. The extract I 1844 stimulates IL-6 production without increasing the production of the other cytokines studied.

This set of data: absence of LPS effect, targeting of only keratinocytes and IL-6-limited stimulation, makes it possible to conclude that the action of the extract I 1844 is specific and that there is no pro-inflammatory effect.

Thus, the extract I 1844 reproducibly stimulates keratinocyte IL-6 production. This effect is observed both on human keratinocytes which have been established as lines and on normal human keratinocytes

derived from primary culture. The property of the extract I 1844 of stimulating IL-6 synthesis by keratinocytes is specific. It is due to a property which is intrinsic to the extract I 1844 and not to an exogenous contamination such as bacterial endotoxin, it does not affect cells of lymphoid origin and, among the cytokines tested, it is observed only for IL-6: the specificity of the extract I 1844 is twofold, both cellular and molecular. The characterization of the stimulatory activity effect of the extract I 1844 on keratinocyte IL-6 made it possible to define IL-6 stimulation indices of between 1.5 and 4.5 as an activity criterion for any sample evaluated on SVK 14 cells.

The characterization of the effect of the extract I 1844 on collagen fibre contraction was carried out as follows.

The aim of this study is to test the effect of the extract I 1844 on collagen fibre contraction. A collagen gel prepared by mixing fibroblasts and collagen is used. Under the effect of tensions exerted by the cells, the collagen is organized into fibres. This phenomenon is quantified by the contraction of the gel.

The cell line used is the MRC5 strain, which is a strain of fibroblasts derived from human embryonic lung. They are cultured in BME medium (Basal Eagle

Medium) with Glutamax I containing 10% of FCS, 1% of penicillin/streptomycin, 1% of fungizone and 1% of NEAA (nonessential amino acids) Gibco®.

The collagen used is type I collagen derived from rat tail tendons, as a solution at 2 mg/ml in acetic acid

Method for obtaining the gels

Preparation of the cell suspension

After trypsinization of an MRC5 culture obtained in a 75-cm^2 Petri dish, the cell suspension is prepared in DMEM containing 10% of FCS and the antibiotics in a proportion of 1×10^6 cells/ml, so as to obtain 500,000 cells per gel.

The preparation of the mixture takes place *on ice* in order to delay gelling.

The various constituents are added with stirring in the following order and proportions:

1.76 X DMEM	2.3 ml
Type I collagen	1.5 ml
0.1N NaOH	0.25 ml
FCS	0.45 ml
Cell suspension	0.5 ml

This well homogenized mixture is distributed into a Petri dish 5 cm in diameter (bacteriology type, NUNC) and incubated at 37°C. The gel forms within 10 minutes. The gel is detached from the wall of the dish approximately 2 hours later using a scalpel blade.

The diameter of the gels is then measured at 24, 48 and 72 hours after formation using a ruler which is graduated in millimetres.

Treatment

The extract I 1844 is diluted in the 1.76 X DMEM medium during the production of the gel so as to obtain the final concentrations of 0.1%, 0.2% and 0.5%. A phenol solution at a final concentration of 6.4 g/l is used as a contraction inhibition control.

Quantitative analysis

The extract I 1844, at and above the concentration of 0.1%, induces a significant increase in contraction. The phenol used as a contraction inhibition control gives 0% contraction. The quantitative analysis by electron microscopy observation of the contracted gels after 72 hours in the presence and absence of the extract I 1844 shows active cells which appear to be in a protein synthesis phase. The treatment with the extract I 1844 does not appear to noticeably modify the number or size of the fibres.

The extract I 1844 is effective at and above the dose of 0.1%. It increases fibroblast activity, which is revealed by an increase in the contraction of the collagen gel and in the number of fibres at the highest concentration only.

The characterization of the effect of the extract I 1844 on collagenase activation was carried out as follows.

The aim of this study is to test the effect of the extract I 1844 on collagenase activation. For this, a collagen gel prepared by mixing fibroblasts and tritiated collagen is used. In this environment, the fibroblasts reorganize, remodel and contract the collagen to produce a dermal-type structure. For this, the fibroblasts produce collagenase which is released into the culture medium in the latent form which can be activated under the action of proteinases. The collagenase activity is measured by its capacity to digest a tritiated collagen substrate into soluble radioactive peptides. The latent collagenase is activated by trypsin treated with 1,1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK). The cell line used is the MRC5 strain, which is a strain of fibroblasts derived from human embryonic lung. They are cultured in BME medium (Basal Eagle Medium) with Glutamax I containing 10% of FCS, 1% of penicillin/streptomycin, 1% of fungizone and 1% of NEAA (nonessential amino acids) Gibco®.

The collagen used is type I collagen derived from rat tail tendons, as a solution at 2 mg/ml in acetic acid. The tritium-labelled collagen is in

solution at 0.3 mg/ml in acetic acid, and its activity is 0.06 mCi/ml (Dupont Net-660).

Method for obtaining the gels

Preparation of the cell suspension

After trypsinization of an MRC5 culture obtained in a 75-cm^2 Petri dish, the cell suspension is prepared in DMEM containing 10% of FCS and the antibiotics in a proportion of 1×10^6 cells/ml.

The preparation of the mixture takes place *on ice* in order to delay gelling.

The various constituents are added with stirring in the following order and proportions:

2 X DMEM	400 µl
Tritiated type I collagen	300 µl
0.1N NaOH	100 µl
FCS	100 µl
Cell suspension	100 µl

200 µl of this well homogenized mixture are distributed into a 24-well plate and incubated at 37°C. The radioactivity is 0.4 µCi/well.

One hour later, 500 µl of DMEM medium + 3% FCS are deposited in each well.

Every 24 hours for 7 days, the activity of the activated and latent collagenase is measured. For this, 500 µl of medium are removed from each well and counted by liquid scintillation. 300 µl of solution of trypsin/TPCK at 25 mg/ml in DMEM are deposited in each

well and incubated at 37°C for 15 minutes. The action of the trypsin is stopped by adding 200 µl of solution of trypsin inhibitor at 100 mg/ml in distilled water. 500 µl of medium are removed and counted by liquid scintillation. 500 µl of DMEM medium + 3% FCS are added again to each well and the plate is again left to incubate for a further 24 hours.

The extract I 1844 is diluted in the 2 X DMEM medium during the production of the gel so as to obtain the final concentrations of 0.1% and 0.5%.

The extract I 1844 does not modify the total collagenase activity. At a given time, the respective proportions of latent and activated collagenase are not modified with respect to the control, although an increase in the latent collagenase is observed with time both in the controls and in the treated samples. The product does not cause any collagenase activation.

In the preparation of the cosmetic compositions according to the present invention, the I 1844 extracts thus constituted are mixed with the conventional diluents and aqueous or nonaqueous solvents which are compatible with topical use, as well as with the active components of the actual composition. Suitable solvents and/or diluents will be chosen according to their capacity to convey the active component of the extract of the invention into the epidermal and dermal skin structures.

The I 1844 extracts of the invention were also shown to be completely free of genotoxicity in Ames and DNA-repair assays.

Their stability is compatible with their use in cosmetic compositions.

The cosmetic compositions of the present invention contain the extract I 1844 in percentages of from 0.00001% to 5% by weight with respect to the total weight of the composition, mixed with the excipients which are commonly used for preparing cosmetic formulations to be applied to the skin.

Said percentages can vary within the range indicated above as a function of the intrinsic activity of the extract I 1844 included in the composition.

Preferably, said extract I 1844 is present in percentages of from 0.0001% to 2% % by weight with respect to the total weight of the composition.

In the preparation of the compositions according to the present invention, the extract I 1844 is mixed with the conventional diluents and aqueous or nonaqueous solvents which are compatible with use on the skin, as well as with other components of the composition itself. Suitable solvents and/or diluents are chosen according to their capacity to deposit the active extract I 1844 of the invention on the skin.

These compositions generally contain excipients or additives which are chosen from the

ingredients conventionally used in compositions which are intended for local application, according to the need of the particular formulations envisaged.

They can contain, for example, thickeners, softener, emollients, stabilizers, preservatives, antifoam agents, surfactants, antioxidants, dyes and/or pigments and/or other types of filler, fragrances, silicones and diverse fatty substances.

The extract I 1844 is always from 0.00001% to 5% by weight, preferably from 0.0001 to 2% by weight with respect to the total weight of the composition, said amount being calculated with respect to the weight of the dry extract.

The compositions according to the present invention comprise, preferably, an extract of the fermentation must of the novel strain I 1844 in proportions, on a weight/weight percentage basis, which depend on the degree of activity of the extract used and thus on the concentration of dry substance and on the specific activity of this substance.

To obtain the cosmetic compositions according to the invention, it may be suitable to use the crude extracts which are obtained directly from the fermentation without any purification step, in proportions of from 0.00001 to 5%, advantageously from 0.0001 to 2%, better still from 0.001 to 2% by weight with respect to the total weight of the composition,

and preferably from 0.01 to 2% by weight with respect to the total weight of the composition.

These percentages by weight are naturally calculated on the basis of the weight of prepared dry extract.

More particularly, the compositions of the present invention contain more or less purified extracts which are dissolved and which can be obtained by fermentation of the strain *Rhodotorula* which is deposited with the C.N.C.M. of the Pasteur Institute under the number I 1844, or of its producer mutants, with excipients which are normally used for formulations of this kind, such as those mentioned above.

The form of the compositions according to the present invention can be an emulsion in which the constituent or the mixture of constituents, with an optional stabilizer, is combined with the excipients which are currently used in cosmetic compositions and which are compatible with said constituents, such as lanolin or plant, mineral or synthetic oils.

The compositions of the invention can also be provided in the form of gel in suitable excipients such as cellulose esters or other gelling agents such as acrylic derivatives, and can contain the active principle in dissolved form or suspended in microgranules.

The compositions according to the invention can also take the form of a lotion or of a solution in which the constituent mixture is dissolved or microdispersed.

The form of the compositions according to the invention can thus be a microdispersion in a liquid containing water, as well as one or more compatible surfactants. The dispersions exhibit the properties of microemulsions, in particular the transparency and the low viscosity, and have practically the appearance of true solutions. They can also be prepared extemporaneously.

One advantageous form of the compositions according to the invention is a fluid which is applied topically via an adhesive support, which is hereafter designated "patch", this patch allowing a controlled diffusion of the active components which is optionally activated by physical phenomena such as electric microcurrents.

The cosmetic compositions of the present invention can also contain other active components having either an effect of the same type as that of collagen synthesis stimulators, collagenase or elastase inhibitors, vasoprotectors or products which contribute towards stimulating keratinocyte proliferation or towards stimulating the synthesis of extracellular matrix constituents (collagen, elastin,

glycosaminoglycans) or products which are useful in this type of topical composition such as cell renewal accelerators, collagenase or elastase inhibitors, vasoprotectors, radical scavengers, active agents for restoring skin barrier function (ceramides, essential fatty acids) or moisturizers.

The compositions of the present invention enjoy good stability and can be conserved for the required time for use, at temperatures of between -10°C and 60°C without there being constituent sedimentation or phase separation, or a decrease in activity which may compromise the use thereof.

These compositions are very well tolerated, they exhibit no phototoxicity and their application to the skin for prolonged periods involves no side effects.

The compositions of the present invention, in their various presentation forms, can be used as preparations intended for preventing and/or combating skin ageing whether it is intrinsic or extrinsic in nature, principally actinic ageing in this case.

The cosmetic compositions of the present invention which are designed for anti-ageing purpose can be brought into contact with the epidermis or the hair or pilous system so as to modify the appearance thereof and to protect them.

It is well known that firmness is an essential quality for keeping a skin young. It in fact constitutes a veritable marker of cell vitality, and its disappearance conditions the evolution of the other signs of ageing such as the formation of wrinkles or the absence of radiance.

As long as the dermis keeps its full density and an elastic suppleness, the epidermis also remains smooth, supple and very tonic. Skin ageing is a complex phenomenon which begins in the prime of youth. Free radicals, genetic factors and the sun are some of the causes thereof, which are, however, manifold and variable. All of these structures of the skin are affected at all levels and, with time, the cells become fewer in number. They produce a tissue of poorer quality which loses its original firmness and its elastic nature.

It has been noted that the metabolism of epidermal cells slows down, and that its basal layer loses its sinuous form and becomes rectilinear. The total keratinocyte population regresses and the horny layer thus loses its effectiveness. In addition, it controls water evaporation poorly. It has also been noted that the activity of fibroblasts, which constitute the key cells of the dermis, decreases as the years go by. The collagen produced then becomes fibrous and rigid. Elastin synthesis drops even

earlier. The nature of the dermis thus changes texture, and it becomes thinner, collapses and becomes less resistant to the tractions of the muscles. This loosening creates cracks which thus constitute the wrinkles.

The smoothing restructuring formula of the cosmetic compositions according to the invention reinstates, at all levels of the skin, the natural bases of the process of firmness.

They respond to the imperceptible microtransformations of the skin which result in the loss of firmness, elasticity and youthfulness of the skin.

The cosmetic compositions according to the invention are also intended for mature skin for which they constitute an anti-ageing care. The extract I 1844 then acts on the reconstruction factor of the skin, thus re-establishing both its structural (firmness and tonicity) and superficial (comfort and radiance) or deep (firmness and tonicity) and surface (radiance and comfort) physiological balance. The vitalizing or restructuring cosmetological compositions according to the invention can be used during the day and/or at night, respectively.

The cosmetic compositions can also contain other additional active agents. They can for example be vitamins or anti-blemish agents.

Their incomparable restructuring qualities are also reinforced and amplified by the synergistic nature of a combination with for example a marine plankton extract. The marine plankton-derived extract makes it possible to ensure optimal retriggering of the moisturization and firmness of the skin.

It is also possible to use phytostimulins such as fruit extracts like, for example, apple phytostimulins which are chosen for their radical-scavenging properties. They constitute particularly active novel radical scavengers which are rich in flavones, sterols, essential fatty acids and vitamins including vitamin E. The skin is then protected to the maximum against the dangers of the free radicals which are generated in particular by ultraviolet rays and stress.

Perfluoro oils can also be used in the cosmetic compositions according to the invention; in addition, a property of these perfluoro oils is a smoothing out of the rough parts and hollows of the microrelief of the grain of the skin. They iron out the lines of the face and visibly embellish the surface of the skin like veritable youthfulness "moulds".

Thus, the cosmetic compositions according to the invention stimulate epidermal cell renewal and collagen and elastin production, ensure optimal retriggering of the natural process of moisturization

and even more completely protect the skin against free radicals which constitute one of the essential factors of its ageing. The epidermis, which is redynamized, then once more has a better moisturization and the skin appears to have become younger, smoother and more tonic. The lines of the face in particular are more sharply redrawn and the skin appears to be restructured from the inside, thus becoming visibly firmer.

The extract I 1844 contained in the cosmetic compositions above acts on the skin as a unifying active agent.

Clinical trials were carried out on 120 30- to 60-year-old women. They tested the qualities of the cosmetic compositions according to the invention for 4 weeks.

In 87%, a visible effectiveness of the product on the firmness, smoothness and moisturization of their skin was noted. For 82%, their major cosmetic qualities were appreciated, for example: ease of application, rapid penetration, total absence of a greasy film, as well as feeling of well-being and of comfort.

Other clinical studies consisted in demonstrating the effectiveness of the cosmetic compositions according to the invention on skin relief, the microdepression network, the biomechanical properties of the skin and its luminosity.

The line which is specifically intended for mature skin made it possible to note that, with a day care for sensitive skin and dry skin, a statistically significant attenuation of the fine lines and of the microrelief, an improvement in the sharpness and appearance of the microdepression network, as well as an improvement in skin tonicity, are obtained.

The day care for normal and mixed skin demonstrated a statistically significant attenuation of the fine lines, an improvement in the sharpness of the microdepression network and an improvement in skin tonicity.

The antiwrinkle/firming night care showed a statistically significant attenuation of deep wrinkles, fine lines and the microrelief, an improvement in the sharpness and appearance of the microdepression network, and an improvement in skin firmness and tonicity.

The regenerating intensive care revealed a statistically significant attenuation of wrinkles and the microrelief, and an improvement in the sharpness and appearance of the microdepression network.

These various clinical results were confirmed by various methods which are well known to persons skilled in the art in cosmetology, such as, for example, the magnetic resonance imaging (MRI) microprobe technique which enables the measurement of

moisturization; transmission microscopy for evaluating skin reconstitution; high resolution echography for measuring skin thickness or phosphorus-31 spectrometry for measuring cellular activity.

A subject of the invention is also a cosmetic treatment method, characterized in that a cosmetically effective amount of the extract I 1844 in a vehicle for cosmetic use is applied to the epidermis and/or the hair or pilous system.

The following EXAMPLES illustrate the invention without however limiting it.

EXAMPLE 1

Preparation of an extract derived from the strain *Rhodotorula* I 1844.

1.1 Fermentation

The fermentation of the strain *Rhodotorula* I 1844 was carried out on various culture media.

1.1.1

a) The strain is propagated in Petri dishes on a subculturing medium.

Autolysate of *Saccharomyces cerevisiae*	10 g
Tryptone	10 g
Glucose	20 g
Agar	15 g
Purified water q.s.	1 l

The culture is incubated for 48 hours at 30°C. A yeast suspension is then obtained by adding to each Petri dish 10 ml of a suitable recovery medium having the following composition:

NaCl	9.00 g
KCl	0.42 g
$CaCl_2$	0.48 g
$NaHCO_3$	0.20 g
Glycerol	150.00 g
3-[N-Morpholino]propanesulphonic acid	3.00 g
Purified water q.s.	1 l

b) 5 ml of this suspension are used to inoculate a 2-litre Erlenmeyer flask containing 500 ml of culture medium having the following composition:

Autolysate of *Saccharomyces cerevisiae*	10 g
Peptonized milk	20 g
Corn steep	10 g
Glucose	30 g
Trace element solution	10 ml
Purified water q.s.	1 l

in which the trace element solution consists of the following compounds:

$FeSO_4 \cdot 7H_2O$	1 g
$MnSO_4 \cdot 4H_2O$	1 g
$CuCl_2 \cdot 2H_2O$	0.025 g
$CaCl_2 \cdot 2H_2O$	0.1 g
H_3BO_3	0.56 g
$(NH_4)_6Mo_{24} \cdot 4H_2O$	0.02 g
$ZnSO_4 \cdot 7H_2O$	0.2 g
Purified water q.s.	1 l

The culture is developed for 48 hours at 30°C in 2-litre Erlenmeyer flasks while stirring at 150 rpm.

1.1.2.

a) 0.2 ml of yeast suspension from the secondary seeding batch is used to inoculate a 500-ml flask containing 100 ml of culture medium having the following composition:

Autolysate of *Saccharomyces cerevisiae*	20 g
Tryptone	20 g
Glucose	20 g
Trace element solution	10 ml
Purified water q.s.	1 l

The trace element solution is identical to that described in EXAMPLE 1.1.1.

The culture is developed for 24 hours at 30°C with stirring at 200 rpm.

b) 100 ml of the culture obtained above are used to inoculate a 20-litre fermenter containing

12 litres of medium whose composition is identical to that of EXAMPLE 1.1.1.b, to which is added 1 ml/l of Struktol®.

The culture is developed at 30°C with an aeration rate of 1 vvm and varying the stirring so as to maintain a dissolved oxygen pressure of 30%. The pH is adjusted to 5.0 with ammonia.

At 25 hours old, a concentrated solution of glucose is added in order to sustain growth.

Culturing is stopped at the growth plateau, at 32 hours old.

1.1.3.+

100 ml of flask culture stirred in the same way as in EXAMPLE 1.1.2. are prepared, and this culture is used to inoculate a 20-litre fermenter containing 12 litres of culture medium having the following composition:

Autolysate of *Saccharomyces cerevisiae*	20 g
Tryptone	20 g
Glucose	30 g
Trace element solution	10 ml
Struktol®	1 ml
Purified water q.s.	1 l

The trace element solution is identical to that described in EXAMPLE 1.1.1.

The culture is developed under the same conditions as in EXAMPLE 1.1.2.b.

At 31 hours old a concentrated solution of glucose is added in order to sustain the growth phase. Culturing is stopped at the growth plateau, at 34 hours old.

1.1.4

Four 2-litre stirred flask cultures are prepared containing 500 ml of medium of the same composition as that of EXAMPLE 1.1.2.a.

The four flasks are each inoculated with 0.75 ml per flask of yeast suspension from the secondary seeding batch. The cultures are developed for 24 hours at 30°C and 200 rpm. They are then pooled to inoculated a 450-litre fermenter containing 230 litres of medium having the following composition:

Autolysate of *Saccharomyces cerevisiae*	30 g
Tryptone	20 g
Roferose	30 g
Trace elements	10 ml
Struktol	1 ml
Purified water q.s.	1 l

The trace element solution is identical to that described in EXAMPLE 1.1.1.

The culture is developed at 30°C with an aeration rate of 1 vvm and varying the stirring so as

to maintain a dissolved oxygen pressure of close to 30%.

The pH is adjusted to 5.5 with ammonia, and at 30 hours old, a concentrated solution of glucose is added in order to sustain the growth phase.

Culturing is stopped at the growth plateau, at 37 hours 30 old.

1.1.5

0.75 ml of yeast suspension from the secondary seeding batch is used to inoculate each of the six 2-litre flasks containing 500 ml of culture medium having the following composition:

Autolysate of *Saccharomyces cerevisiae*	20 g
Casein hydrolysate	20 g
Glucose	20 g
Trace elements	10 ml
Purified water q.s.	1 l

The trace element solution is identical to that described in EXAMPLE 1.1.1.

The culture is developed for 24 hours at 30°C with stirring at 220 rpm.

The six flasks are then pooled to inoculate a 450-litre fermenter containing 300 litres of medium having the following composition:

Autolysate of *Saccharomyces cerevisiae*	30 g
Casein hydrolysate	20 g
Glucose	20 g
Trace elements	10 ml
Struktol	1 ml
Purified water q.s.	1 l

The trace element solution is identical to that described in EXAMPLE 1.1.1.

The culture is developed at 30°C with an aeration rate of 1 vvm and varying the stirring so as to maintain a dissolved oxygen pressure of close to 30%.

The pH is adjusted to 5.5 with ammonia, and at 35 hours old, a concentrated solution of glucose is added in order to sustain the growth phase.

Culturing is stopped at the growth plateau, at 40 hours old.

1.2 Extraction

1.2.1

The extraction is carried out on 9 litres of must obtained according to EXAMPLE 1.1.1. (18 2-litre flasks containing 500 ml of must).

Centrifugation is carried out at 9000 rpm (14,000 g) for 10 min, and 154 g of wet biomass are obtained.

This biomass is brought into contact with 6 litres of dichloromethane/methanol (v/v) mixture at room temperature with stirring overnight.

The biomass is then removed by filtration and the organic phase is evaporated to dryness under vacuum at 50°C.

The evaporation residue (9.8 g) constitutes the dry extract and it is redissolved in 112 ml of methanol.

The fraction thus obtained is filtered through 0.2 μm and evaluated in the keratinocyte stimulation biological assay; it exhibits a stimulation index (SI) for keratinocytes of 1.9 when it is diluted 200-fold (1/200).

1.2.2

The extraction is carried out on 12 litres of culture must from EXAMPLE 1.1.2.

The same protocol as in EXAMPLE 1.2.1. is followed, but the extraction is carried out on the 1900 g of wet biomass obtained with 80 litres of dichloromethane/methanol (50/50) mixture. i.e. using a solvent mixture/wet biomass proportion of close to 40 litres of solvent mixture per 1 kg of wet biomass.

After bringing into contact overnight, the biomass is removed by filtration and the organic phase is evaporated to dryness under vacuum at 50°C.

93.8 g of dry extract are obtained, which are taken up with 375 g of the 50/50 (v/v) ethanol/water mixture.

The 0.2-μm-filtered solution containing 0.2 g/g of dry extract thus obtained exhibits a stimulation index (SI) of 2.1 for a 1/200 dilution.

1.2.3

The extraction is carried out on 11 litres of culture from EXAMPLE 1.1.3. The same protocol as in EXAMPLE 1.2.2. is followed, and the 2.2 kg of wet biomass obtained are extracted with 80 litres of the 50/50 (v/v) dichloromethane/methanol mixture.

The biomass is then removed via filtration and the organic phase is evaporated to dryness under vacuum at 50°C.

98 g of dry extract are obtained, which are taken up with 392 g of ethanol/water (50/50), (v/v) mixture so as to obtain a solution containing 0.2 g/g.

After filtration through 0.2 μm, this solution is evaluated in the keratinocyte stimulation biological assay; it exhibits a stimulation index (SI) of 2.8 for a dilution of 1/200.

1.2.4

The extraction is carried out on 12 litres of culture prepared in the same way as that of EXAMPLE 1.1.3, but in this case, a continuous centrifuge is used to separate the biomass. 1.6 kg of

wet biomass are obtained; this biomass is treated with 80 litres of 50/50 (v/v) dichloromethane/methanol mixture.

After bringing into contact overnight, the biomass is removed by filtration and the organic phase is evaporated to dryness under vacuum at 50°C.

83 g of dry extract are obtained, which are taken up with 747 g of 50/50 (v/v) ethanol/water mixture so as to obtain a solution containing 0.1 g/g of dry extract.

The solution obtained is filtered through 0.2 μm and exhibits a stimulation index (SI) of 3.5 for a dilution of 1/100.

1.2.5

The extraction of 230 litres of culture from EXAMPLE 1.1.3. is carried out; after separation of the biomass with a continuous centrifuge, 29 kg of wet biomass are obtained. 8 kg of this biomass are treated with 80 litres of 50/50 (v/v) dichloromethane/methanol mixture, i.e. using a solvent mixture/wet biomass proportion of close to 10 litres of solvent mixture per 1 kg of wet biomass.

After bringing into contact overnight, the biomass is removed by filtration and the organic phase is evaporated to dryness under vacuum at 50°C.

After evaporation of the organic phase, 314 g of dry extract are obtained, which are taken up with 2826 g of 50/50 (v/v) ethanol/water mixture.

The solution thus obtained (0.1 g/g of dry extract) exhibits a stimulation index (SI) of 2.8 for a dilution of 1/100.

1.2.6

The extraction of 330 litres of culture from EXAMPLE 1.1.5. is carried out. Using a continuous centrifuge, 54.4 kg of wet biomass are obtained. 27.7 kg of this biomass are treated with 272 litres of 50/50 (v/v) dichloromethane/methanol mixture, i.e. using a solvent mixture/wet biomass proportion of close to 5 litres of solvent mixture per 1 kg of wet biomass.

After bringing into contact overnight, the biomass is removed by filtration and the organic phase is evaporated to dryness under vacuum at 50°C.

After evaporation of the organic phase, 1447 g of dry extract are obtained, which are taken up with 13,023 g of 50/50 (v/v) ethanol/water mixture so as to obtain a solution containing 0.1 g of dry extract. After filtration through 0.2 g, this solution is evaluated in the keratinocyte stimulation biological assay; it exhibits a stimulation index of 2.6 for a dilution of 1/100.

In the following examples, the amounts are indicated as percentages by weight.

EXAMPLE 2: DAY CREAM FOR THE FACE

CARBOMER	0.15
TRIETHANOLAMINE	0.1
PROPYLENE GLYCOL	5
XANTHAN GUM	0.2
GLYCEROL	3
GLYCERYL STEARATE	3
PEG STEARATE	2
STEARETH-20	2
OCTYL PALMITATE	10
CETYL ALCOHOL	2
STEARYL ALCOHOL	0.25
PERFLUORO OIL	0.2
CHOLESTEROL	0.5
SYNTHETIC BEESWAX	5
DIMETHICONE	5
MARINE PLANKTON EXTRACT	0.5
APPLE EXTRACT	3
BISABOLOL	0.5
TOCOPHERYL ACETATE	1
GRAPE PIP EXTRACT	0.5
PRESERVATIVE	0.8
FRAGRANCE	0.5
EXTRACT I 1844	0.1
DEMINERALIZED WATER q.s.	100

EXAMPLE 3: CARE CONCENTRATE FOR THE FACE

BUTYLENE GLYCOL	5
CARBOMER	0.2
TRIETHANOLAMINE	1
POLYSORBATE-60	0.8
SORBITAN STEARATE	0.6
LIQUID PETROLEUM JELLY	2
LANOLIN	0.3
[lacuna] DIPELARGONATE	5
[lacuna] TRIGLYCERIDE	5
CETYL ALCOHOL	0.25
[lacuna] DICAPRYLATE/ DICAPRATE	5
GLYCERYL STEARATE	3.00
PLANT STEROL	0.5
DIMETHICONE	0.5
STEARIC ACID	2
PANTHENOL	0.5
SODIUM HYALURONATE	0.05
BISABOLOL	0.1
TOCOPHERYL ACETATE	1
SOYBEAN EXTRACT	0.5
PRESERVATIVE	0.8
FRAGRANCE	0.5
EXTRACT I 1844	0.25
DEMINERALIZED WATER q.s.	100

EXAMPLE 4: FLUID EMULSION FOR THE FACE

BUTYLENE GLYCOL	5
XANTHAN GUM	0.2
GLYCERYL STEARATE	1
PEG STEARATE	1
[lacuna] TRIGLYCERIDE	5
CETYL ALCOHOL	0.5
TITANIUM OXIDE	1
DIMETHICONE	3
POLYACRYLAMIDE	5
CERAMIDES	1
PANTHENOL	1
SODIUM HYALURONATE	0.05
BISABOLOL	0.1
TOCOPHERYL ACETATE	1
PRESERVATIVE	0.7
FRAGRANCE	0.5
EXTRACT I 1844	0.15
DEMINERALIZED WATER q.s.	100

EXAMPLE 5: LOTION

PEG-32	5
PHOSPHATE BUFFER	0.1
DIMETHICONE COPOLYOL	5
POLYSORBATE-20	0.1
POLYPROPYLENE GLYCOL	10
MARINE PLANKTON EXTRACT	1
APPLE EXTRACT	5
BISABOLOL	0.5
TOCOPHERYL ACETATE	0.5
WHEAT PROTEIN EXTRACT	2
PRESERVATIVE	1
FRAGRANCE	0.3
EXTRACT I 1844	0.1
DEMINERALIZED WATER q.s.	100

EXAMPLE 6: GEL

CARBOMER	1
NEUTRALIZER	0.25
PROPYLENE GLYCOL	5
PANTHENOL	1
ALOE VERA EXTRACT	1
CORNFLOWER EXTRACT	5
YEAST EXTRACT	0.5
WHEAT EXTRACT	0.25
SODIUM HYALURONATE	0.01
PRESERVATIVE	0.7
EXTRACT I 1844	0.05
DEMINERALIZED WATER q.s.	100

EXAMPLE 7: CREAM MASK

CARBOMER	0.5
TRIETHANOLAMINE	0.5
SORBITAN STEARATE	1.5
PEG STEARATE	3
CETYL ALCOHOL	5
PLANT OIL	20
TITANIUM OXIDE	0.7
GLYCEROL	7
TOCOPHEROL	0.75
APPLE EXTRACT	3
BISABOLOL	0.5
RETINYL PALMITATE	0.5
SODIUM HYALURONATE	0.02
PRESERVATIVE	1
FRAGRANCE	0.3
EXTRACT I 1844	0.1
DYES	
DEMINERALIZED WATER q.s.	100

EXAMPLE 8: RINSE-OFF MASK

ACRYLIC COPOLYMER	1
TRIETHANOLAMINE	1
GLYCEROL	15
C14-C16 OLEFIN SULPHONATES	5
PEG ETHER AND [lacuna] COCOATE	2
MARINE PLANKTON EXTRACT	1
APPLE EXTRACT	5
SOYBEAN EXTRACT	0.5
[lacuna] ASCORBYL PHOSPHATE	3
SILK PROTEIN EXTRACT	2
PRESERVATIVE	0.8
FRAGRANCE	0.5
EXTRACT I 1844	0.15
DEMINERALIZED WATER q.s.	100

CLAIMS

1. Cosmetic composition comprising, mixed with an excipient for cosmetic preparations, an extract with stimulatory activity on IL-6 production by keratinocytes which is a product originating from the fermentation of a microorganism.

2. Composition according to Claim 1, characterized in that the extract with stimulatory activity on IL-6 production by keratinocytes is a product originating from the fermentation of a yeast.

3. Composition according to Claim 2, characterized in that the extract with stimulatory activity on IL-6 production by keratinocytes is obtained by fermentation of a strain of *Rhodotorula*.

4. Composition according to Claim 3, characterized in that the extract with stimulatory activity on IL-6 production by keratinocytes can be obtained by fermentation of the strain *Rhodotorula* SEBR 2002 which is deposited with the C.N.C.M. of the Pasteur Institute under the number I 1844, or of its producer mutants.

5. Strain *Rhodotorula* SEBR 2002, which is deposited with the C.N.C.M. of the Pasteur Institute under the number I 1844, and its producer mutants.

6. Method for preparing an extract product with stimulatory activity on IL-6 production by

keratinocytes, characterized in that a strain according to Claim 5 is cultured on a fermentation medium and under conventional conditions until a stimulatory activity on IL-6 production by keratinocytes is obtained in the fermentation must.

7. Composition with stimulatory activity on IL-6 production by keratinocytes, obtained according to the method of Claim 6.

8. Composition according to Claim 7 obtained according to the method of Claim 6, in which the biomass is used in unmodified form or concentrated.

9. Composition according to Claim 7, characterized in that it exhibits a stimulatory activity on IL-6 production by keratinocytes with a stimulation index SI of between 1.2 and 6.

10. Composition according to one of Claims 7 to 9, characterized in that it is obtained by extraction of the biomass, evaporation of the solvents and water, and dilution of the dry extract in a solvent which is compatible with cosmetic use.

11. Use of a compound with stimulatory activity on IL-6 production by keratinocytes, originating from the fermentation of a microorganism, for manufacturing a cosmetic composition intended for combating skin ageing.

12. Use according to Claim 11, characterized

in that the compound is obtained by fermentation of a yeast.

13. Use according to Claim 12, characterized in that the compound is obtained with a strain of *Rhodotorula*, in particular *Rhodotorula* SEBR 2002 which is deposited with the C.N.C.M. of the Pasteur Institute under the number I 1844, or of its producer mutants.

14. Use of a strain of *Rhodotorula*, in particular *Rhodotorula* SEBR 2002 which is deposited with the C.N.C.M. of the Pasteur Institute under the number I 1844, or of its producer mutants, for preparing a compound with stimulatory activity on IL-6 production by keratinocytes.

15. Cosmetic treatment method, characterized in that a cosmetically effective amount of a compound with stimulatory activity on IL-6 production, originating from the fermentation of a microorganism, in a vehicle for cosmetic use, is applied to the epidermis.