Nattokinase(Powder of extract natto culture mixture)for health foods

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Abstract 1

Nattokinase is discovered to be a potent fibrinolysis enzyme and has a function to dissolve blood clots associated with heart and brain stroke. Organo Food Tech will introduce "Powder of extract natto culture mixture "for Health foods and Supplement. Natto contents a lot of functional ingredients for health. One of them is Nattokinase, which is an enzyme produced by bacillus natto.

We have developed powder of extract from bacillus natto culture mixture included nattokinase, which everyone can take in without feeling unique smell. Fibrinolytic activity of the products was estimated by standard fibrin plate method and synthetic substrate method according to previously reported standardized procedures (Sumi et al., Experientia 43:1110, 1987; Acta Haematol., 84:139, 1990).

Abstract 2

Nattokinase has a fibrinolytic activity, but correct titer official approval can't be done. The strong activity of synthetic substrate I (Bz-Ile-Glu-(OR)- Gly - Arg - pNA) was indicated as a result of so checking the resolution to the synthetic amide matrix, and next we found out that it's the order of synthetic substrate II (Suc - Ala - Ala - Pro - Phe - pNA). On the other hand because an enzyme of withered Bacillus subtilis (subtilisin) indicates the singularity in a way of synthetic substrate II, using this special quality, distinction of whether it's real nattokinase becomes possible, and various products are being analyzed at present. More titer official approval by synthetic substrate I is also contributing to quality control very much. Because it also operates on a elastase way and circulation improvement, nattokinase will be the very interesting one as healthy food material from now on.

Market size of each health food

Health food	Market size
Aojiru(Green juice)	1,01B JPY
Health tea	56B JPY
Fermented botanical food product	51B JPY
Health vinegar	50B JPY
Royal jelly	45B JPY
Blueberry	40B JPY
Hyaluronic acid	39B JPY
Turmeric	35B JPY
Noni	35B JPY

Health food	Market size
Natto supplement	**30B JPY**
Propolis	30B JPY
Nucleic acid	30B JPY
Millet	30B JPY
Placenta	25B JPY
Sasa bamboo	20B JPY
Asisn ginseng	20B JPY

Market size of Natto supplement is 30 billion JPY.
Accounting for 2.5% of the health food market.（1.187 trillion JPY/2015）

References: 健康産業新聞, March 2, 2016

The history of Nattokinase

1980 Dr. Hiroyuki Sumi discovered that natto which he put on artificial clots greatly dissolve them, working as researcher in Chicago Michael Reese Institute.

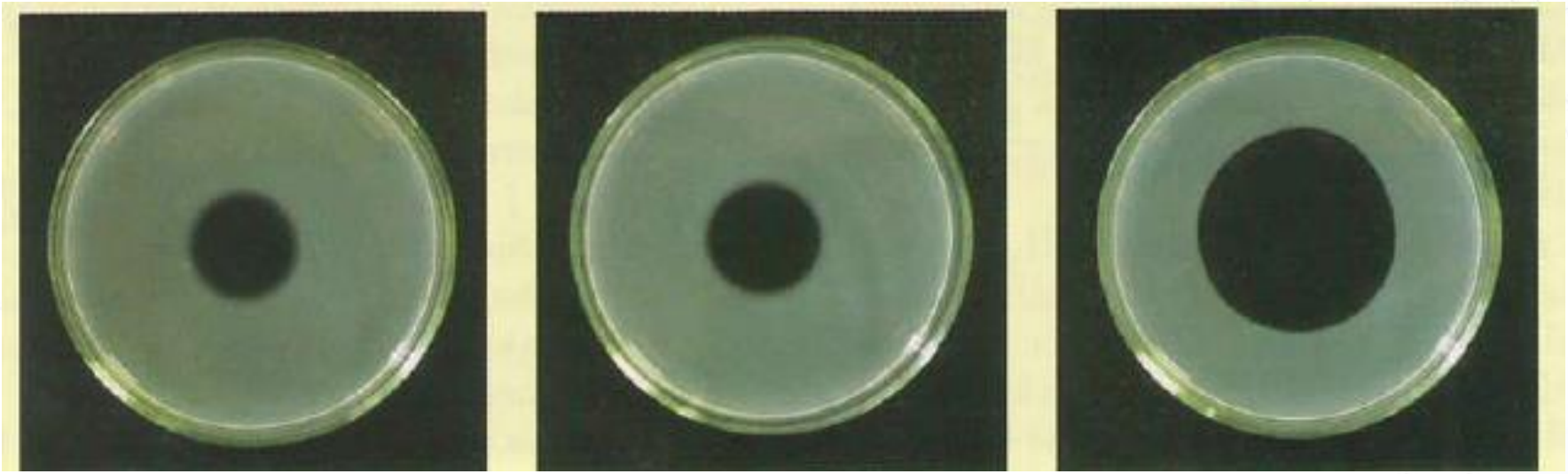
1982 Dr. Sumi analyzed an enzyme in natto and confirmed that it was the protein consisting of 275 amino acid and named the enzyme nattokinase.

H2N–Ala–Gln–Ser–Val–Pro–Tyr–Gly–Ile–Ser–Gln–Ile–Lys–Ala–Pro–Ala–Leu–His–Ser–Gln–
Gly–Tyr–Thr–Gly–Ser–Asn–Val–Lys–Val–Ala–Val–Ile–Asp–Ser–Gly–Ile–Asp–Ser–Ser–His–
Pro–Asp–Leu–Asn–Val–Arg–Gly–Gly–Ala–Ser–Phe–Val–Pro–Ser–Glu–Thr–Asn–Pro–Tyr–Gln–
Asp–Gly–Ser–Ser–His–Gly–Thr–His–Val–Ala–Gly–Thr–Ile–Ala–Ala–Leu–Asn–Asn–Ser–Ile–
Gly–Val–Leu–Gly–Val–Ala–Pro–Ser–Ala–Ser–Leu–Tyr–Ala–Val–Lys–Val–Leu–Asp–Ser–Thr–
Gly–Ser–Gly–Gln–Tyr–Ser–Trp–Ile–Ile–Asn–Gly–Ile–Glu–Trp–Ala–Ile–Ser–Asn–Asn–Met–
Asp–Val–Ile–Asn–Met–Ser–Leu–Gly–Gly–Pro–Thr–Gly–Ser–Thr–Ala–Leu–Lys–Thr–Val–Val–
Asp–Lys–Ala–Val–Ser–Ser–Gly–Ile–Val–Val–Ala–Ala–Ala–Ala–Gly–Asn–Glu–Gly–Ser–Ser–
Gly–Ser–Thr–Ser–Thr–Val–Gly–Thr–Pro–Ala–Lys–Tyr–Pro–Ser–Thr–Ile–Ala–Val–Gly–Ala–
Val–Asn–Ser–Ser–Asn–Gln–Arg–Ala–Ser–Phe–Ser–Ser–Val–Gly–Ser–Glu–Leu–Asp–Val–Met–
Ala–Pro–Gly–Val–Ser–Ile–Gln–Ser–Thr–Leu–Pro–Gly–Gly–Thr–Tyr–Gly–Ala–Tyr–Asn–Gly–
Thr–Ser–Met–Ala–Thr–Pro–His–Val–Ala–Gly–Ala–Ala–Ala–Leu–Ile–Leu–Ser–Lys–His–Pro–
Thr–Trp–Thr–Asn–Ala–Gln–Val–Arg–Asp–Arg–Leu–Glu–Ser–Thr–Ala–Thr–Tyr–Leu–Gly–Asn–
Ser–Phe–Tyr–Tyr–Gly–Lys–Gly–Leu–Ile–Asn–Val–Gln–Ala–Ala–Ala–Gln–COOH

Fig1. Amino acid structure of the nattokinase

Activity of Nattokinase (FU)

This activity is evaluate the degree of decomposition a substrate as the fibrin.



Nattokinase **Subtilisin** **Trypsin**

Fig2. Fibrinolytic activity of fiblin plate

It is possible to measure the activity of nattokinase with a decomposition of fibrin. But for other enzymes to decompose of fibrin, we must be careful.

References: 和光純薬時報　Vol.78, No.4(2010)pp.8-10

Activity of Nattokinase (IU)

This activity is determined using a specific, characteristically decompose synthetic amide substrate.

Synthetic amide substrates I ：Bz-Ile-Glu-(OR)-Gly-Arg-pNA

Synthetic amide substrates II：Suc-Ala-Ala-Pro-Phe-pNA

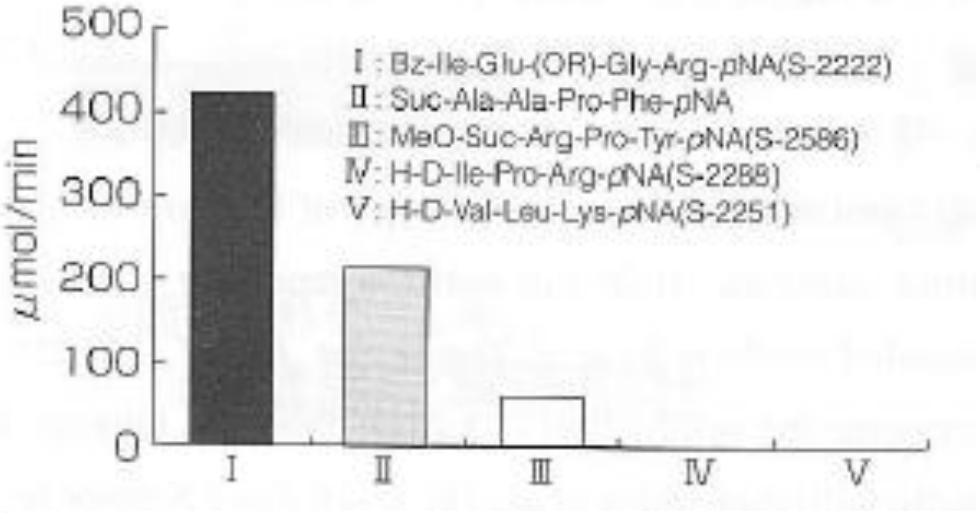


Fig3. Comparative amidolytic activity of nattokinase with several synthetic substrates

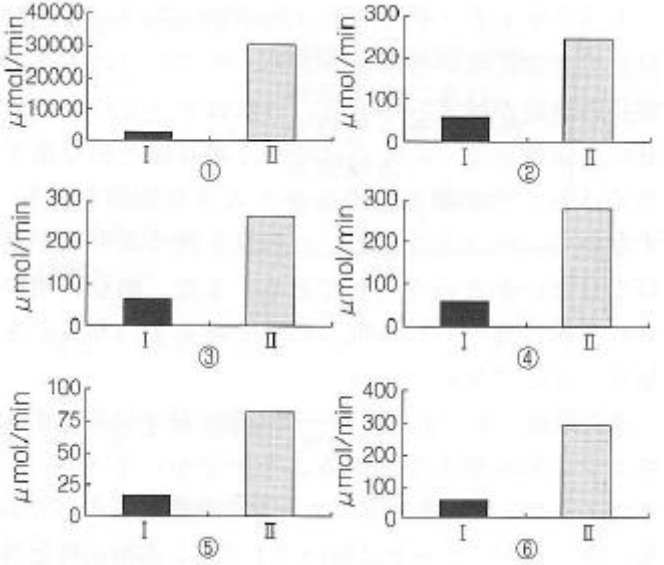


Fig4. Comparative amidolytic activity of 6 kinds subtilisins with two synthetic substrates

In difference in decomposing power of the synthetic amide substrate, we can distinguish nattokinase from other enzymes.

References: FOODSTYLE21 Vol.14 No.3(2010)pp.52-55

Introduction of Nattokinase HTNK-J

We supply bacillus natto cultured broth extract powder containing nattokinase. It is easy to process this into supplements, which even the person who does not like natto takes it in easily.

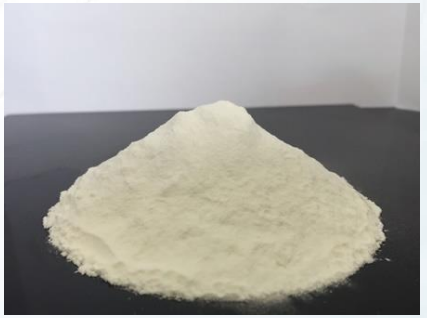
Product features

Having a activity of Nattokinase (confirmed by FU and IU)
Non-Genetically Modified Organism*
Non-animal
Removal of Vitamin K_2
Reduction of the characteristic odor of natto

*Limited to agricultural products as a target of GMO labeling obligation

Product specification

Activity of Nattokinase : >20,000 FU/g
Loss on drying : <10%
Heavy metal : <40 μg/g
Arsenic : <4 μg/g
General viable bacteria : <3,000 cfu/g
Coliform bacteria : Negativity



Safety of Nattokinase HTNK-J

- **Single dose oral toxicity test in rats**
  Lethal dose is more than 2,000 mg/kg in male and female rats

- **28-day repeat dose oral toxicity test in rats**
  No observable adverse effect level is more than 1,000 mg/kg in male and female rats

- **Reverse mutation test in bacteria**
  Mutagenicity was negative

- **In vitro mammalian chromosome aberration test**
  Not induce chromosome aberration

The safety is confirmed by these studies.

Examine in outsourcing

Fibrinolytic activity of Nattokinase HTNK-J

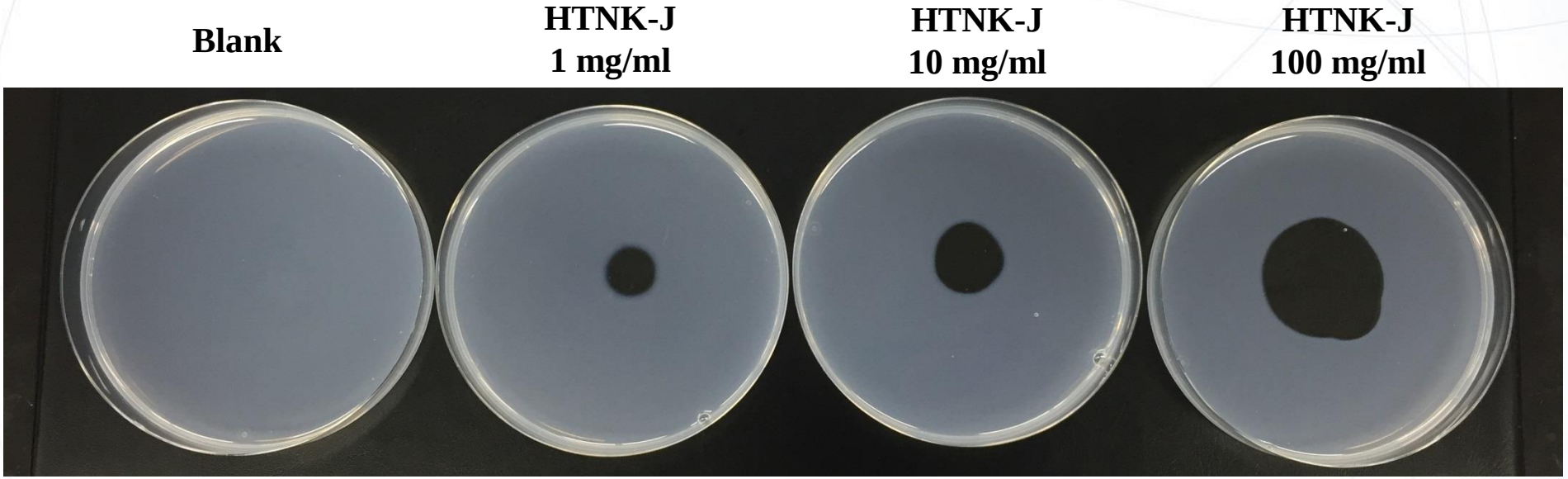


Fig5. Fibrinolytic activity of fiblin plate
Nattokinase solution of each density was applied to fiblin plate.
After incubation at 37° C for 3 hrs.

Nattokinase HTNK-J has fibrinolytic activity.