Research Article **AJBB (2018) 2:8**

American Journal of Biotechnology and Bioscience (ISSN:2572-8768)

Enhanced Expression of Dermal Aquaporins by Polysaccharide from *Camellia sinensis*

Byeong Mun Kwak, Lulu Han, Dong Sun Lee[1], Young Woon Ju[1] and Sang Yo BYUN*

Cosmetic Science Major, Applied Biotechnology Program, Graduate School, Ajou University, Suwon, 16499; [1]DadangAnd Co. Ltd., Sanhak Venture Hall, Ajou University, Suwon, 16499

ABSTRACT

The green tea polysaccharide was extracted from leaves of *Camellia sinensis* and hydrolyzed with enzyme. Skin moisturization effect of green tea polysaccharide was studied with 2D- PAGE analysis of epidermal hydration factors. Among up-regulated proteins extracted from Hacat cell treated with Green tea polysaccharide on 2DE gel, 5 aquaporins were observed. Among them the aquaporin 5 and 8 were increased 3.4 and 5.3 times respectively. The green tea polysaccharide was found to be effective for skin moisturization by enhanced expression of aquaporins.

Keywords: green tea, polysaccharide, moisturization, aquaporin

***Correspondence to Author:**
Prof. Sang Yo Byun, Ph.D.
Head, Cosmetic Science Major, Applied Biotechnology Department, Graduate School, Director, Cosmetic Business R&D Center, Ajou University, Suwon, Korea. 16499
Tel: +82-31-219-2451
Mobile: +82-10-2767-2410

How to cite this article:
Byeong Mun Kwak, Lulu Han, Dong Sun Lee, Young Woon Ju and Sang Yo BYUN.Enhanced Expression of Dermal Aquaporins by Polysaccharide from Camellia sinensis. American Journal of Biotechnology and Bioscience, 2018; 2:8.

eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

Introduction

The epidermis is the outmost part of the skin and it is made up of five layers. The outmost layer of those skin layers is stratum corneum and acts as protective skin barriers. Before the 70's in last century, the stratum corneum was regarded as bio-inert tissue, seemed to be a thin plastic film that protects the lower layers of the skin. In the past a few years, scientists have found that the stratum corneum has biological and chemical activity that is quite complicated. The stratum corneum has a "brick and mortar" structure in which the "bricks" are corneocytes consisted of protein complexes. Corneocytes are composed of small threads of keratin in an organized matrix. The keratin contains a lot of water among the fibers. The stratum corneum consists of around 12~16 layers of corneocytes and the thickness of corneocyte is average 1μm.

For a long time, scientists believed that the cells are offered with their own channels for water transportation. Water can pass easily and rapidly through those channels. With several years of efforts and studies, in 1992, scientists who made breakthrough eventually discovered water channels in the cell membrane, and then named them aquaporins. [1-5]

The epidermis is the most top layer of skin that does not contain blood vessels. Besides, it does not like most of the other organs of the body which absorb nutrients from the blood, but epidermis through the water circulating to nourish itself. Water not only supplies inorganic salts and other nutrients, but also moisturizes and smooth the skin. 13 different aquaporins (AQP0 to AQP12) have been identified in the human body, but until recently it has been determined that AQP 3 is existed in the epidermis and very effective. AQP3 regulating water transportation plays a key role in influencing skin moisture, skin elasticity and skin barrier function [6-8].

This study was carried out to evaluate the moisturization efficacy of polysaccharide from green tea, *Camellia sinensis*. Expressions of epidermal aquaporins analyzed to evaluate the skin hydration efficacies by green tea polysaccharides.

Experimental

Green tea polysaccharide was extracted from leaves of *Camellia sinensis*. Prior to extraction, caffeine and catechins were removed. Polysaccharides extracted were hydrolyzed with viscozyme L. Medium chain polysaccharides were collected and dried. Hacat cell was seeding and after 6 hours cultivation, it was treated with polysaccharide at concentration of 1000 ppm. After 24 hours treatments, cells were harvested. 10^7 cells washed with PBS were isolated by centrifugation. Proteins were extracted by the lysis of cells. The lysis buffer solution was prepared with 7M urea, 2M Thiourea, 4% CHAPS, 1% DTT, 2% Carrier ampholyte, 40 μL/mL PIC, 1 μg/mL Benzonase, 0.002% BPB. Cells in lysis buffer were sonicated for 1 min and maintained for 30 min at the room temperature. The solution was centrifuged at 4000 x g for 5 min. The supernatant was collected and preserved at -20°C. The amount

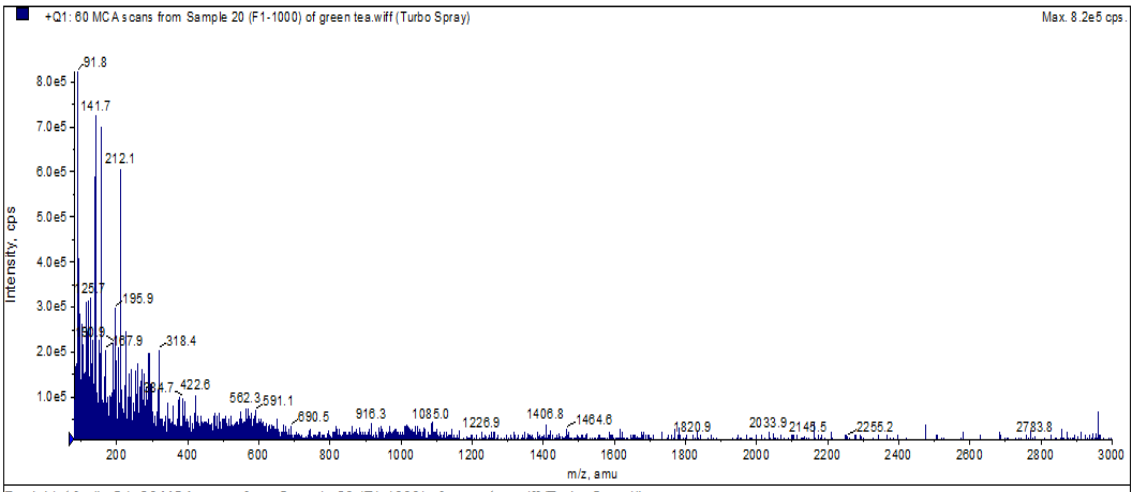
of total protein extracted was measured with modified Bradford method. Bovine γ-globulin was used as standard protein. Immobiline Dry strips (13cm, pI 3-10 NL, Amersham Biosciences, Sweden) were used with IPGphore fixed length strip holder. The strip was rehydrated with 250 µl of the rehydration solution for 12 hours and 100 µg of the sample proteome was injected simultaneously. The rehydration solution was prepared with 7M urea, 2M Thiourea, 2% CHAPS, 1% DTT, 2% Carrier ampholyte, 10% glycerol, 0.002% BPB. Isoelectric focusing was done in IPGphore (Amersham Bioscience, Sweden). After 12 h of rehydration, stepwise focusing was made, 1 h at 500 V, 1 h at 2000 V, then increased to 8000 V and maintained until no current change was observed. Before the SDS electrophoresis, focused strips were equilibrated in buffer solutions. After the focusing, the strip was immersed in 10 ml of the equilibrium buffer solution (7M urea, 2M Thiourea, 2% SDS, 50mM Tris-HCl, 30% glycerol) with 1% DTT and maintained under the mild shaking for 15 min. Then it was immersed again in the equilibrium buffer solution containing 2.5% iodoacetamide and maintained with mild shaking for 15 min. 10.5% acrylamide homogenous gels (T-13%, C-2.5%, 18x24 cm) were made in 1.5 mm thickness. For stacking, 0.5% low melting agarose was used. The IPG strip was located on the top of the SDS gel with the size marker and the stacking gel was poured. For the stacking, 10 mA was applied for 25 min. For the electrophoresis 35 mA/gel was applied until the bromophenol blue marker striped out. The gel was washed for 1 h with dH2O. It was fixed for 1 h in the fixing solution prepared with 50% methanol and 5% acetic acid. After the repeated washing for 1.5 h in dH2O, the gel was maintained for 5 min in 0.02% sodium thiosulfate solution and then washed twice for 10 min. The staining was made in the refrigerated 0.1% silver nitrate solution for 30 min. After the duplicate washing for a minute, the gel was maintained for 10 min in the developing solution composed of 2% sodium carbonate, 0.014% formaldehyde, and sodium thiosulfate. Once protein spots were recognized, 1% acetic acid was added and maintained 10 min for the fixation. The stained gel was scanned and protein spot images were analyzed with 2D Elite (Amersham Biosciences, Sweden) image analysis software.

Results and Discussion

Composition of green tea polysaccharide

Polysaccharide extracted and enzyme hydrolyzed was analyzed to evaluate its composition. Analysis was made with tandem mass spectrometer(API-150). The polysaccharide used in this study was composed of various saccharides, monosaccharide, disaccharide, oligosaccharide, and polysaccharide as shown in Fig 1.

The quantitative composition of various saccharides were also analyzed according to their m/z value. As shown in Fig 2, oligosaccharide was the most abundant components in the green tea polysaccharide used for studies.



Peak List for "+Q1: 60 MCA scans from Sample 20 (F1-1000) of green tea.wiff (Turbo Spray)"

	m/z (amu)	Intensity (cps)	Centroid mass (amu)	Charges	% Intensity	% Centroid
14	90.8000	1.5185e5	90.8071	1	18.4397	50.0000
15	91.1000	5.2562e4	91.1000	Undefined	6.3830	100.0000
16	91.4000	5.2562e4	91.4000	Undefined	6.3830	50.0000
17	91.8000	8.2347e5	91.8000	1	100.0000	50.0000
18	92.6000	2.9201e4	92.6000	2	3.5461	100.0000
19	92.9000	1.9857e5	92.9000	1	24.1135	50.0000
20	93.2000	2.3361e4	93.2333	2	2.8369	50.0000
21	93.5000	2.9201e4	93.5000	Undefined	3.5461	100.0000
22	93.9000	1.5185e5	93.8857	Undefined	18.4397	50.0000
23	94.8000	7.5923e4	94.7789	1	9.2199	50.0000
24	95.4000	3.5041e4	95.4000	Undefined	4.2553	50.0000
25	95.8000	1.0512e5	95.8105	Undefined	12.7660	50.0000
26	96.6000	2.8617e5	96.6985	1	34.7518	50.0000

Fig. 1. ESI-MS analysis of green tea polysaccharide. Various saccharides were analyzed according to their m/z values.

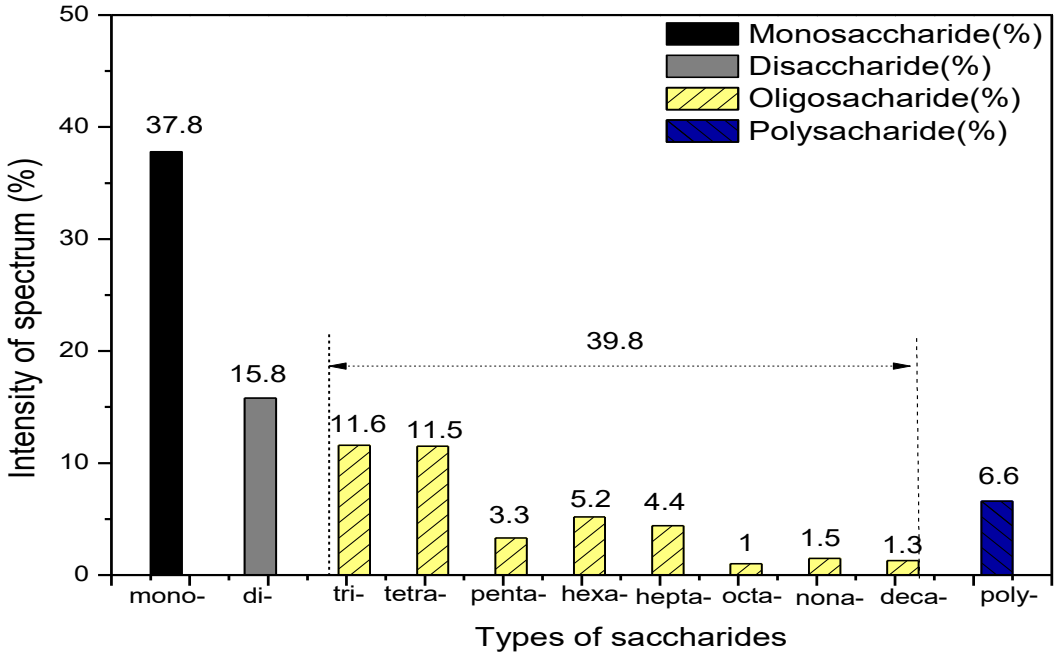


Fig. 2. Composition of green tea polysaccharide. ESI-MS analysis showed oligosaccharides were most abundant saccharides.

Proteome analysis for expression of aquaporins

Proteome technique provides an overview of various aquaporins related to skin moisturization. So it can be an efficient tool to find out complicated skin hydration caused by expression of aquaporins. The protein profiling was performed as comparison to expressed protein with and without green tea polysaccharide. Protein spot images in the 24x24 cm gels are shown. Fig. 3. is protein spots from the cell not treated with green tea polysaccharide. Fig. 4, on the other hand, is proteins spots with green tea polysaccharide.

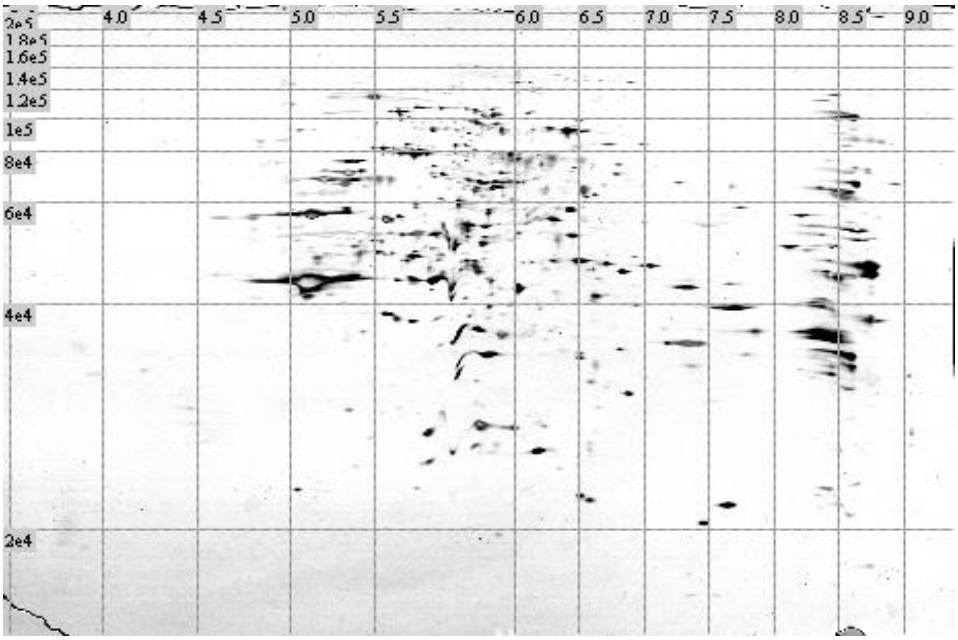


Fig. 3. Proteins spots of control 2DE gel.

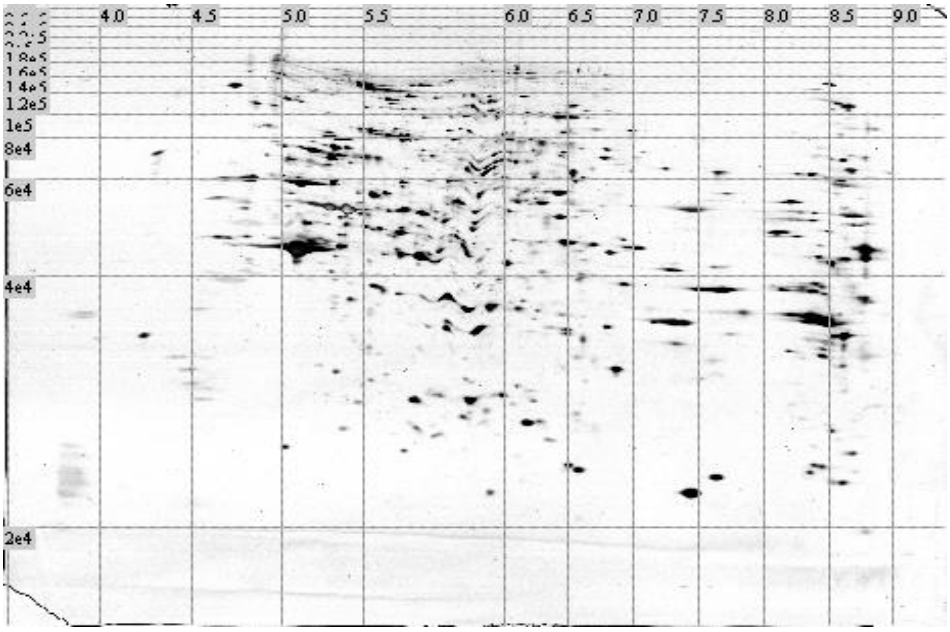


Fig. 4: Image of proteins extracted from Hacat cell treated with Green tea polysaccharide on 2DE gel

In the control gel, 1050 spots were identified. And in the gel with green tea polysaccharide, 1376 spots were identified. When the comparison between with and without green tea polysaccharide, 633 spots were matched. Among them 203 spots were down-regulated and 430 spots were up-regulated. Among those up-regulated proteins, we found 5 aquaporins as shown in Fig. 5.

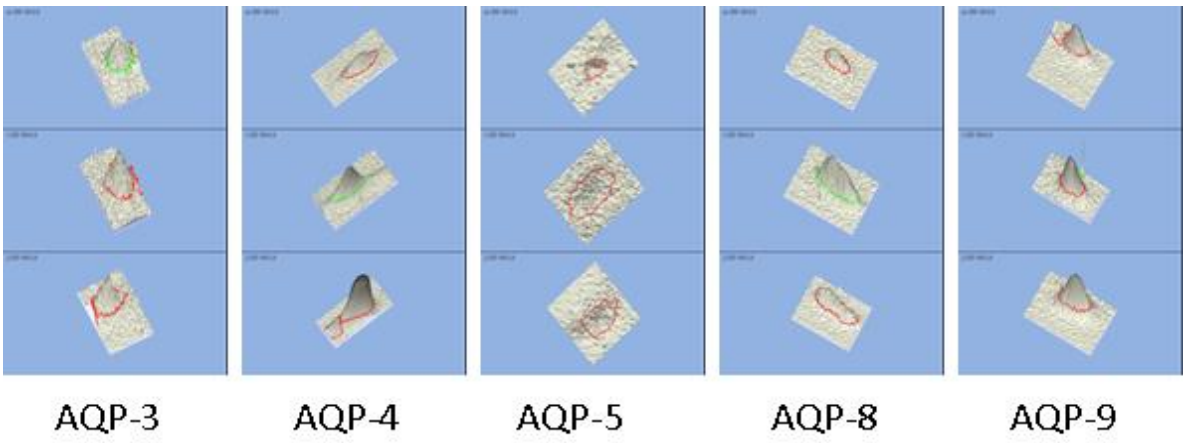


Fig. 5: Three dimensional images of aquaporins expressed by green tea polysaccharide.

Table. 1. Summary of increased expression of aquaporins by green tea polysaccharide.

2DE spot proteins		Expression Increased (% of up-regulation compared to the control)
Aquaporins	AQP-3	161.9
	AQP-4	158.5
	AQP-5	343.0
	AQP-8	534.1
	AQP-9	138.6

Auaporins are related to epidermal hydration. The enhanced expression of 5 aquaporins represents the green tea polysaccharide is efficient to epidermal skin moisturization. Among various aquaporins, AQP5 was the most much expressed by green tea polysaccharide as shown in Table 1. Up-regulated expression of other aquaporins was also believed to increase epidermal hydration.

Acknowledgements

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea.(Grant No.:HN14C0087).

References

1. M. Hara-Chikuma and A.S. Verkman, Role of Aquaporin 3 in the epidermis, Journal of Investigative Dermatology, 128, 2145(2008)
2. J.W. Fluhr, R. Darienski, C. Surber, Glycerol and the skin: historic approach to its origin andfunction, British Journal of Dermatology. 159(1), 23(2008)
3. M. Olsson, A. Broberg, M. Jernas, L. Carlsson, M. Rudemo, M. Suukula, PA. Svensson, M. Benson, Increased expression of aquaporin 3 in atopic eczema, Allergy, 61, 1132(2006)
4. J. Li, H. Tang, X. Hu, M. Chen, H. Xie, Aquaporin-3 gene and protein expression in sun-protected human skin decreases with skis aging, The Australian journal of Dermatology, 51, 106(2010)
5. Anatomy & Physiology, Connexions Web site. http://cnx.org/content/col11496/1.6/, (2013)
6. http://www.kanebo.com/science/skin/aquaporin.html
7. H. Tanii, T. Hasegawa, N. Hirakawa, M. Suzuki, S. Tanaka, Molecular and Cellular Characterization of a Water-Channel Protein, AQP-h3, Specifically Expressed in the Frog Ventral Skin, Journal of Membrane Biology, 188, 43(2002)
8. M. Hara-Chikuma, K. Takahashi, S. Chikuma, A.S. Verkman, Y. Miyachi, The expression of differentiation markers in aquaporin-3 deficient epidermis, Arch Dermatol Research, 301, 245(2009)