ISSN : 0974 - 7435 Volume 9 Issue 7

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 9(7), 2014 [285-291]

Effect of bacterial exopolysaccharide and human hair proteinon recovery of damaged hair

Sun Hee Lee[1], Bo Young Jeon[2], Doo Hyun Park[2]*

[1]Department of Beauty Art, Seokyeong University, (KOREA)

[2]Department of Chemical and Biological Engineering, Seokyeong University, 16-1 Jungneung-dong, Sungbuk-gu, Seoul 136-704 (KOREA)

E-mail : baakdoo@skuniv.ac.kr

ABSTRACT

This study was performed to evaluate effect of natural compounds that are exopolysaccharide (EPS) and human hair protein (HHP) on recovering of damaged hairs. Exopolysaccharide (EPS) was extracted from culture fluid of *Weissella hellenica* SKkimchi3. HHP was obtained from human hair keratin that was chemically extracted and modified to be water-soluble. Molecular weight of EPS and HHP was approximately 250, 000 and 100, 000, respectively. Normal hairs were obtained from a woman who never treated her hairs for permanent wave or dyeing. Damaged hairs were obtained from a woman who treated her hairs for permanent wave two times and dyeing one time. One % of EPS solutionor 1% HHP was sprayed on surface of damaged hairs. Both EPS and HHP filled gaps between cuticle scales according to SEM images. However, tensile strength of HHP-treateddamaged hairs was significantly higher than that of EPS-treateddamaged hairs. Especially the tensile-strength of HHP-treateddamaged hairs was very similar to that of normal hairs. These results are a clue that HHP may connect between fibrous proteins of cuticle and cortex.

KEYWORDS

Hair keratin;
Fibrous protein;
Exopolysaccharide;
Damaged hair;
Hair recovery.

INTRODUCTION

Human hair that is structurally composed of cuticle layer, cortex, and medulla, and chemically constituted of lipids, keratin-associated proteins, and melanin has to be naturally changedand damaged by aging and environmental variations[1]. Especially structure of the hair cuticles exposed to outside may bemore largelyinfluenced than inside molecules (keratin-associated protein, intermediate filamentous proteins, sulfur proteins, and melanin) by environmental factors that are temperature variation, ultraviolet radiation, moisture variation, and physical contact[2,3]. However, beauty treatments that are bleaching, coloring, and permanent waving can more strongly damage all of cuticle, keratin-associated proteins, and melanin than the environmental factors[4-9],. The damaged hair can't be restored owing to the natural phenomena that are irreversible denaturation of keratin-associated proteins, physical breaking of cuticle layers, and the destructive (decol-

orized) denaturation of melanin[10-12]. Accordingly, the damaged hairs are required to be temporarily or partially recoveredby filling of local lesion site using chemical or natural polymers. Chemically synthesized polymers may be another cause to damage hair structure owing to their different structure and function from biopolymers.

EPS that is a typical carbohydrate polymer produced by diverse bacteria is structurallyvarious based on molecular weight and constituent sugar monomer[14]. EPS has been applied in food and nonfood industries as viscosifying, stabilizing, emulsifying, gelling, or water-binding agents[15]. In particular, the EPS produced by lactic acid bacteria are generally recognized as safe food additive for human because lactic acid bacteria are widely used for the production of numerous fermented foods[16,17]. *Weissella hellenica* is one of the lactic acid bacteria that are responsible for kimchi fermentation produces EPS consisting of glucose[18]. The EPS produced by the*W. hellenica* was applied to evaluate recovery effectof damaged hair. The EPS may be effectively attached to surface of damaged hair by the function of viscosifying and gelation.

Human hair proteins (HHPs) are composed of keratin-associated proteins, intermediate filament proteins, high-sulfur proteins, high-tyrosine proteins, and high glycine-tyrosine proteins[1]. Hair keratin-associated proteins are major component of the hair structure, and crucial roles in forming a stable hair shaft through a cross-linked network with keratin intermediate filaments, which are produced from the hair keratin[19]. Hair fibers are keratinized proteins that are elongated structure and heavily cross-linked. Each fiber is divided into three components for structure of cuticle, cortex, and medulla. Cuticle fiberis composed primarily of beta-keratins that function to protect the cortex fiber from physical and chemical damage. Major body of the hair fiber constitutes the cortex, which is composed of many spindle-shaped cells that contain keratin filaments. Occasionally, medulla that is a central part of hair structure consists of a column of loosely connected keratinized cells[20]Generally, hair proteins are elongated fibrous structure that was reported to be chemically separated from intact hairs by treatment of urea, thiourea, and mercaptoethanol under alkaline (pH 9.5) condition[21].

In this study, two-types of biopolymers that are fibrous carbohydrate (EPS) and fibrous protein (HHP) were separated from bacterial culture fluid and human hairs, respectively. The culture fluid of *W. hellenica*was employed as a source for EPS extraction and virgin hair of woman was used as a source for HHP extraction. EPS and HHP were directly applied to damaged hair to evaluate effect of the biopolymers for recovery of the damaged hairs.

EXPERIMENTAL

Preparation of EPS

EPS was isolated from culture fluid of *Weissella hellenica* SKkimchi3 that was cultivated in MRS medium containing sucrose (100g/L) instead of glucose at 30°C for 72 hr. Bacterial cells were removed from bacterial culture by centrifugation at 8, 000g for 40 min. Cell-free culture fluid (supernatant) was mixed with same volume of 99% cold ethanol (4°C) and then incubated at 4°C for 24 hr. EPS coagulated by ethanol was then separated by centrifugation at 4°C and 5, 000×g for 20 min and washed twice with 99% ethanoland then suspended in double-distilled water. Molecular weight of EPS was determined by gel filtration chromatographyusing dextran as a molecular mass standard. A standard dextran (Fluka, molecular mass, 25, 000, 50, 000, 150, 000, 410, 000) was used to calibrate the relationship between retention time and molecular mass. Concentration of the EPS suspension was determined based on dry weight.

Preparation of HHP

Human hair of a Korean woman was soaked in 99% ethanol for 4 hr to remove contaminants and soaked again in a mixture of chloroform and methanol (2:1) for 24 hr to remove external lipids. The washed and delipidized hair (3kg) was soaked in a solution composed of 10 L distilled water, 25 mM Tris-HCl buffer (pH 10), 8 M urea, 1 M thiourea, and 5% 2-mercaptoethanol at 50°C for 5 days. Liquid part was separate from solid part by filtration and centrifuged at 15, 000×g for 60 min at 4°C. The precipitant was washed with distilled water twice by centrifugation at 15, 000×g for 60 min at 4°C. The extracted hair proteins were treated with 300mM NaOH in distilled water for 24 hr at 60°C under anoxic condition and neu-

tralized with 300mM HCl using pH meter. The proteins solubilized by the alkaline and heat treatment were separated from insoluble proteins by centrifugation at 15, 000 ×g for 60 min at 4°C. The insoluble proteins were repeatedly treated under same condition. Protein concentration was determined by the colorimetric method of Bradfordusing the Bio-Radprotein assay (Bio-Rad, USA). The solubilized HHP was separated by differences of molecular weight using SDS-PAGE. Molecular weight of HHP was determined by comparison with molecular weight standard proteins (10, 000~225, 000).

Sources of damaged hair

Two Korean women contributed their hairs for experimental purpose. One of them treated chemically her hair two times for permanent wave and one time for coloring in 6 months, which was used as a damaged hair group, but other of them never treated chemicallyher hair for 1 year, which was used as a normal hair group.

Treatment of damaged hair with EPS or HHP

EPS and HHP were dissolved in 2% (w/w) glycerol solution. Final concentration of EPS and HHP was commonly adjusted to 0.2%(w/v) based on dry weight. Fifty strands of damaged hairs, which were previously washed with neutral detergent and dried in the air, were immersed in EPS or HHP solution for 30 sec and then dried separately in the air.

Scanning electron microscopy (SEM)

Completely dried normal hair, damaged hair, and damaged hair treated with EPS or HHP were employed as a sample for SEM. Each hair samples in 4 groups were vacuuming coating for 5 min so that silver ion could be attached to surface regularly using ion sputtering device (SCD005, BAL-REC, Germany) by fixing sample treatment plate (silver fasten). Then, scanning electron microscope (SUPRA™ 55VP; ZEISS, Germany) was observed by ×5000.

Measurement of tensile strength

Normal hair, damaged hair, and damaged hair treated with EPS or HHP were commonly dried at 45°C in a drying oven for 3 hr. The dried hairs were employed as a sample for measurement of tensile strength. Each side of one hair strand was fixed on clamp of apparatus. Length of hair strand fixed between each clamp was adjusted to 60±2 mm. The strength was measured with a force measurement apparatus (model, DS2-500N, IMADA, Japan). The tensile strength was determined with force ($N=kgmS^{-2}$) recorded at hair strand-snapping moment by pulling from both side.

RESULTS

Molecular weight of EPS

Molecular mass of EPS determined by 4 times-repeated tests was 215, 000-235, 000 as shown in Figure 1. Approximate molecular weight of EPS is 222, 000, which may induce hydrophilic interaction between EPS and cuticle protein fiber because number of functional group of EPS alcohol residue) is proportional to the molecular weight.

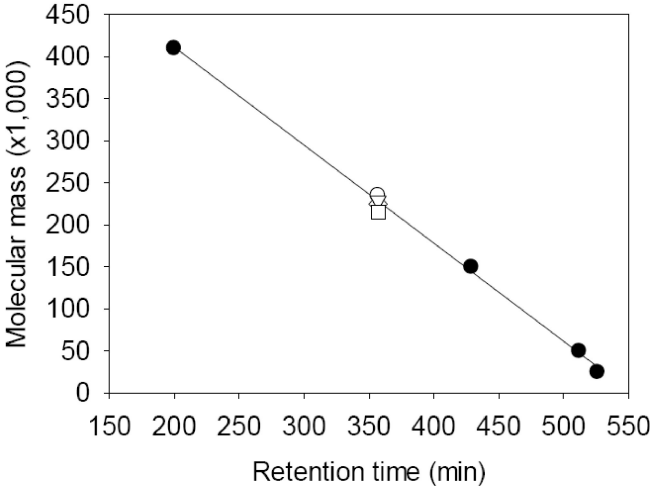

Figure 1 : Relative molecular weight of EPS determined by gel filtration chromatography.A standard dextran (Fluka, molecular mass, 25,000, 50,000, 150,000, 410,000) was used to calibrate the relationship between retention time and molecular mass (closed symbols). Molecular mass of EPS was determined by 4 times-repeated tests (open symbols).

Molecular weight of HHP

Molecular weight of HHP that was extracted from human hair and partially hydrolyzed by alkaline and heat treatment was determined by SDS-polyacrylamide gel electrophoresis as shown in Figure 2. The solubilized HHP was composed of at least 9 different proteins based on the number of protein bands separated by SDS-PAGE. Molecular weight (MW) of major protein was approximate 100, 000 and that of minor proteins was higher than 225, 000 or lower than 100, 000.

FULL PAPER

Mixture of various MW proteins may be useful to induce molecular interaction between cuticle protein fiber and HHP by hydrogen and ionic bonds.

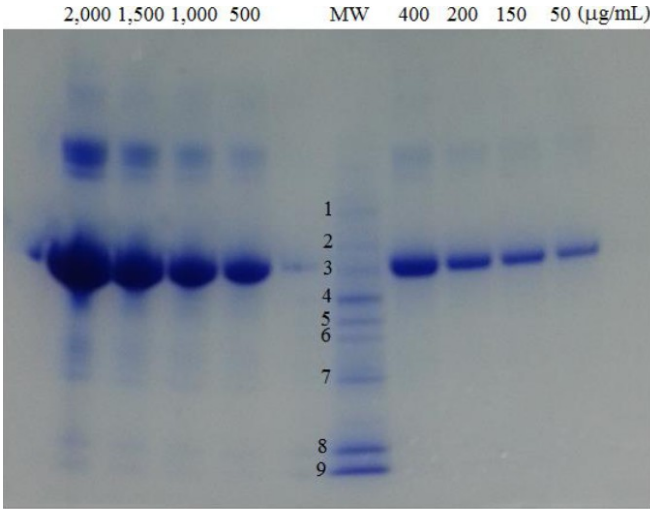

Figure 2 : SDS-PAGE image of solubilized human hair proteins (HHP) obtained by heat treatment under alkaline condition. Protein concentration of samples for electrophoresis was adjusted to 2,000~50 mg/mL to visualize bands of minor proteins and obtain clear band of major proteins. MW indicates protein markers that are separated based on molecular weight of standard proteins (1, 225,000; 2, 150,000; 3, 100,000; 4, 75,000; 5, 50,000; 6, 35,000; 7, 25,000; 8, 15,000; 9, 10,000)

Effect of EPS and HHP on structural recovery of damaged hair

Structural observation of hair cuticle may be a useful indicator to evaluate lesion degree and recovery state of damaged hair. SEM image of normal hair, damaged hair, EPS-treated damaged hair, and HHP-treated damaged hair was structurally different each other as shown in Figure 3. Cuticle scales of normal hair (Figure 3A) were relatively more regular than those of damaged hair (Figure 3B). Gaps between cuticle layers of damaged hair were commonly filled by treatment of EPS and HHP (Figure 3C and 3D). The effect of EPS and HHP on filling in the gap of cuticle layers was not significantly different based on the SEM images. From this result, it may be supposed that EPS and HHP are enough to be viscous to attach to gap between cuticle layers of human hair biopolymers are structurally recovered

Effect of EPS and HHP on functional recovery of damaged hair

Tensile strength of normal hair, damaged hair, EPS-treated damaged hair, and HHP-treated damaged hair may be a valid indicator to evaluate functional recovery of damaged hair. Tensile strength of damaged hair was

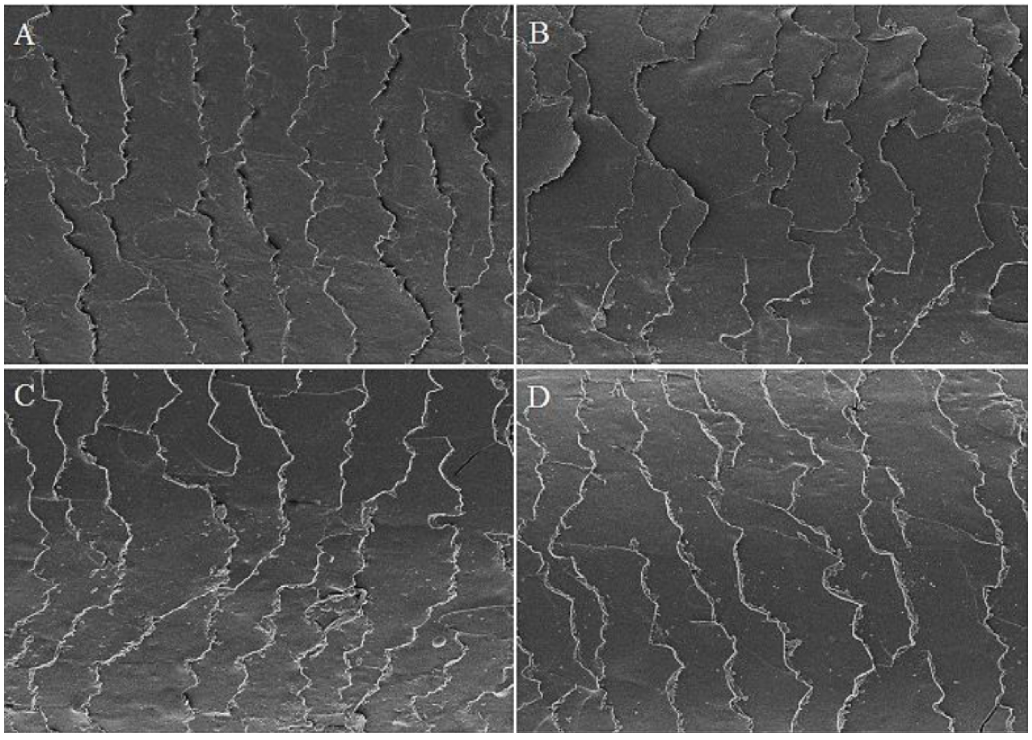

Figure 3 : Effect of EPS and HHPon structural recovery of damaged hair which was evaluated based on SEM images (×5,000) of normal hair (A), damaged hair (B), EPS-treated damaged hair (C), and HHP-treated damaged hair (D).

approximate 1.56, which was very similar to that of EPS-treated damaged hair (1.58). This result is a clue that the EPS was not effective to recover function of damaged hair. However, the tensile strength of normal hair was very similar to that of HHP-treated damaged hair as shown in TABLE 1. This result is a clue that EPS may fill in gap between cuticle scales but may not connect fibrous proteins. On the other hand, it is possible that HHP may not only fill in the gap but also chemically and physically connect between cuticle proteins.

TABLE 1 : Effect of EPS and HHPon functional recovery of damaged hair which was evaluated based on tensile strength (N=kgmS^{-2}) of normal hair, damged hair, EPS-treated damaged hair, and HHP-treated damaged hair.

No. of Hair	Normal hair	Damaged hair		
		Not treated	Treated with EPS	Treated with HHP
1	1.6	1.5	1.7	1.5
2	1.8	0.9	1.6	2.0
3	1.9	1.5	1.8	1.8
4	1.6	1.5	1.4	1.4
5	1.5	1.7	1.4	1.6
6	1.5	1.2	1.3	1.8
7	1.5	1.3	1.4	1.7
8	1.8	1.9	1.2	1.9
9	1.6	1.4	1.6	2.1
10	1.8	1.8	1.6	1.6
11	1.7	1.6	1.9	1.5
12	2.0	1.8	1.8	1.6
13	1.6	1.6	1.6	1.9
14	1.7	2.0	1.4	1.9
15	1.6	1.4	1.6	1.5
16	1.9	1.6	1.8	1.6
17	1.9	1.9	1.7	1.4
18	2.0	1.7	1.3	1.7
19	1.6	1.5	1.2	1.8
20	1.6	1.7	1.4	1.7
21	1.8	1.2	1.7	1.5
22	1.8	1.5	1.8	1.8
23	2.1	1.7	1.6	1.9
24	1.6	1.5	1.4	2.0
25	1.9	1.7	1.7	2.0
26	1.6	1.8	2.0	1.7
27	1.8	1.3	1.3	1.5
28	1.3	1.4	1.5	1.7
29	1.8	1.9	1.9	1.8
30	1.6	1.3	1.7	1.3
Mean ±SD	1.72±0.18	1.56±0.25	1.58±0.22	1.71±0.21

DISCUSSION

Generally, human being growsphysiologically and cuts (trims) intentionally their hair owing to specific reasons that are beauty treatments, vocations, customs, and social activities. Hair is lengthened in inversely proportion to cutting (trimming) frequency and structurally changed in proportional to the hair length. Structure of human hair has to be physically or chemicallychanged by various causes that are shampooing, heatdrying, rubbing, contacting, and exposing to UV. Meanwhile, beautytreatments for permanent waving and coloringare a cause to damagehair structure[22-25]. In particular, repeated beauty treatments may be a cause to harshly damage not only cuticle layers but also cortex[26, 27]. The damaged hair is impossibleto be structurally restored to the original state because grown hair isa mixture of lifeless compounds like a corneous tissue.

The chemical denaturation of hair proteins and physical breakage of cuticle layers are a major cause to damage hairs. The denatured proteins and broken cuticle layers can't be chemically or biochemically substituted by other biological compounds. The local lesion site (cuticle layer) of damaged hairs has been chemically fixed by treatment of synthesized polymers on hair[28-30]. The synthesized polymers are copolymer of vinylacetate and crotonic acid, silicone polymers, polyvinylpyrrolidone (PVP), copolymer of PVP-vinylacetate, octylacrylamide acrylates butylaminoethyl methacrylate, and polyurethane-1. The synthetic polymers are effective for fixation of damaged hairs but safety concerns due to contamination with monomeric impurity. Monomers are sometimes highly toxic or even carcinogenic or highly irritating to skin[30]. On the other hand, biopolymers (carbohydrate and protein) are biologically safe but are not verified to be effective for recovery of damaged hair.

SEM image of normal or damaged hair limitedly shows exocuticle layers, which may be one of the naturalfactorsfor determination of hair texture. Textureof damaged hair may be worsen in proportion to size of gaps between cuticle layers and irregularity of cuticle

scales but gaps between cuticle layers or scales were filled by treatmentwith the biopolymers (EPS and HHP). EPS and HHP may commonly be a filler for the gaps between cuticle layers based on the SEM image (Figure 3); however, HHP may bea linker for connecting thedamaged fibrous proteins constituted of cuticle layer or cortex based on the tensile strength (TABLE 1). The tensile strength was reported to be influenced byfibrous proteins constituted of cortex but not by those of cuticle layer[31-34]. Accordingly, it can be supposed that the HHP may put into cortex and recover the damaged fibrous proteins by connecting or attaching but EPS may not. Most parts of human hair are composed of fibrous and filamentous proteins, which are chemicallyaffinity more with HHP than EPS based on the difference of tensile strength (TABLE 1). The HHP is very possible to be a mixture of various fibrous and filamentous proteins because it was non-selectively extracted from normal human hair and converted to solubilized polymers of which molecular weight is approximate 100, 000 (Figure 2). Viscosity, gelling activity, and molecular attraction may be proportional to molecular weight of polymer under condition without other molecular interactions that are ionic and hydrogen bonds. The tensile strength may be more objective than SEM image to evaluate effect of EPS and HHP for damaged hair because the recovery of damaged hair has to be determined by function rather than structure.

Chemical interaction between fibrous protein and HHP may be stronger than that between fibrous protein and EPS based on the chemical difference between protein and carbohydrate. Binding force between structurally homologous proteins is possible to be generated by the hydrogen bond, ionic bond, and hydrophobic bond but that between protein and carbohydrate may be generated by only random hydrogen bond. The functional group of EPS is only alcohol but those of HHP are carboxy, amine, imine, hydroxide, thiol, and alkyl group.

Human hairs may have physiological function that is protection of scalp from UV-radiation, temperature variation, and physical impact and cultural function that is beauty treatment for styling, permanent waving (curling), coloring, and bleaching. The beauty treatment of hair may be a cause to damage hair structure; meanwhile, the damage of hair may be a cause to deplete effect of beauty treatments and also to weaken physiological function. Accordingly, biochemical technique for recovery of damaged hair is required to be developed using natural polymers. Various natural polymers that are produced by plants, animals, fungi, and bacteria may be applicable for recovery of damaged hair; however, the protein extracted from human hair may be more structurally affinity with human hair cuticle than proteins that can be produced by other organisms except human. Conclusively, structurally and functionally damaged human hairs may be effectively recovered by using protein extracted from human hair.

REFERENCES

[1] R.R.Clarence; Chemical and physical behavior of human hair, 4th Edition, Springer; New York, **(2002)**.
[2] J.A.Swift; J.Cosmet.Sci., **50**, 23 **(1999)**.
[3] J.A.Swift, A.C.Bews; J.Cosmet.Sci., **23**, 695 **(1972)**.
[4] C.Earland, D.J.Raven; Nature, **191,** 384 **(1961)**.
[5] N.Fair, B.S.Gupta; J.S.Cosmet.Chem., 33, 229 **(1982)**.
[6] F.Gjesdal; Acta Pathol.Microbiol.Microbiol.Scand., **133**, 12 **(1959)**.
[7] P.J.Flesch; Soc.Cosmet.Chem., **19**, 675 **(1968)**.
[8] M.G.JrEckstrom; J.Soc.Cosmet.Chem., **2**, 244 **(1951)**.
[9] A.Schwartz, D.Knowles; J.Soc.Cosmet.Chem., **14**, 455 **(1963)**.
[10] D.Slawinska, J.Slawinska; Physiol.Chem.Phys., **14**, 363 **(1982)**.
[11] T.Sarna, I.A.Menon, R.C.Sealy; Phtochem.Phtobiol., **39**, 805 **(1984)**.
[12] K.Takada, A.Nakamura, N.Matsuo, A.Inoue, K.Someya, H.Shimogaki; J.Oleo Scie., **52**, 541 **(2003)**.
[13] G.H.vanGeel-Schutten, E.J.Faber, E.Smit, K.Bonting, M.R.Smith, B.Ten Brink, J.P.Kamerling, J.F.G.Vliegenthart, L.Dijkhunizen; Appl.Environ.Microbiol., **65**, 3008 **(1999)**.
[14] S.A.F.T.vanHijum, S.Karlj, L.K.Ozimek, L.Dijkhuizen, I.G.H.vanGeel-Schutten; Mol.Biol.Rev., **70**, 157 **(2006)**.
[15] S.Kraij, G.H.vanGeel-Schuttern, H.Rahaomi, R.J.Leer, E.J.Faber, M.H.E.C.van der Maarel, L.Dijkhuizen; Appl.Environ.Microbiol., **68**, 4283**(2002)**.
[16] P.J.Looijesteijn, I.C.Boels, M.Kleerebezem,

J.Hugenholtz; Appl.Environ.Microbiol., **65**, 5003 **(1999)**.
[17] M.Korakli, M.Pavlovic, M.G.Gänzle, R.F.Vogel; Appl.Environ.Microbiol., **69**, 2073 **(2003)**.
[18] M.J.Kim, H.N.Seo, T.S.Hwang, S.H.Lee, D.H.Park; J.Microbiol., **46**, 535 **(2008)**.
[19] Y.Shimomura, M.Ito; Symposium Proceeding.European Society for Dermatological Research, **10**, 230 **(2005)**.
[20] W.G.Crewther, R.D.B.Fraser, F.G.Lennox, H.Lindley; The Chemistry of Keratins, in C.B.Anfinsen, M.L.Anson, J.T.Edsall, F.M.Richards, (Eds); 'Advances in Protein Chemistry', Academic Press (New York), 191-347 **(1965)**.
[21] A.Nakamura, M.Arimoto, K.Takeuchi, T.Fujii; Biol. Pharm.Bull., **25**, 569 **(2002)**.
[22] S.C.Kelly, V.N.E.Robinson; J.Soc.Cosmet.Chem., **33**, 203 **(1982)**.
[23] J.A.Swift, B.Bews; J.Soc.Cosmet.Chem., **25**, 13 **(1974)**.
[24] M.Gamez-Garcia; J.Cosmet.Sci., **49**, 141 **(1988)**.
[25] R.McMillen, J.Jachowicz; J.Cosmetic.Sci., **49**, 223 **(1988)**.
[26] Y.Kamath, D.Weigmann; J.Soc.Cosmet.Chem., **35**, 21 **(1984)**.
[27] J.G.Gould, R.L.Sneath; J.Soc.Cosmet.Chem., **36**, 53 **(1985)**.
[28] J.Jachowicz, K.Yao; J.Soc.Cosmet.Chem., **47**, 73 **(1996)**.
[29] R.Ayer, J.Thompson; J.Soc.Cosmet.Chem., **23**, 617 **(1972)**.
[30] J.K.Wagoner; Ann, N.Y.Acad.Sci., **246**, 1 **(1975)**.
[31] A.Elliot; Textile Res.J., **22**, 783 **(1952)**.
[32] L.J.Wolfram, M.Lindemann; J.Soc.Cosmet.Chem., **22**, 839 **(1971)**.
[33] C.R.Robbins, R.Crawford; J.Soc.Cosmet.Chem., **42**, 59 **(1991)**.
[34] W.J.Simpson; Textile Inst., **51T**, 675 **(1965)**.