Primary Antioxidant

NPK SOD Enzyme

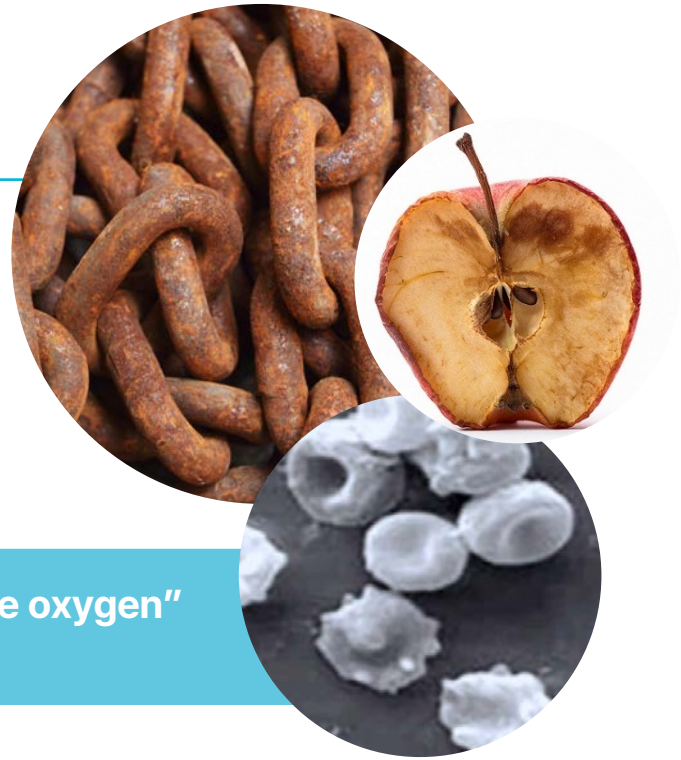
Oxidation

What is Oxidation?

1) Combination with O2 2) Loss of a Hydrogen 3) Loss of an Electron

▶ Strong Chemical Reactivity ▶ Strong Energy
▶ Strong Cell Destruction

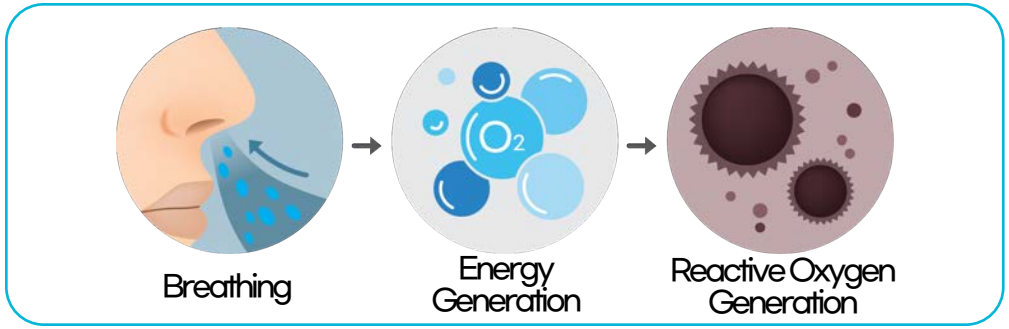
Due to oxidative stress, prehistoric lifeforms either died out completely or moved away from oxygen into environments like the ground or the anaerobic conditions of intestines.



"More than 90% of all diseases are caused by reactive oxygen"

- Johns Hopkins University School of Medicine Research Team -

Oxidative Stress (Generation of Reactive Oxygen Species)



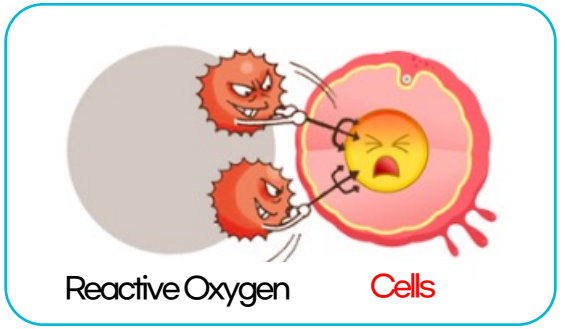


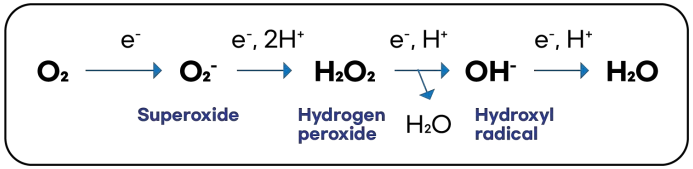
그림 출처 : 브레이크 뉴스(현대판 불로초 비타민 C와 E)

Reactive Oxygen Species are unstable and damage normal cells in an attempt to gain electrons.

This phenomenon is called "oxidative stress."

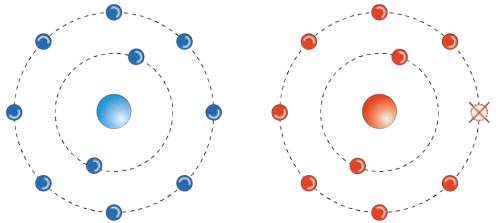
When there is an excessive amount of active oxygen, it is chemically reactive and damages DNA and cells, resulting in aging and chronic diseases.

Reactive Oxygen Species (Free Radicals)



[Common Types of Reactive Oxygen]

Superoxide,
Hydrogen Peroxide,
Hydroxyl Radical



Normal Molecule
Fully Paired

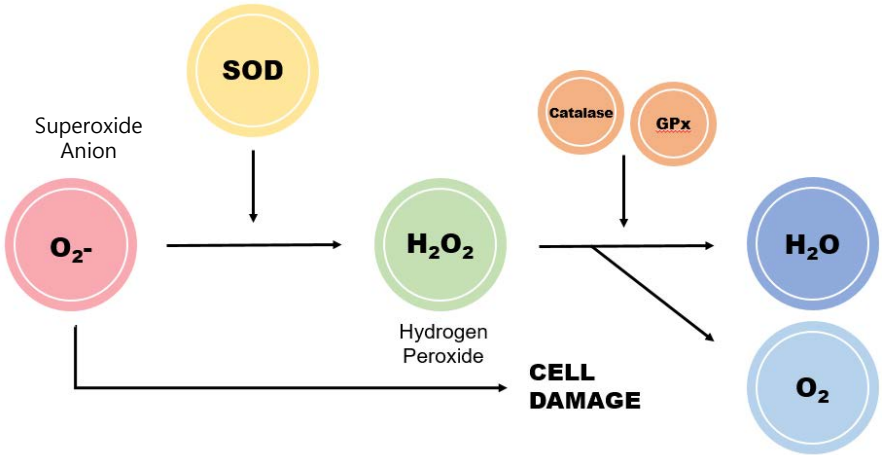
Free Radical
Missing Electron

There is an **antioxidant system** in the human body that removes oxidizing substances, but if the balance between production and removal is broken due to metabolic imbalance in the body, problems such as cancer, aging, and obesity occur.

Antioxidant System

1) Enzymatic Antioxidants

a) **SOD :** Superoxide Dismutase
b) **Catalase**
c) **GPx :** Glutathione Peroxidase
d) **GR :** Glutathione Reductase



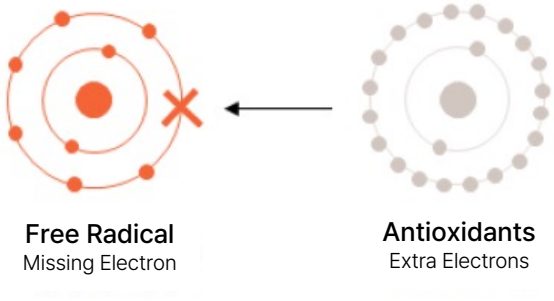
- ✓ Destroys Reactive Oxygen Produced by the Body
- ✓ Moderate Exercise and Nutrition Help to Produce Enzymatic Antioxidants

"The SOD Enzyme is the First Agent of the Antioxidant System."

Antioxidant System

2) Non-Enzymatic Antioxidants

Vitamins A, C, and E, and Glutathione convert or combine with reactive oxygen to remove them from the body



a) Glutathione : a type of amino acid produced in the liver.

b) Ascorbic Acid (Vitamin C) : a cofactor for enzymes, a radical scavenger, and involved in the transfer of electrons across cell membranes.

c) Carotenoids : yellow, orange, or red pigments that are widely found in the plant kingdom, that are precursors of Vitamin A, and are effective radical scavengers or singlet oxygen scavengers.

d) Vitamin E : aids in the formation of tocopheroxyl radicals through the donation of a hydrogen atom.

e) CoEnzyme Q10 : exists in mitochondria to participate in electron transport and ATP production.

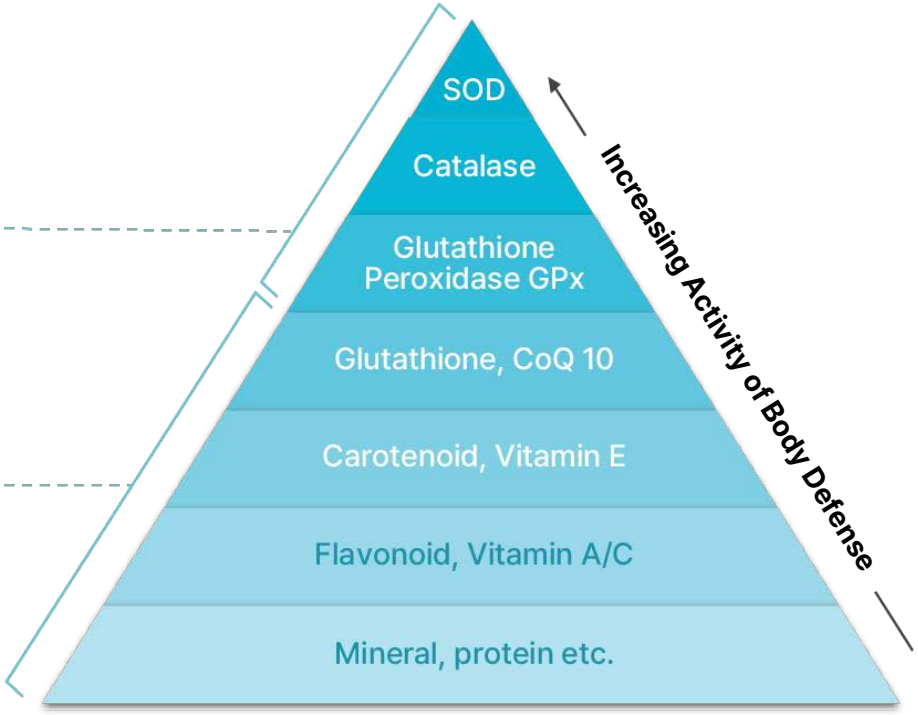
Antioxidant System

Enzymatic Antioxidants

- **Primary** Antioxidants
- **Continuous** Action
  (not consumed after reaction)
- Higher Stability than Other Antioxidants

Non-Enzymatic Antioxidants

- **Secondary** Antioxidants
- **Single** Action(consumed in reaction)
- Questionable Stability in Gastrointestinal Tract

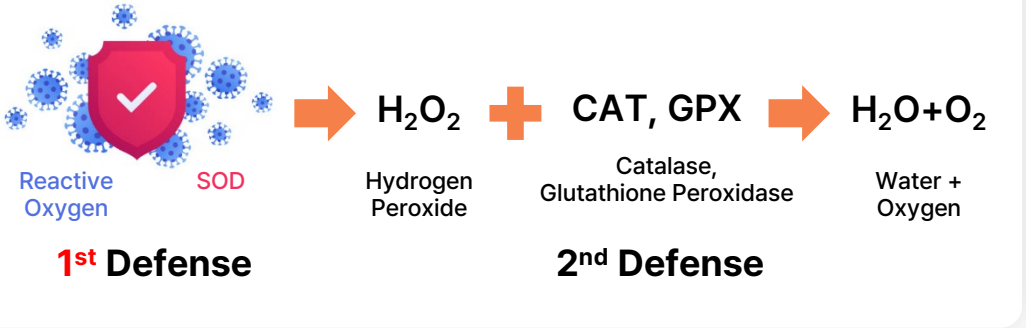


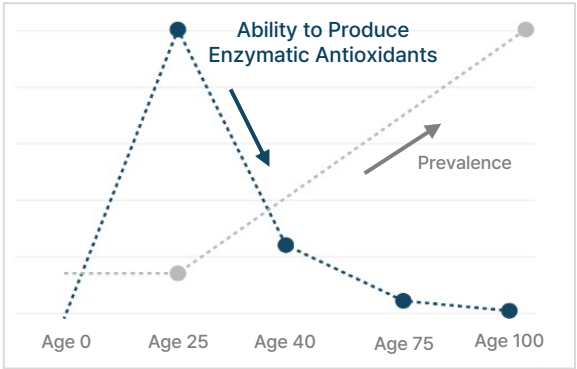
SOD & Catalase Enzyme Necessity

The SOD Enzyme is the first line of defense against free radicals!

The Catalase Enzyme is the second line of defense against free radicals!

Like other enzymes in the body, the body's production of SOD Enzymes decreases drastically after the age of 30.





The History of the SOD Enzyme

1968

- Discovered by Professors McCord and Fridovich

  Initially, extracted from bovine blood and used by injection

2000

- The first plant-derived SOD Enzyme was extracted from cantaloupe

2008

- The SOD Enzyme was selected as Europe's best antioxidant

2024

- NPK's successful production of the SOD Enzyme via soybean fermentation

Research

- SOD-deficient mice die within days (Li Y, 1995)
- SOD-deficient mice develop liver cancer (Elchuri S, 2005)
- SOD-induced lifespan extension in fruit flies (Sun J, 2002)
- Differences in SOD expression are responsible for lifespan differences between queen ants and worker ants, not differences in nucleotide sequences (Lucas ER, 2018)
- SOD-deficient yeast have 5-fold higher rate of DNA damage (Muid, 2014)

NPK SOD Enzyme Specifications

Analysis Item	Certified Analysis and Internal Analysis
SOD Enzyme Activity (IU)	**"500,000 IU/g Guarantee"** Analysis Results : When processing 100 mg/mL, 206,176 ± 6,892 (certified)
Catalase Enzyme Activity (IU)	**2,000 IU/g Guarantee** Analysis Results : When processing 100 mg/mL, 843.89 ± 59.87 (certified)
DPPH Radical Scavenging Ability (%)	When processing 100 mg/mL, **89.36%** ± 0.19 (certified), 91.52% ± 9.30 (internal)
α-Amylase (Unit/g)	184,717 (certified), 343,053 (internal)
Protease (Unit/g)	5,934 (certified), 1,653 (internal)
Fibrinolytic Enzyme Activity (Unit/g)	10,859 (certified), 15,565 (internal)
Poly-Gamma Glutamic Acid (mg/g)	50.16 (certified)
Acid Resistance	Both SOD and Catalase enzymes were confirmed to **maintain enzyme activity at pH 2 (the pH of stomach acid)**

※ IU = Unit/L = mU/mL

Certified Analysis Results

SOD Activity

Analysis Items	SOD Antioxidant Activity Evaluation
Sample Name	SOD Activity (mU/mL)
NPK SOD Enzyme 10 mg/mL	15748.98 ± 841.71 mU/mL
NPK SOD Enzyme 50 mg/mL	86345.75 ± 6080.89 mU/mL
NPK SOD Enzyme 100 mg/mL	206176.09 ± 6892.49 mU/mL

Catalase Activity

Analysis Items	Catalase Antioxidant Activity Evaluation
Sample Name	Catalase Activity (mU/mL)
NPK SOD Enzyme 100 mg/mL	843.89 ± 59.87 mU/mL

DPPH Radical Scavenging Activity

Analysis Items	DPPH Antioxidant Activity Evaluation
Sample Name	DPPH Activity (%)
NPK SOD Enzyme 10 mg/mL	13.24 ± 4.05 %
NPK SOD Enzyme 50 mg/mL	56.34 ± 1.05 %
NPK SOD Enzyme 100 mg/mL	89.36 ± 0.19 %

Antioxidant Effect Test (Iodine Reaction) Our SOD vs Other SOD

Principle

- The principle of a redox reaction is that it acts as an antioxidant and oxidizes itself and reduces other substances.
- When povidone and vitamin C meet, the iodine molecule in povidone is reduced by vitamin C and changes into a colorless iodide ion.

Restoration

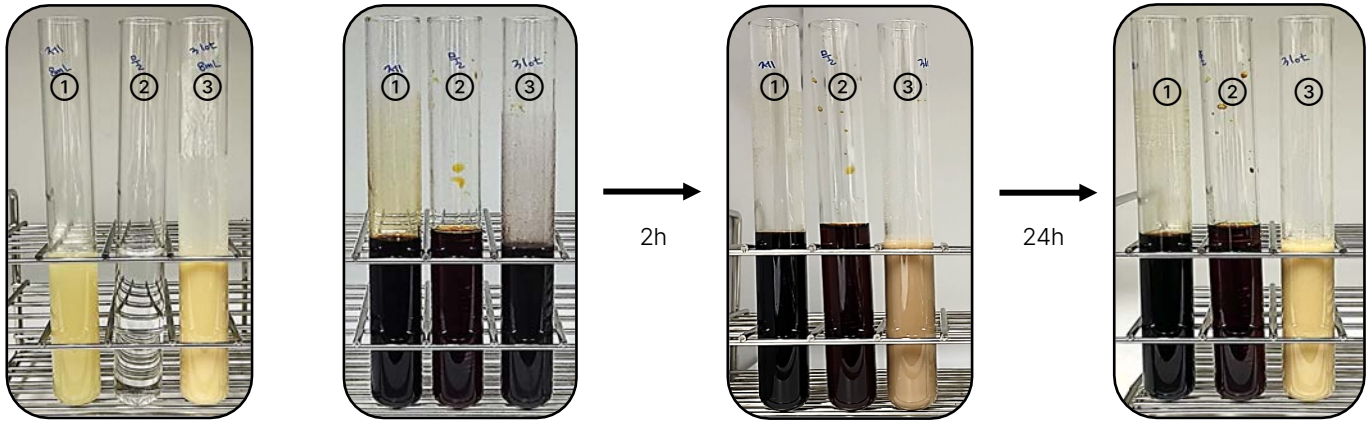
Iodine (I_2) + Electrons ($2e^-$) → Iodide Ions ($2I^-$)

Maroon → Colorless

Experimental Results

✓ Catalase enzyme activity confirmed only in NPK SOD enzyme

① Other SOD Enzyme (30%)
② Water
③ **NPK SOD Enzyme (30%)**



Original Sample Color

Immediately After Addition of Iodine

Catalase Activity (Hydrogen Peroxide Decomposition)

Our SOD vs Other SOD

Principle

- Oxygen is generated from hydrogen peroxide by Catalase(as seen when foam is generated when hydrogen peroxide is sprayed on a wound)

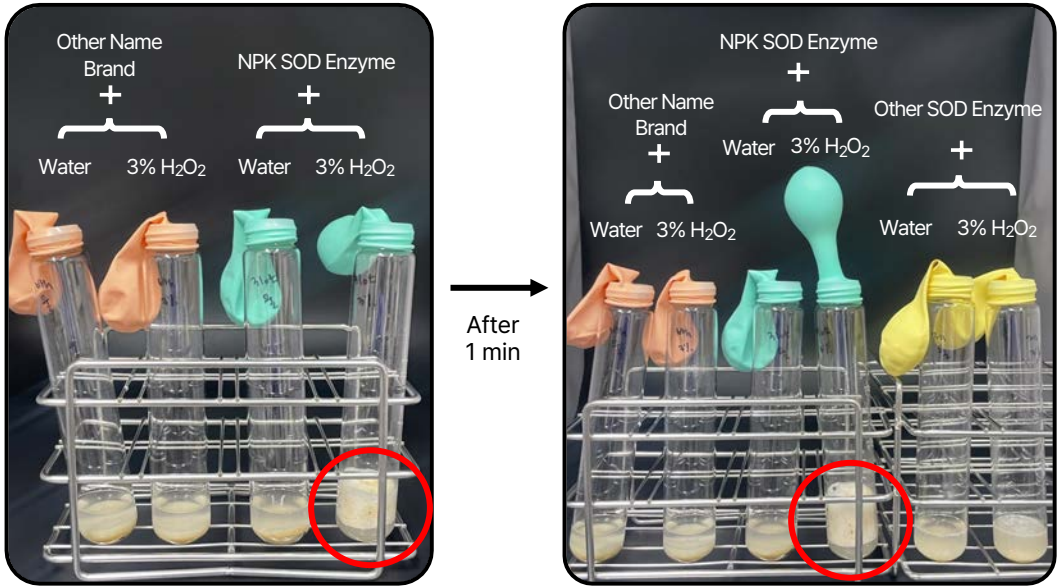
H_2O_2 + CAT → H_2O+O_2

Catalase

Water + Oxygen

Experimental Results

✓ Catalase enzyme activity confirmed only in NPK SOD Enzyme



Catalase Activity (Hydrogen Peroxide Decomposition Reaction)

Glutathione vs Vitamin C vs NPK SOD Enzyme

Experimental Results

✓ Vitamin C ≒ Glutathione << NPK SOD Enzyme

<Experimental Process>

- 5mL of diluted 3.45% hydrogen peroxide was added to 1g of sample each

<Experimental Results>

- **Only the NPK SOD Enzyme showed catalase enzyme activity (confirmed via foaming due to oxygen)**

VitaminC Clutathione SOD Enzyme

SOD Enzyme Activity Acid Resistance Test

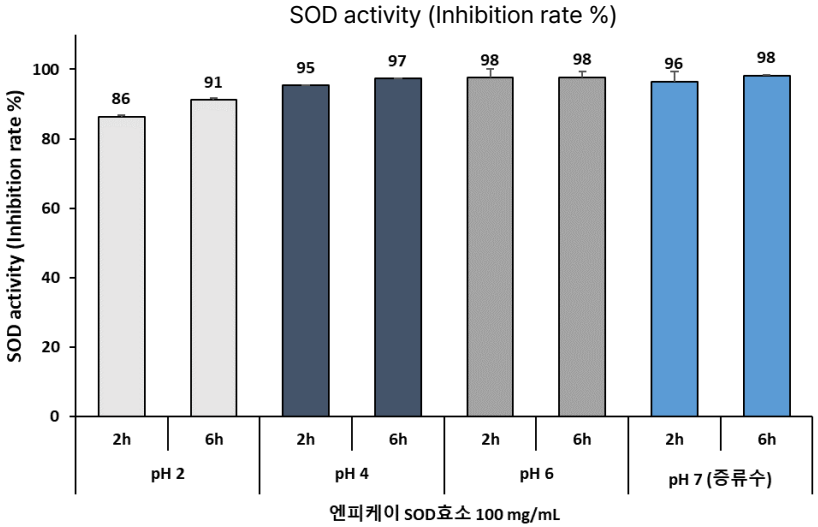
(whether the SOD Enzyme is destroyed during digestion)

Experimental Results

- **SOD Enzyme Activity Retention, according to 2h and 6h tests under various pH conditions(internal)**
- **SOD Enzyme Activity Maintained even at pH 2-6(internal)**

✓ Experiment Principle : When the SOD Enzyme is active, it converts O_2 produced by xanthine oxidase into O_2 and H_2O_2, thereby inhibiting the formation of WST-formazan.

✓ SOD Activity (Inhibition Rate %) is the inhibition rate (the degree to which O_2 is converted to O_2 and H_2O_2) that inhibits the formation of WST-formazan.

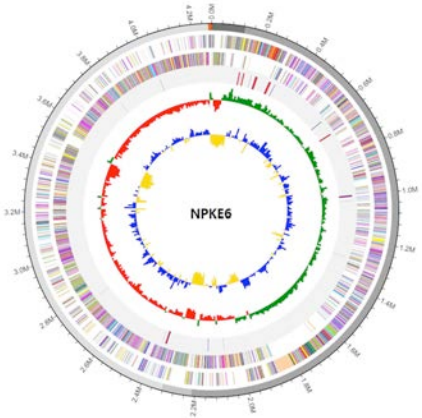


Obtaining Safety Data for Strains (CJ Bioscience)

Resulst of GRIIS Essential whole-genome analysis of *B. amyloliquefaciens* NPKE6 (including antibiotic resistance / virulence gene analysis)

- Genome Size: Approximately 4.3 Mb; G+C Content: 45.1%; Functional Genes (CDS) Number: 4,412
- 10 antibiotic resistance genes not detected; 4 toxicity genes not detected
- *B. amyloliquefaciens* NPKE6 strain whole genome and safety data acquisition

[Genome Map]



[Antibiotic Resistance Gene Detection Results]

순위	항생제	검출여부 (YES/NO)	유사도
1	Clindamycin	NO	Lincosamides 계열
2	Kanamycin	NO	Aminoglycoside 계열
3	Erythromycin	NO	Macrolide 계열
4	Tylosin	NO	Macrolide 계열
5	Tetracycline	NO	Tetracycline 계열
6	Gentamicin	NO	Aminoglycoside 계열
7	Chloramphenicol	NO	Chloramphenicol 계열
8	Ampicillin	NO	Penicillin 계열
9	Streptomycin	NO	Aminoglycoside 계열
10	Vancomycin	NO	Glycopeptide 계열

[Toxicity Factor Gene Analysis Results]

Function	Gene	독성인자	검출여부 (YES/NO)
Toxin	*cylA*	Cytolysin	NO
Adherence	*asa1*	Aggregation substance	NO
Exoenzyme	*hyl*	Hyaluronidase	NO
Exoenzyme	*gelE*	Gelatinase	NO

GRIIS Essential Whole Genome Analysis Results

(B. amyloliquefaciens NPKE6)

Confirmation of the Functionality of *B. amyloliquefaciens* NPKE6

- Confirmation of presence of antioxidant enzyme related genes (SOD, Catalase, GPx)

[Superoxide dismutase]

CDS name	Other name(s)	KEGG ID	Product	Function	Length
NPKE6_03433	**SOD2**	K04564	**Superoxide dismutase**	Iron; Metal-binding; Oxidoreductase.	846
NPKE6_03441	**SOD1**	K04565	**Superoxide dismutase**	Cell membrane; Copper; Disulfide bond; Lipoprotein; Membrane; Metal-binding; Palmitate; Signal; Zinc.	591
NPKE6_04025	**SOD2**	K04564	**Superoxide dismutase**	Iron; Manganese; Metal-binding; Oxidoreductase; Stress response.	606

[Catalase]

CDS name	Other name(s)	KEGG ID	Product	Function	Length
NPKE6_03260	katE|CAT|catB|srpA	K03781	**Catalase**	Cytoplasm; Heme; Hydrogen peroxide; Iron; Metal-binding; Oxidoreductase; Peroxidase.	1614
NPKE6_03286	katE|CAT|catB|srpA	K03781	**Catalase**	Heme; Hydrogen peroxide; Iron; Metal-binding; Oxidoreductase; Peroxidase; Sporulation.	2052
NPKE6_01086	katE|CAT|catB|srpA	K03781	**Catalase**	Cytoplasm; Heme; Hydrogen peroxide; Iron; Metal-binding; Oxidoreductase; Peroxidase.	1446

[Glutathion peroxidase]

CDS name	Other name(s)	KEGG ID	Product	Function	Length
NPKE6_03520	gpx	K00432	**Glutathione peroxidase**	Oxidoreductase; Peroxidase.	483

Patent Application & PCT International Application

(using B. amyloliquefaciens NPKE6 strain)

SOD/Catalase Enzyme Patent Application / Priority Examination Request

출원번호통지서

출원일자	2023.11.21
특기사항	심사청구(유) 공개신청(무) 참조번호(12557)
출원번호	10-2023-0162534 (접수번호 1-1-2023-1299683-07) (DAS접근코드:BAF6)
출원인 명칭	엔피케이(주)(1-2013-031231-7)
대리인 성명	황이남(9-1998-000610-1)
발명자 성명	손성오 조운희 전소헌
발명의 명칭	슈퍼옥사이드 디스뮤타아제 활성 및 카탈라아제 활성을 가지는 팽화곡물 발효효소, 이의 제조방법 및 이를 포함하는 식품 조성물

SOD/Catalase Enzyme PCT International Application

PCT2318

1/4

PCT 출원서

(전자적 형태가 원본)

0	수리관청 전용	
0-1	국제출원번호	PCT/KR2023/018975
0-2	국제출원일자	2023년 11월 23일 (23.11.2023)
0-3	수리관청 명칭 및 "PCT 국제출원"	대한민국 특허청 PCT 국제출원
0-4	서식 PCT/RO/101 - PCT 출원서	
0-4-1	우측에 기재된 바와 같이 작성되었다.	ePCT-Filing Version 4.12.005 MT/FOP 20231109/1.1
0-5	신청 이러 서명인은 본 국제 출원서가 특허협력조약에 의해 처리될 것을 청구합니다.	
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0-7	출원인 또는 대리인의 서류참조기호	PCT2318
I	발명의 명칭	슈퍼옥사이드 디스뮤타아제 활성 및 카탈라아제 활성을 가지는 팽화곡물 발효효소, 이의 제조방법 및 이를 포함하는 식품 조성물
II	출원인	
II-1	이 사람은	오직 출원인 (applicant only)
II-2	우측 지정국에 관한 출원인	모든 지정국 (all designated States)
II-4ko	성명	엔피케이 주식회사
II-4en	Name:	NPK INC.
II-5ko	주소	대한민국 57309 전라남도 담양군 담양읍 에코길 61
II-5en	Address:	61, Eco-gil, Damyang-eup Damyang-gun Jeollanam-do 57309 Republic of Korea
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II-10	이메일 주소	ibsy9046@nate.com
II-10(a)	이메일 사용동의 수리관청, 국제조사기관, 국제사무국, 국제예비심사기관이 발급 시 이 이메일 주소를 사용하여 이 국제 출원과 관련하여 발행된 통지서를 송부할 것에 동의한다.	오직 전자적 형태의 통지서만 송부 (서면 통지서는 미발송)
II-11	출원인 코드	1-2013-031231-7

Thank you

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