

PRESS RELEASE (2017/7/28)

**The evolution of oral care agents that are safe to swallow -
Antibacterial effect against oral candida of the natural antibacterial agent
derived from vegetable lactic acid bacteria peptides (protein) and
rose essential oil
- Covers bacteria causing infectious diseases in the oral cavity**

[Overview]

Trife Inc. (Headquarters: Yokohama, Yokohama Business Grand Prix 2014 Best Company), through industrial-academic collaboration with Kyushu University Faculty of Agriculture (Fukuoka Prefecture) and Yasashii Laboratory Inc. (Fukuoka Prefecture), has confirmed that the natural antibacterial peptide (protein) Nisin A (*1), produced by vegetable lactic acid bacteria, in an original combination of Damask Rose essential oil from Bulgaria to form the natural antibacterial agent Neonisin-e, has strong antibacterial effect against the fungus (yeast) which causes oral candidiasis (*2).

This research group announced Neonisin at the Kyushu University Press Club in December 2012, as a natural antibacterial agent which combines high-purity Nisin A with plum extract in a unique ratio. It is a natural formulation that is made of only edible ingredients and is safe to swallow, which has gained attention as the world's first formulation with excellent bactericidal effect on gram-positive and gram-negative bacteria which are causative of dental caries, periodontal disease, and aspiration pneumonia. However, it had a weak point in that it was not effective against fungi (yeast), which is a causal organism for oral candidiasis.

Now Neonisin-e at this time expands the antibacterial spectrum to not only the bacteria of the conventional Neonisin, but also to fungus (yeast), and this lactic acid bacteria peptide preparation which is safe to swallow will cover most of the pathogenic bacteria that cause infections in the oral cavity. In the autumn of this year, we are planning to release products covering the causal bacteria responsible for infections in the oral cavity, effective not only for dental caries, periodontal disease and aspiration pneumonia, but also for oral candidiasis, which is commonly found in elderly people, infants, disabled people and cancer patients with weak immunity.

[Background]

Both dental caries and periodontal disease are infections caused by bacteria, it is said that 90% of Japanese people have dental caries and 70% suffer from periodontal disease. In addition, increase in bacteria in the oral cavity is major problem as a risk factor for aspiration pneumonia (*3), which is the cause of death of as many as 300 people a day, mainly elderly people.

Candida is a fungus that lives on human skin and mucous membranes, and can cause infections in people with an underlying disease (cancer, blood disease, immunodeficiency disease such as AIDS, diabetes etc.), or who are taking

immunosuppressive agents or antibiotics, and in infants and elderly people who have weak immunity. Especially in the elderly, oral candidiasis (*2), in which the surface of the tongue is covered with white spots, is often a problem.

Treatment of oral candidiasis is usually done by oral care, and the use of antifungal agents (potent synthetic fungicides) or natural formulations such as tree-derived essential oils which cause concerns about safety if swallowed. However, oral care alone cannot disinfect or cure oral candidiasis once it has developed, and since the use of antifungal drugs and conventional natural preparations has problems with resistant bacteria and side effects (such as stomach trouble, teratogenic effects), new treatments that are effective and safe even when swallowed have been sought all over the world.

[Content and Research Results]

1 The Development and Practical Application of Neonisin

Discovered by Professor Kenji Sonomoto of Kyushu University Faculty of Agriculture, Department of Bioscience and Biotechnology, the leading researcher on lactic acid bacteria, Nisin A, a natural antibacterial peptide (protein) produced by lactic acid bacteria in okara, and a technology to extract it at high purity was developed by a bio-venture over 10 years of research. Furthermore, in 2012 we developed a lactic acid bacteria peptide preparation "Neonisin" (Patent No. 5750552) that combines our own high purity Nisin A with plum extract, which exhibits excellent bactericidal effect against oral disease bacteria that cause dental caries (*Streptococcus mutans*, Gram positive bacteria) (*4) and periodontal disease-causing bacteria (*Aggregatibacter actinomycetemcomitans*, Gram negative bacteria) (*5). In July 2013, it was launched as the oral care product Oralpeace, which contains Neonisin, and the world's first practical application of Neonisin was achieved.

2. Development of Neonisin-e

Neonisin shows excellent antibacterial effect, but had a weak point in that it is less effective against fungi (yeast). Therefore, in order to compensate for the weak point of Neonisin, selection tests of various natural substances were conducted, and as a result, we found a synergistic effect with Nisin against yeast in a small amount of Damask rose essential oil. Although it was said that Damask rose essential oil has antibacterial activity, a certain concentration is necessary, and little effect was seen in trace amounts. However, the lactic acid bacteria peptide preparation Neonisin-e, which combines high-purity Nisin with a small amount of Damask rose essential oil in a unique formulation ratio, showed enhanced antibacterial effect. Neonisin-e is an evolved version of the previous Neonisin. This lactic acid bacteria peptide formulation which is safe to swallow will cover most bacteria that cause infectious diseases in the oral cavity.

[Features of Neonisin-e]

1. Excellent antibacterial activity against candida, dental caries and periodontal disease bacteria
2. Highly safe with natural origin
3. Excellent biodegradability and safety after decomposition (environmentally and human friendly)

Synergistic Effect of Antibacterial Activity (Antibacterial peptide + rose oil)

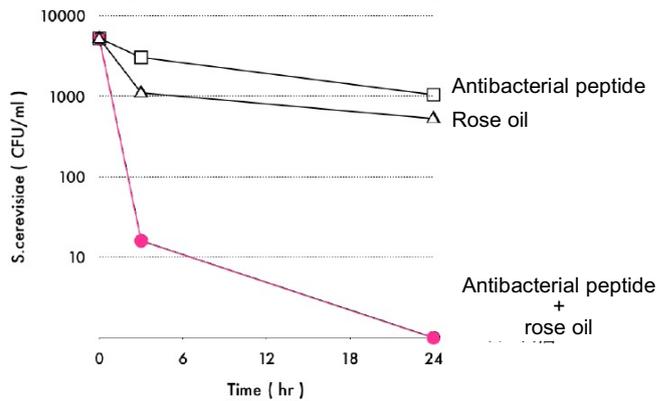


Figure 1. Antibacterial activity of antibacterial peptide (Nisin A) + rose oil (Damask rose essential oil) against yeast

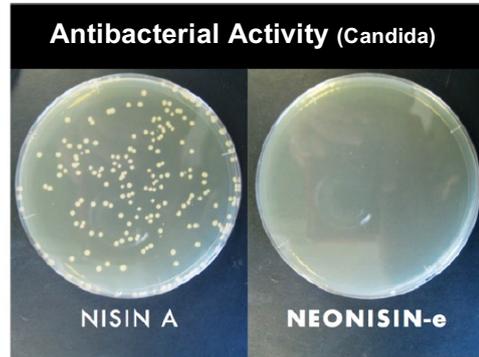


Figure 2. Antibacterial activity of the lactic acid bacteria peptide formulation Neonisin-e against yeast

Table 1. Antibacterial Activity of the Lactic Acid Bacteria Peptide Formulation Neonisin-e (Rose Oil) against Bacteria and Fungi

Antibacterial Activity Test (1-minute sterilization rate)		Without	Rose oil
Aggregatibacter actinomycetemcomitans IDH 781	Gram negative periodontal disease	57	100
Aggregatibacter actinomycetemcomitans Y 4	Gram negative periodontal disease	10	100
Streptococcus sobrinus	Gram positive dental caries	55	99
Streptococcus mutans UA 159	Gram positive dental caries	56	100
Candida albicans	Candida yeast	9	85
Candida glabrata	Candida yeast	17	100
Candida tropicalis	Candida yeast	45	100

(Data provided by Kagoshima University Graduate School of Medical and Dental Sciences, Komatsuzawa Laboratory)

[Future Development]

Neonisin-e is a new 100% natural antibacterial agent that combines high-purity antibacterial peptides (Nisin A) produced by the lactic acid bacteria with Damask rose essential oil in a unique ratio. While safe to swallow, it is expected to be used effectively as a natural ingredient with antibacterial effects on dental caries, periodontal disease bacteria and candida. This autumn, we plan to launch an oral care product containing Neonisin-e for people suffering from infections in the oral cavity.

Reference Materials

Table 2. Comparison of Oral Antibacterial Agents

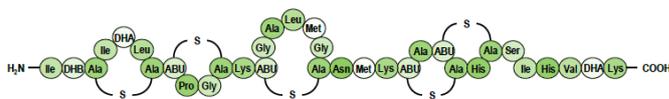
Antibacterial component	Gram positive bacteria Dental caries / Aspiration pneumonia	Gram negative bacteria Periodontal disease	Fungus (yeast) Candida	Natural substance
Alcohol	○	○	△	×
Cetylpyridinium chloride (CPC)	○	○	△	×
Chlorhexidine (CHX)	○	○	×	×
Benzethonium chloride	○	○	△	×
Hypochlorous acid water	○	○	○	×
Lactic acid bacteria	△	△	×	○
Plant extract	△	△	×	○
Hinokitiol (*)	○	○	○	○
Nisin A	○	×	×	○
Neonisin	○	○	×	○
Neonisin-e	○	○	○	○

(*) Strong odor affects taste × Suspected teratogenicity ×

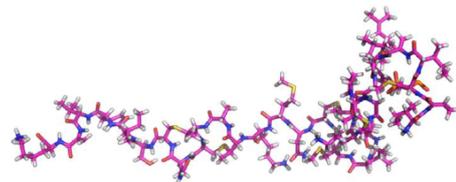
[Glossary]

*1 Nisin A

Nisin A is an antibacterial peptide (protein) produced by lactic acid bacteria (*Lactococcus lactis*), consisting of 34 amino acids. Nisin is naturally contained in traditional fermented foods such as yoghurt, cheese and rice bran pickles, which people have eaten since ancient times. It was discovered in 1928, and since it was approved by WHO and FAO in 1969, it has been used in food as a safe preservative in over 50 countries around the world. In Japan, it was approved as a food additive by the Ministry of Health, Labour and Welfare in 2009. Nisin A is water soluble and exerts its effect at a concentration of one part per billion. All negative results have been obtained in genotoxicity, carcinogenicity and other tests, and its safety has been demonstrated. It is easily broken down and digested with enzymes in the human intestinal tract, and is biodegradable so that release to the natural world is possible without environmental burden.



Structural formula of Nisin A



3D molecular model of Nisin A

*2 Oral Candidiasis

Oral candidiasis is an opportunistic infection caused by *Candida*, a resident fungus in the oral cavity. It is caused by systemic factors such as an underlying disease (such as cancer, a blood disease, immunodeficiency disease such as AIDS, or diabetes), the weak immunity of infants and elderly people, or taking immunosuppressive agents or antibiotics, and appears as milky white mossy spots in mucous membranes. Treatment of oral candidiasis is usually done by oral care, and the use of antifungal agents (potent synthetic fungicides) or natural formulations such as tree-derived essential oils which cause concerns about safety if swallowed. However, oral care alone cannot disinfect or cure oral candidiasis once it has developed, and since the use of antifungal drugs and conventional natural preparations has problems with resistant bacteria and side effects (such as stomach trouble, teratogenic effects), new treatments that are effective and safe even when swallowed have been sought all over the world.



(Photo: Wikipedia)

*3 Aspiration Pneumonia

Aspiration pneumonia is pneumonia caused by bacteria flowing into the lungs with saliva and gastric juice. It occurs frequently in the elderly, and is characterized by repeated recurrence. As recurrence is repeated, bacteria develop resistance to antibiotic treatment, leading to the deaths of many elderly people. Currently, pneumonia is the third leading cause of death in Japanese. About 95% of those who die are over 65 years old, and prevention is being called for.

*4 *Streptococcus mutans*

Streptococcus mutans is a kind of gram-positive streptococci that causes dental caries. This bacterium breaks down sucrose and creates a sticky substance called insoluble glucan (biofilm). It proliferates in the biofilm, produces acid, and causes dental caries.

*5 *Aggregatibacter actinomycetemcomitans*

Aggregatibacter actinomycetemcomitans is a kind of gram-negative bacteria that is a cause of periodontal disease. It also has virulence that affects tissues such as white blood cells.

*6 *Candida albicans*

Candida albicans is a kind of yeast that is a cause of oral candidiasis. Originally living in the human body surface, gastrointestinal tract and vaginal mucosa, it can become an opportunistic infection which causes lesions when the physical condition becomes poor.

The Oralpeace Project

The research and development of Oralpeace was based in the world's leading lactic acid bacteria biotechnology research of government, industry and academia. Released in July 2013, Oralpeace is an oral care product containing the globally innovative patented formulation Neonisin, that is safe to swallow while having excellent disinfection effect on trouble-causing bacteria in the oral cavity. For reducing nursing care expenses of the elderly, which exceed 9 trillion yen in Japan, and efforts to create work and income for disabled people nationwide, it was awarded the Yokohama Business Grand Prix 2014 Grand Prize and the highest Japan Venture Award 2015. It is attracting attention as a bio-social venture that is leading Japan and Yokohama in driving the world's aging society, welfare and health care industry. The project features a structure of three employees and the efforts of more than 100 highly motivated pro bono members.

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