



From *Pinctada
margaritifera* in Tahiti

Promois[®] BLACK PEARL

Hydrolyzed Conchiolin



Seiwa Kasei Co., Ltd.

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1. Introduction

· Amino Acid Composition

Amino Acid	mol%	Amino Acid	mol%
Glycine	45.0	Proline	5.0
Alanine	11.0	Methionine	1.7
Serine	2.6	Cystine/2	-
Threonine	0.7	Cysteic Acid	0.2
Valine	4.4	Arginine	-
Leucine	5.8	Histidine	0.8
Isoleucine	3.1	Lysine	1.6
Phenylalanine	2.6	Aspartic Acid	9.2
Tyrosine	2.9	Glutamic Acid	3.4

 : Acidic Amino Acid  : Basic Amino Acid
 : Neutral Amino Acid

2. Advantage

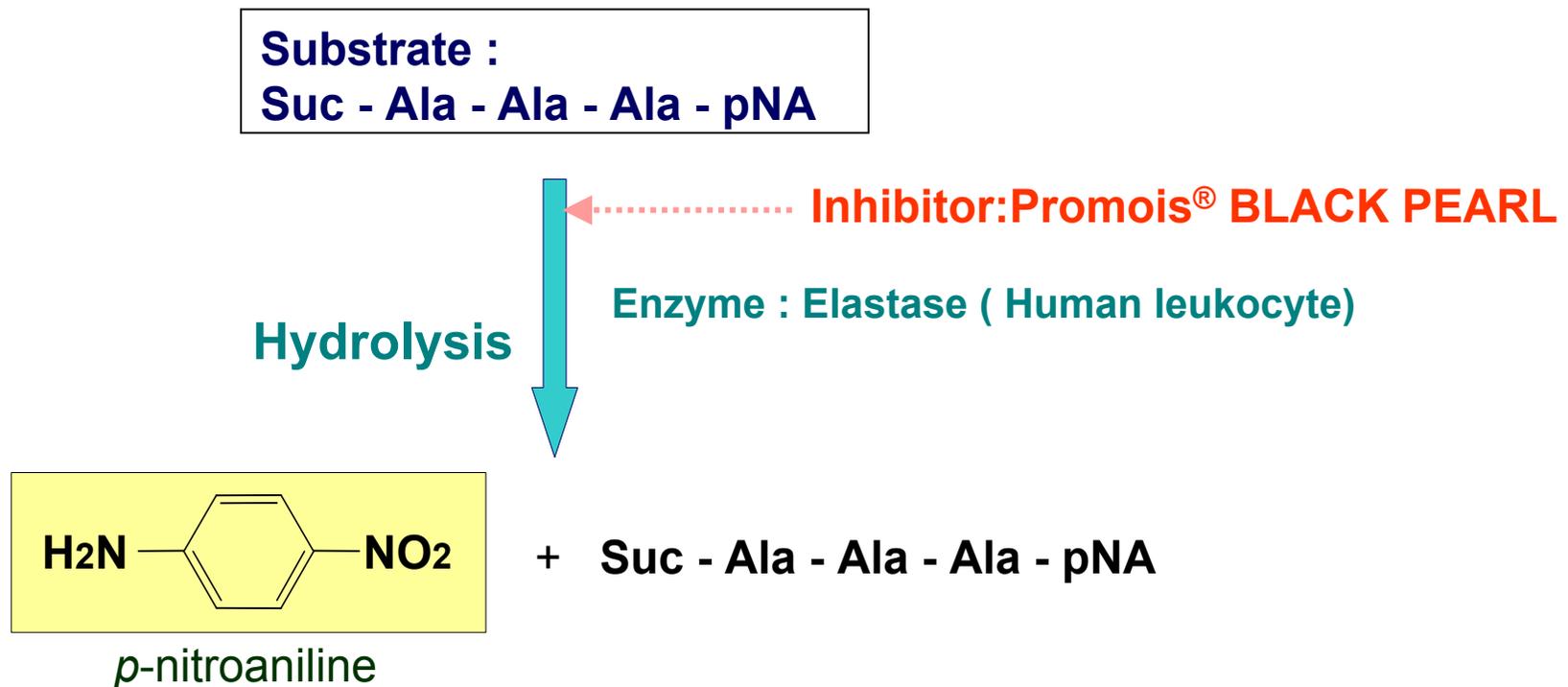
Promois® BLACK PEARL has following effects for keeping skin & hair healthy .

- Alleviation effect for skin stresses
 - Anti-elastase activity
 - Anti-tyrosinase activity
- Repairing effect for damaged hair
 - Keeping moisture
 - Smoothing cuticle

2. Advantage : Alleviation effect for skin stresses

- Anti-elastase activity (*in vitro*)

To confirm anti-elastase activity, the amount of *p*-nitroaniline produced in the following reaction systems was measured with a spectrophotometer at 410 nm.



2. Advantage : Alleviation effect for skin stresses

- Anti-elastase activity (*in vitro*)

Reaction system was consisted of as follows :

Enzyme	: Elastase from H.L. (1 U/mL)	0.05 mL
Substrate	: 125 mM Suc - Ala - Ala - Ala - pNA	0.02 mL
Inhibitor	: Promois [®] BLACK PEARL	X mL
Buffer solution	: 100 mM Tris-HCl pH 7.5	2.63 - X mL
		2.7 mL

- X varies with the concentration of the inhibitor.
- The temperature under the measurement is kept on 40 °C.

The inhibition rate is calculated according to the following equation.

$$\text{Inhibition rate(\%)} = \frac{b}{a} \times 100$$

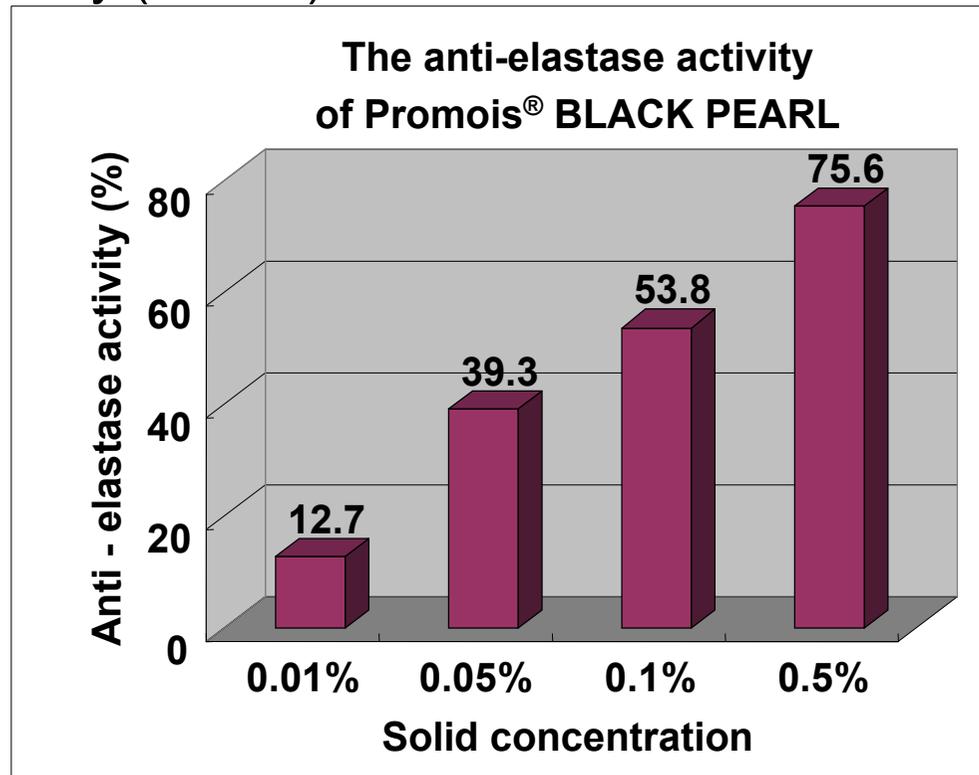
a : $\Delta A_{410\text{nm}}$ without inhibitor , b : $\Delta A_{410\text{nm}}$ with inhibitor

$\Delta A_{410\text{nm}} = (\text{Final absorbance} - \text{Initial absorbance}) / \text{time of measurement}$

The absorbance is a value measured with a spectrophotometer at 410 nm.

2. Advantage : Alleviation effect for skin stresses

- Anti-elastase activity (*in vitro*)



Anti-elastase activity raised as concentration of Promois® BLACK PEARL increased.

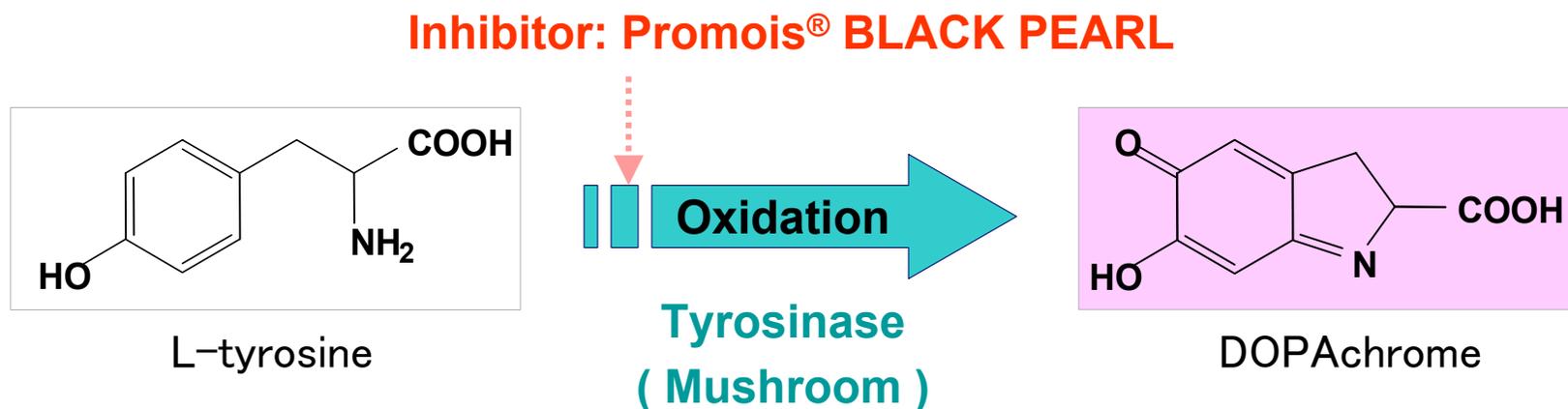


Promois® BLACK PEARL lowers elastase activity. Promois® BLACK PEARL protects Elastin which is the elastic component of the skin.

2. Advantage : Alleviation effect for skin stresses

- Anti-tyrosinase activity (*in vitro*)

To confirm anti-tyrosinase activity, the amount of DOPAchrome produced in the following reaction systems was measured with a spectrophotometer at 475 nm.



2. Advantage : Alleviation effect for skin stresses

- Anti-tyrosinase activity (*in vitro*)

Reaction system was consisted of as follows :

Enzyme	: Tyrosinase from mushroom (1000 U/mL)	0.1 mL
Substrate	: L-tyrosine (0.5 mg/mL)	1.2 mL
Inhibitor	: Promois [®] BLACK PEARL	X mL
Buffer solution	: 100 mM PBS pH 6.8	1.4 -X mL
		<hr/>
		2.7 mL

- X varies with the concentration of the Inhibitor.
- The temperature under the measurement is kept on 40 °C.

The Inhibition rate is calculated according to the following equation.

$$\text{Inhibition rate(\%)} = \frac{b}{a} \times 100$$

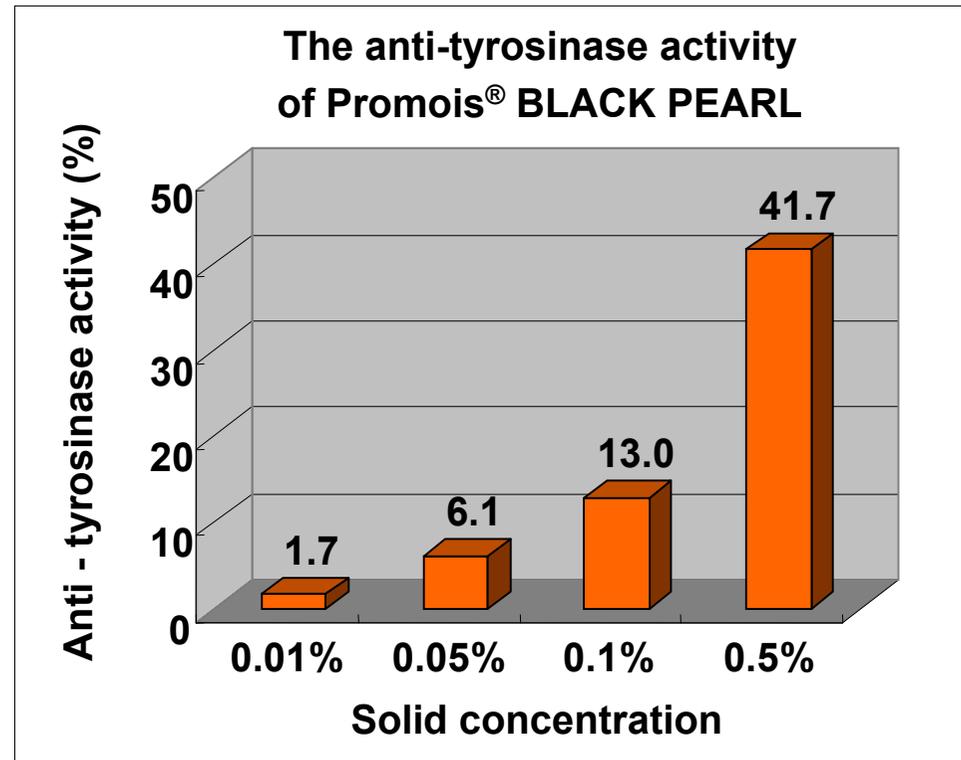
a : $\Delta A_{475\text{nm}}$ without Inhibitor , b : $\Delta A_{475\text{nm}}$ with Inhibitor

$\Delta A_{475\text{nm}} = (\text{Final absorbance} - \text{Initial absorbance}) / \text{time of measurement}$

The absorbance is a value measured with a spectrophotometer at 475 nm.

2. Advantage : Alleviation effect for skin stresses

- Anti-tyrosinase activity (*in vitro*)



Anti-tyrosinase activity raised as concentration of Promois® BLACK PEARL increased.



Promois® BLACK PEARL lowers tyrosinase activity, which means Promois® BLACK PEARL inhibits melanin production that is generated by the exposure to ultraviolet rays etc.

2. Advantage : Repairing effect for damaged hair

- Measurement of secondary transpiration moisture

Japanese hair



Bleaching

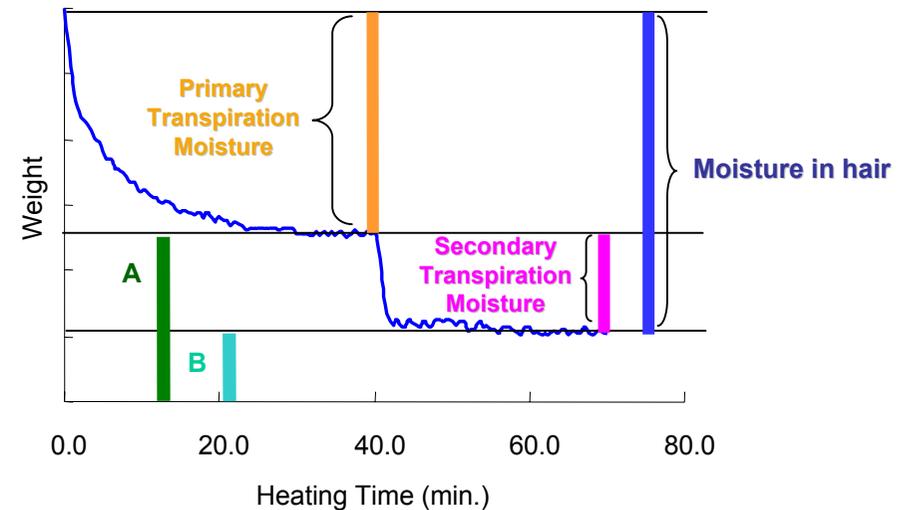


Treatment with peptide solution (10 times)

Hair tress was soaked in 1% solid content water solution with agitation at 40°C for 10 min. Control was soaked in deionized water.



Measurement of secondary transpiration moisture



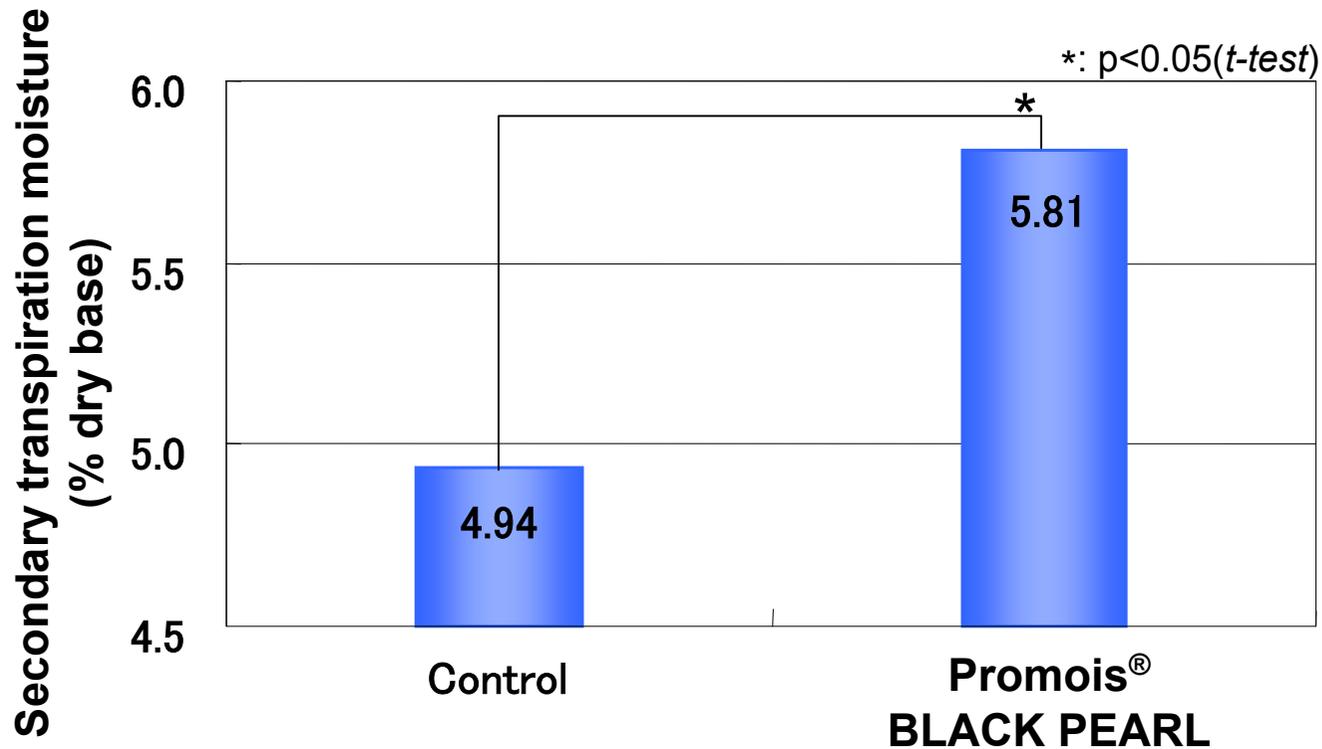
$$\text{Secondary transpiration moisture (\% dry base)} = \frac{(A-B)}{B} \times 100$$

Moisturizing effect was evaluated by measurement of moisture inside the hair. Water loss was measured with electric moisture balance (model: EB-340MOC, Shimadzu Co., Ltd.). Shredded hair fragments (300 mg, 1cm length) were placed on a saucer of the balance, and weight change in the hair was measured. The sample hair was heated at 65 °C (assumed the temperature of hair-dryer) for 40 min, and then the hair was heated at 180 °C (assumed the temperature when whole hair moisture transpires) for 30min.

As shown in Figure, based on the difference of weights between A and B, the secondary transpiration moisture was calculated according to the above equation.

2. Advantage : Repairing effect for damaged hair

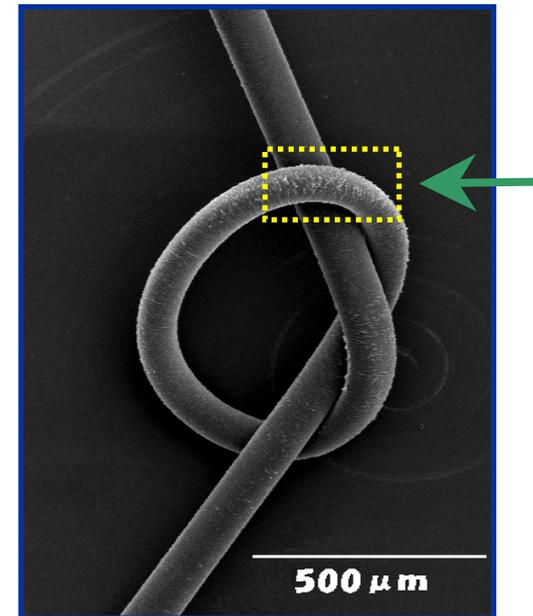
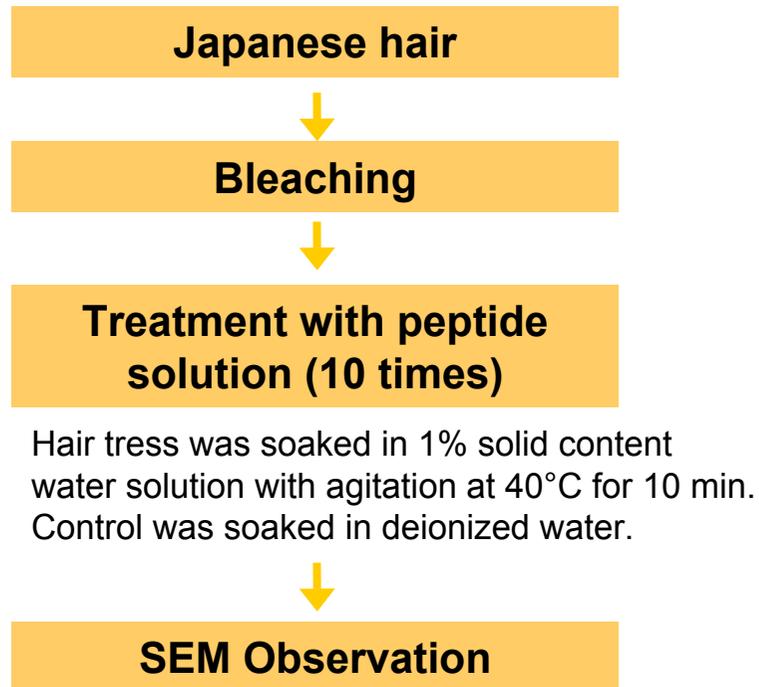
- Measurement of secondary transpiration moisture



Promois® BLACK PEARL has an excellent moisturizing property and restores hair moisture.

2. Advantage : Repairing effect for damaged hair

- SEM Observation



scanning electron microphotograph

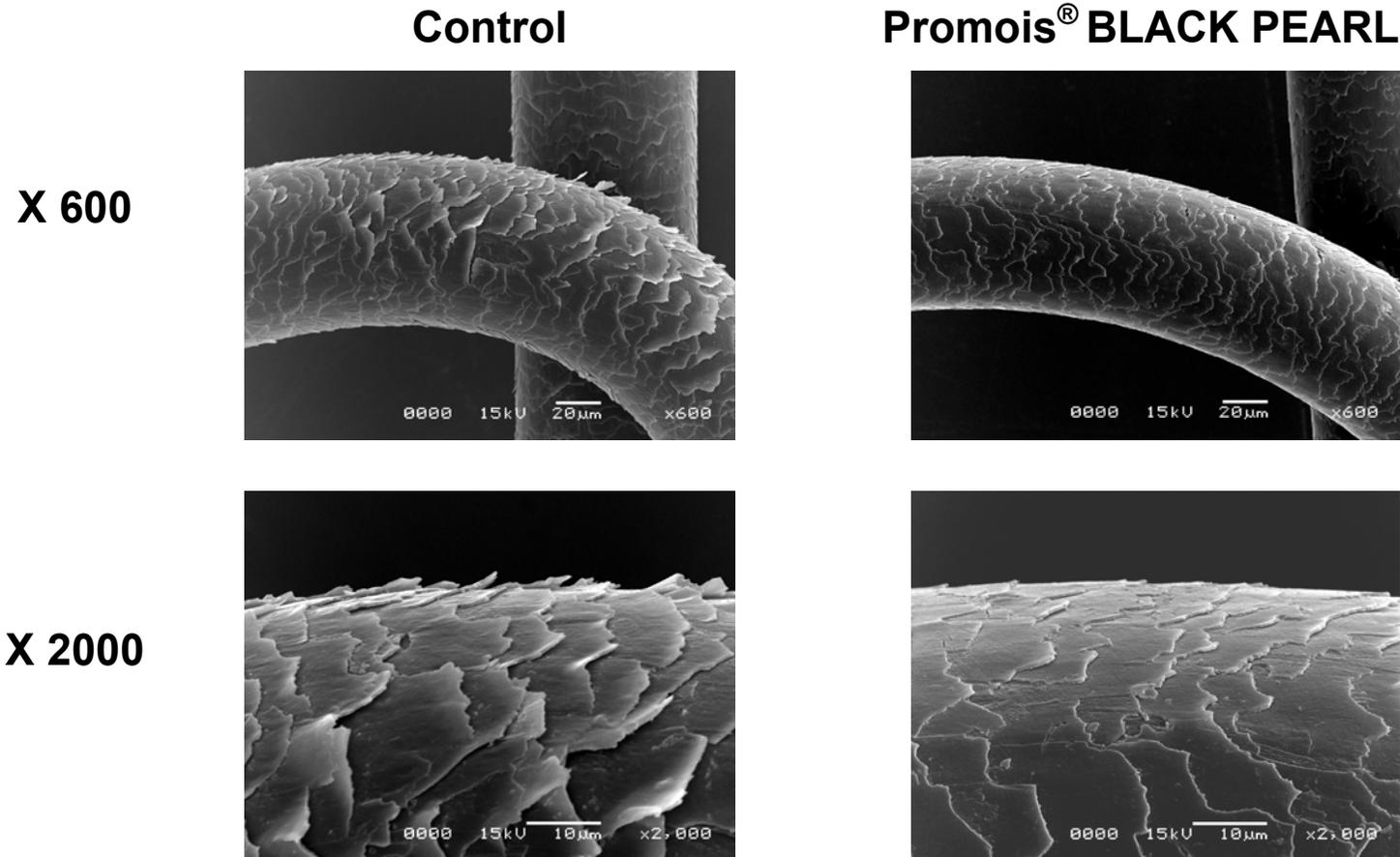
Equipment : JEOL JSM-5800LV (JEOL Ltd.)

Condition : Photographed a tied knot in a hair strand.

(x600, x2000)

2. Advantage : Repairing effect for damaged hair

- SEM Observation



Pormois® BLACK PEARL repairs hair surface damaged by bleaching, and smoothes hair surface.

3. Product information

- Product information

Promois® BLACK PEARL	
INCI name	Hydrolyzed Conchiolin Protein
Appearance	Yellowish brown transparent liquid
Number – average molecular weight	600
Active substance	3 %
Solvent	Water
Origin	Hydrolyzed conchiolin : Black-lipped Pearl Oyster (from Tahiti)
Package	1kg / 15 kg

3. Product information

- Toxicological studies

Promois® BLACK PEARL	
HET-CAM Test	Non-irritant
AMES Test	Non-mutagenic
Human Repeated Insult Patch Test	Non-sensitizer
Patch Test	Non-irritant