LeeonIPL

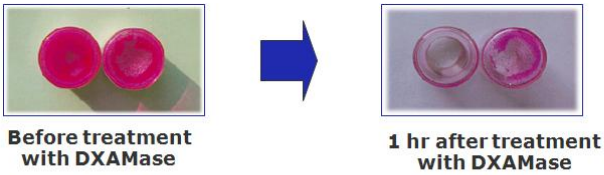
PROJECT NAME (Ingredient Trade Name): DXAMase
Active Ingredient Scientific Name: Multi-functional enzyme extracted from Lipomyces starkeyi KSM22
Submission Date: 15. April

Description of Product:

This technology relates to DXAMase purified from a strain of Lipomyces starkeyi KSM22. Specifically, this technology revealed that the enzyme isolated from the Lipomyces starkeyi KSM22 strain shows high ability to degrade substrates related to plaque formation, such as mutan, dextran, starch, sugar and levan. The enzyme was named “DXAMase”.

[Process for obtaining DXAMase enzyme]

- **DXAMase enzyme has high ability to degrade insoluble glucan, a major component of plaque, and thus can prevent tooth decay, tartar buildup, gingivitis, periodontitis, etc.**

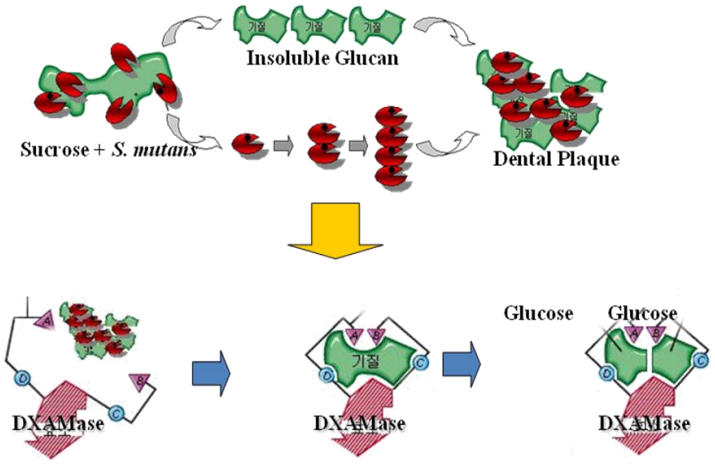


[Results of visual observation for ability to degrade insoluble glucan]

- **DXAMase was tested for toxicity in the Korea Research Institute of Chemical Technology, and the results thereof did not reveal any toxicity in terms of mortality, general symptoms, body weight change, or autopsy findings.**
- **DXAMase is stable at pH 4.5~8 and -30~60 ℃ and showed a significantly improved synergistic effect when used in combination with existing products (chemicals). Thus, DXAMase can be included in tooth-related products, such as mouthwash, toothpaste, milk powder, milk and gum for dental care.**
- **Maximum prevention of plaque thickening, superior plaque degradation effect, minimization of spread of periodontitis, excellent adhesion to teeth, and maximization of synergistic effect with mouse rinse.**

1. Supporting Evidence (Efficacy/Functionality):

1) Mechanism of Action/s (MOA)



- Dental plaque consists of 70% mocroorganisms and 30% insoluble glucan.

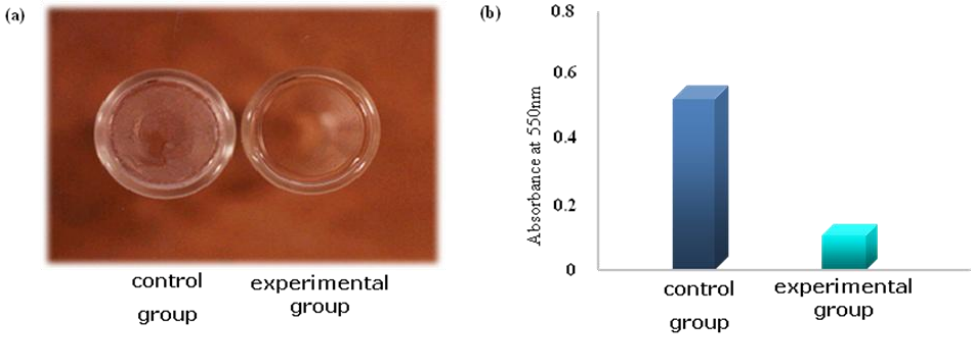
- DXAMase of this technology has a function of suppresing plaque formation by degrading insoluble glucan into water-soluble glucose.

2) Chemistry/Characterization data

This technology relates to an enzyme, and although there are no data on the chemical characteristics thereof, there are sequence data for the enzyme.

3) *In vitro* bioassay data

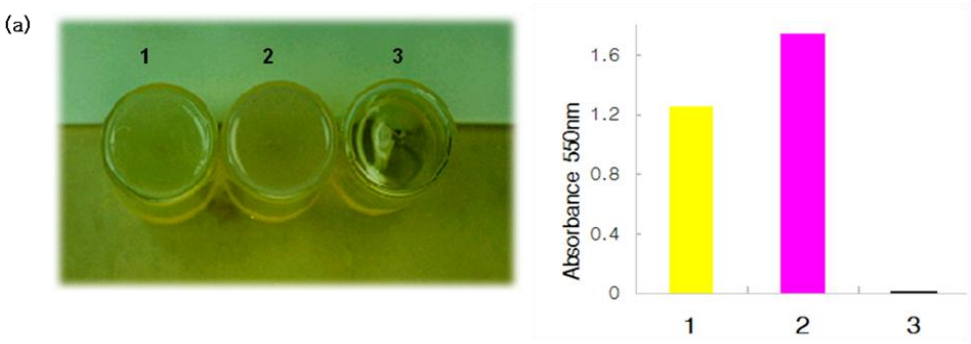
(1) Test for the ability of DXAMase to degrade insoluble glucan



(a), (b) control group - No DXAMase, experimental group - DXAMase

- The enzyme in this technology was tested at a concentration of 3 units/㎖.

(2) Measurement of synergistic effect of mouthwash containing DXAMase



(a) Measurement of synergistic effect of mouthwash containing DXAMase
1. Sucrose+mutansucrase
2. Sucrose+mutansucrase+mouthwash
3. Sucrose+mutansucrase+mouthwash containing DXAMase

(b) Measurement of absorbance (A_{550})

- It could be seen that DXAMase had a synergistic effect on the degradation of insoluble glucan when used in a mixture with existing mouthwash.

4) Clinically tested

(1) First clinical trial (measurement of effect of single substance)

- Subjects: general persons

- Conditions: percent of volunteers who were smokers: 10% (a total of about 40 persons)

- Method: Volunteers were made to gargle for 30 sec with 20 cc of each of a DXAMase-containing solution (3 units/㎖), a Chlorhexidine wash (0.5% general wash), and distilled water, and then the effects of these mouthwashes on the inhibition of plaque accumulation and gingival inflammation were comparatively evaluated.

- Period: 6 months

- Measures: tooth discoloration, degree of plaque accumulation, and degree of gingival inflammation

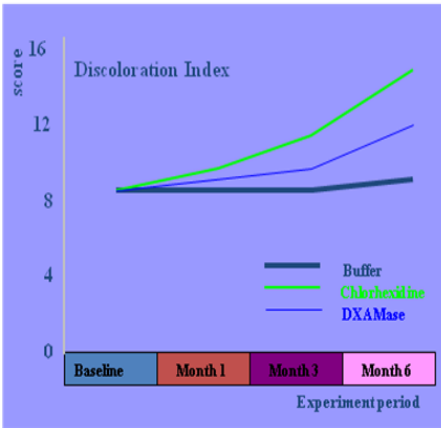
- Carried out in: College of Dentistry (head of research: Professor Hyun-Joo Jung), Chonnam National University

- Results:

tooth discoloration

		Baseline	Month 1	Month 3	Month 6
Buffer	n=724	8.371 ▪0.754	8.842 ▪0.777	9.227 ▪0.749	9.820 ▪0.755
Chlorhexidine	n=712	8.245▪0.720	9.705 ▪0.806	11.614 ▪0.685 *a	14.122▪0.499 ¶a
DXAMase	n=726	8.418 ▪0.787	9.255 ▪0.617	10.102 ▪0.621 *	11.885 ▪0.686*ab

Discolorationl index score during the experiment period



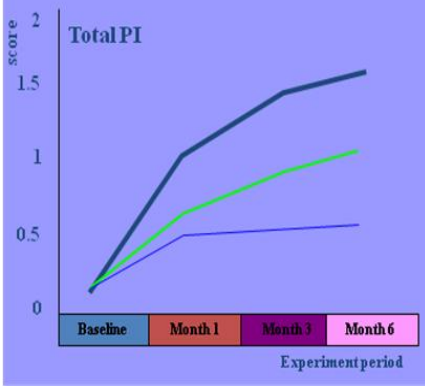
Values are expressed in Mean ▪ SE.
* Significantly different from Baseline : P<0.001
# Significantly different from Month 1 : P<0.001
¶ Significantly different from Month 2 : P<0.001
a Significantly different, compared to Buffer group : P<0.001
b Significantly different, compared to Chlorhexidine group : P<0.001

tooth discloration refers to the extent to which the teeth change color after washing

Change in degree of plaque accumulation

		Baseline	Month 1	Month 3	Month 6
Distilled water	n=2172	0.319 ▪0.012	1.140 ▪0.021 *	1.496 ▪0.024 #	1.746 ▪0.024 ¶
Chlorhexidine	n=2136	0.334▪0.012	0.848 ▪0.021 *a	1.157 ▪0.024 #a	1.343 ▪0.025 ¶a
DXAMase	n=2177	0.336 ▪0.013	0.446 ▪0.019 *ab	0.479 ▪0.019 #ab	0.534 ▪0.019 ¶ab

Plaque index score during the experiment period

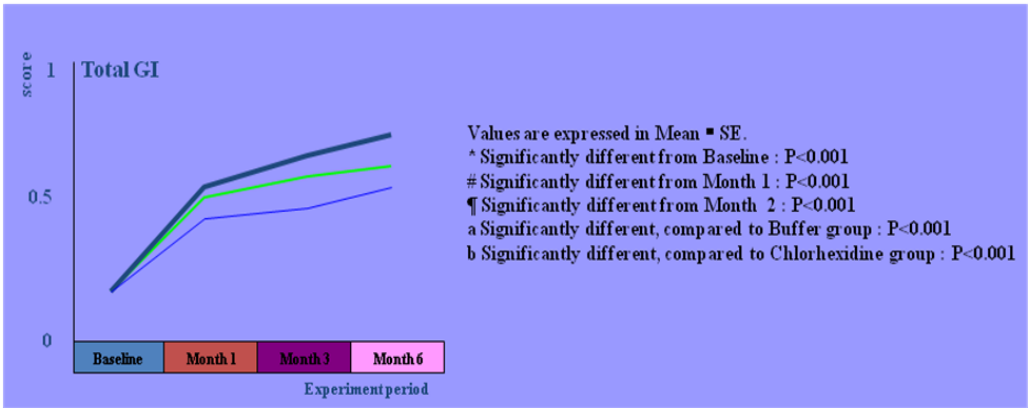


Values are expressed in Mean ▪ SE.
* Significantly different from Baseline : P<0.001
# Significantly different from Month 1 : P<0.001
¶ Significantly different from Month 2 : P<0.001
a Significantly different, compared to Buffer group : P<0.001
b Significantly different, compared to Chlorhexidine group : P<0.001

Change in degree of gingival inflammation

		Baseline	Month 1	Month 3	Month 6
Distilled water	n=2172	0.159 ▪0.008	0.532 ▪0.014 *	0.712 ▪0.015 #	0.856 ▪0.015 ¶
Chlorhexidine	n=2136	0.159▪0.008	0.501 ▪0.014 *a	0.616 ▪0.014 #a	0.696 ▪0.013 ¶a
DXAMase	n=2177	0.158 ▪0.008	0.454 ▪0.012 *ab	0.530 ▪0.012 #ab	0.619 ▪0.013 ¶ab

Gingival index score during the experiment period



Tooth discoloration: the DXAMase group showed low tooth discoloration compared to the general mouthwash group

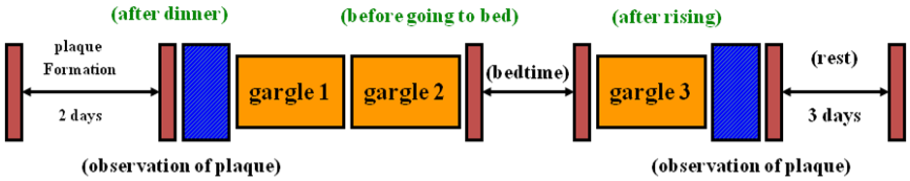
Degree of plaque accumulation: the DXAMase group showed the best effect on the inhibition of plaque accumulation.

Degree of gingival inflammation: the DXAMase group showed the best effect on the inhibition of gingival inflammation.

(2) Second clinical trial (measurement of synergistic effect of composite substance)

- Subject: general persons
- Conditions: percent of volunteers who were smokers: 10% (a total of about 6 persons)
- Method:

Experiment on synergistic effect with commercial gargle solution
(Samsung Medical Center)



1. plaque formation for 2 days
2. Observation and recording of plaque after dinner
3. gargle (30 ml, 1 min)
4. gargle (ml, 1 min) before going to bed
5. bedtime
6. gargle (ml, 1 min) after rising
7. observation of plaque
8. rest for 3 days

SAMSUNG Samsung Medical Center

<Control group: Listerine from Johnson & Johnson (hereinafter referred to as "gargle solution"), and experimental group: gargle solution + DXAMase (3 units/㎖)>

- Measures: Comparative measurement of plaque removal ability

- Carried out in: Samsung Medical Center (Professor Dong-Sung Park, Sungkyunkwan University)

- Results

- When dental plaque was examined before and after the use of the existing gargle solution, it could be seen that no substantial removal of dental plaque occurred.

- In contrast, it could be seen that the gargle solution + DXAMase mixture easily removed dental

plaque.

- Thus, this technology has an advantage in that it can be added to existing gargle solutions.

5) Conclusions from the studies

① Reduction in plaque area

② Reduction in plaque thickness

③ Inhibition of plaque production

The above results suggest that the enzyme of this technology has an excellent effect of inhibiting dental plaque.

6) Published or not published, and in which journal/s?

- Cloning and characterization of a dextranase gene from Lipomyces starkeyi and its expression in Saccharomyces cerevisiae (Yeast 2005; 22: 1239–1248)

- Biochemical Analysis of Recombinant Fungal Mutanases (THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 275, No. 3, Issue of January 21, pp. 2009–2018, 2000)

- Cloning and expression of Lipomyces starkeyi a-amylase in Escherichia coli and determination of some of its properties (FEMS Microbiology Letters 233 (2004) 53–64)

- Demonstration of Two independent Dextranase and Amylase Active Sties on a Single enzyme Elaborated by Lipomyces starkeyi KSM 22 (J. Microbiol, Biotechnol, (2003), 13(2) 313-316)

- Characterization of a Novel Carbohydrase from Lipomyces starkeyi KSM 22 for Dental Application ((J. Microbiol, Biotechnol, (1999), 9(3) 260-264)

- Purification and Partical Characterization of a Novel Glucanhydrolase from Lipomyces starkeyi KSM 22 and its Use for Inhihition of Insoluble Glucan Formation (Biosci. Biotechnol. Biochem., 64(2), 223-228, 2000)

7) Recommended Delivery form/s?

- DXAMase may be in any form that can be used as an additive to gargle solutions, patches or other foods.

8) Recommended dose/s? (mg per day)

- The recommended concentration of DXAMase is 3 units/㎖ (the recommended concentration relative to body weight is irrelevant, because the enzyme is not for dietary use, but for use as a mouthwash).

9) Collaborating Organizations, professors or University Affiliations

- Professor Chul-Ho Yoon (Chonnam National University)
- Professor Woong-Jin Kim (California Institute of Technology)
- Professor Do-Man Kim (Chonnam National University)
- Professor Do-Won Kim (Kangwon National University)
- Dr. Yoon-Suk Park (British Colombia University)
- Medical specialist Dong-Sung Park (dentist, Seoul National University)
- Dr. Kwang-Hoon Kong (Tokyo University)

Additional information about the professors can be provided upon request.

2. Intellectual Property / Exclusivity

1) Provide patent information

Title of Invention	Korean Patent Registration No.	Filing Date	Foreign Patent Registration No.
Enzyme capable of hydrolyzing plaque, microorganism producing the same, and a composition comprising the same	10-0358376	2000-03-08	US 6485953
Protein having activity of degrading amylopectin, starch, glycogen and amylase, a gene encoding the protein, a cell expressing the protein, and a method for producing the protein	10-0604401	2004-01-30	
Protein degrading mutan, inulin and levan, a gene encoding the protein, a cell expressing the protein, and a method for producing the protein	10-0809090	2006-08-30	

2) Describe exclusivity options (MLM, all markets, global, etc.)

- All possibilities are open and will be discussed later on.