NEW DIETARY INGREDIENT NOTIFICATION FOR

Weissella confusa WIKIM51 (Wilac D001)

Prepared for:

Park Sang Min (Position: Head of Innovation R&D Department) (Signature)
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Pharmsville Co., Ltd.
37, Magokjungang 8-ro 3-gil, Gangseo-gu, Seoul, 07793, Republic of Korea

September 06, 2022
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I. CONTACT INFORMATION

1. Submitter of the Notification

Type of Submitter (Select all that apply)
■ Manufacturer of NDI
■ Distributor of NDI
■ Manufacturer of Dietary Supplement Containing NDI
■ Distributor of Dietary Supplement Containing NDI
□ Agent/Attorney/Consultant

Company Name: Pharmsville Co., Ltd.
Address: 37, Magokjungang 8-ro 3-gil, Gangseo-gu, Seoul, 07793, Republic of Korea

2. Owner of the Notification

Is the owner of the notification the same as the submitter?
■ Yes
□ No

Type of Manufacturer or Distributor (Select all that apply)
□ Manufacturer of NDI
■ Distributor of NDI
■ Manufacturer of Dietary Supplement Containing NDI
■ Distributor of Dietary Supplement Containing NDI

Company Name: Pharmsville Co., Ltd.
Address: 37, Magokjungang 8-ro 3-gil, Gangseo-gu, Seoul, 07793, Republic of Korea

3. Primary Contact

Type of Contact (Select all that apply)
■ Submitter of the Notification
■ Owner of the Notification
□ Agent/Attorney/Consultant

Contact Name: Park Sang Min (Position: Head of Innovation R&D Department)
Company Name: Pharmsville Co., Ltd.
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Email Address: plan1@pharmsville.com
Physical Address: 37, Magokjungang 8-ro 3-gil, Gangseo-gu, Seoul, 07793, Republic of Korea
NDIN for Weissella confusa WIKIM51 (Wilac D001)
Prepared for Pharmsville Co., Ltd.

4. Signature

Park Sang Min, Head of Innovation R&D Department
Pharmsville Co., Ltd.

September 06th, 2022
Date
II. GENERAL ADMINISTRATIVE INFORMATION

1. Name of the New Dietary Ingredient

*Weissella confusa* WIKIM51 (Wilac D001)

2. Does Notification Contain Confidential Information

- Yes, see attached designation of confidential information
- Yes, information is designated at the place where it occurs in the notification
- No

3. Are You Providing a Redacted Copy of the Notification

- Yes, redacted copy of complete notification
- Yes, redacted copy of part(s) of the notification
- No

4. Are All Citations to Published Information Accompanied by Reprints or Full Photostatic Copies of the Publications?

- Yes
- No

5. Are the Notification and All Publications Submitted in English or Accompanied by a Complete and Accurate English Translation?

- Yes
- No
III. DESCRIPTION OF NDI AND DIETARY SUPPLEMENT CONTAINING THE NDI

1. New Dietary Ingredient Type (Check all that apply)

- Vitamin
- Mineral
- Herb or other botanical
- Amino acid
  ■ Dietary substance for use by man to supplement the diet by increasing the total dietary intake
- Concentrate, metabolite, constituent, extract, or combinate of any ingredient described above

2. Name of the New Dietary Ingredient and Related Information

Maximum level of new dietary ingredient in each serving of dietary supplement (include units): 1.0X10^{10} CFU/day by “Weissella confusa WIKIM51 (Wilac D001)”

NDI Name: Weissella confusa WIKIM51 (Wilac D001)

Latin Binomial Name (LBN): Weissella confusa

Common name: Lactobacillus confusus, Lactobacillus coprophilus subsp. confusus

Synonyms and Trade Name: Weissella confusa WIKIM51 (Wilac D001)

Author of LBN: Collins

NDI Type: Live microbial dietary ingredients

Strain: Weissella confusa WIKIM51

CAS Registry Number: Not Available

3. Dietary Supplement Serving Form (Check all that apply)

- Tablet
- Capsule
- Powder
- Softgel
- Liquid
- Gelcap
- Sachet
- Sachet
- Other

-4-
Other in the following cases: Bulk ingredient

4. Description of Dietary Supplement

In accordance with the Dietary Supplement Health and Education Act of 1994 (DSHEA), 21 U.S.C. 350b(a)(2), and with final regulations published in the Federal Register (1997, 62:49886-49892, 21 D.F.R. 1906) “Requirement of Premarket Notification”, the following information is submitted by Pharmsville Co., Ltd. in support of a New Dietary Ingredient Notification for “*Weissella confusa* WIKIM51 (Wilac D001)”.

5. Conditions of Use of the Dietary Supplement

5a. Serving Instructions (e.g., “Take with Food”, “Take Before Bed”, “Dissolve in a Glass of Water”, etc.)

The dietary supplement can be administered directly or with water.

5b. Dietary Supplement Serving Size (Weight or Volumetric Measure), Serving Frequency (# of Servings/Day, Interval Between Servings), Duration of Use and Maximum Total Daily Intake Level

1.0X10^{10} CFU/day by “*Weissella confusa* WIKIM51 (Wilac D001)”

5c. Target Population/Excluded Populations/Other Restrictions

General adult (care is required for pregnant, lactating women, infants, and children.

○ Evidence: No adverse events have been identified when consuming 1.0X10^{10} CFU/day of NDI for 12 weeks for general adults in human clinical test. Human clinical test did not include, pregnant, lactating women, infants, and children, and therefore did not confirm any side effect.
1. NEW DIETARY INGREDIENT IDENTITY INFORMATION

1.1 Description of the Identity of the NDI

*TRADE SECRET: All information on Section 1.1.


<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Latin name</th>
<th>Author</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weissella confusa WIKIM51 (Wilac D001)</td>
<td>Weissella confusa</td>
<td>Collins</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

Identity of the new dietary ingredient

NDI is a light grayish-yellow powder with unique flavor and no odor obtained by freeze-drying a lactic acid bacteria strain (Weissella confusa WIKIM51) isolated and identified from kimchi, a traditional Korean fermented food.

The level of the new dietary ingredient in the dietary supplement

Daily serving level is $1.0 \times 10^{10}$ Colony Forming Units (CFU).

Background

Weissella is Lactic Acid Bacteria (LAB) belonging to the Leuconostocaceae family, which is gram-positive, catalase-negative, non-motile and non-spore-forming, and has a spherical-shape or irregular rod-shape. Currently, 22 species belonging to the Weissella spp. have been identified and it is widely found in animals (milk, skin and feces), humans (saliva, breast milk, vaginal and feces), vegetables, plants and fermented foods, and etc. Many strains of the Weissella spp. are involved in traditional fermentation because they have characteristics that can improve food quality, safety, nutrition, sensory and texture. Recently, the species Weissella confusa and Weissella cibaria have been isolated from the Weissella spp., they have probiotics characteristics (1-1), (1-2), (1-3), (1-4).

Lactic Acid Bacteria (LAB) produces antibacterial substances such as organic acids and bacteriocins by degrading sugars during the fermentation process, thereby improving the flavor of
food and inhibiting the growth of putrefactive bacteria to prolong the shelf life of food. Also, it is known to normalize the intestinal flora by inhibiting the growth of harmful bacteria through acidification of intestinal pH, and it makes the intestinal environment healthy\(^1-5\).

Probiotics are widely accepted as dietary supplements, and adverse events are rare despite their widespread use.

Kimchi contains various Lactic Acid Bacteria (LAB), and the main LAB in kimchi are *Weissella* spp., *Leuconostoc* spp., *Lactobacillus* spp., and *Pediococcus* spp.\(^1-6\). According to a recent study, a high level of *Weissella* spp. in kimchi is found in kimchi. In addition, several studies have reported the effects of *Weissella* strains isolated from kimchi for improving obesity and health functional effect such as exopolysaccharide (EPS) production\(^1-7\).

*<Annex 01> Reference of Weissella*

In addition, the strain, *Weissella confusa*, is listed in “Possible ingredient lists in human food”\(^2-4\) of the MFDS (Ministry of Food and Drug Safety) which is Korean FDA in South Korea and “Inventory of microbial food cultures with safety demonstration in fermented food products(Bulletin of the IDF N°495-2018)\(^2-2\)” of the IDF (International Dairy Federation).

*<Annex 02> Weissella confusa listed in human food*

*Weissella confusa* WIKIM51 (Wilac D001) was isolated from kimchi, there have been no reports of adverse reactions or side effects from consumers. You can more information on Section 3.1.5.

*Weissella confusa* WIKIM51 (Wilac D001) is registered as a food ingredient in South Korea.

*<Annex 03> Certificate and statement of WIKIM51 (Wilac D001) in South Korea*
1.2 Description of the Evidence Verifying the Identity of the NDI

※TRADE SECRET: All information on Section 1.2.

The NDI Strain ' (b) (4)

<Annex 04> Receipt in the case of an original deposit

(b) (4)

<Annex 05> Report of 16S rRNA gene sequence and DNA genomic analysis

(b) (4)
(b) (4)
1.3 NDI Manufacture

1.3.1 Formulation ingredients

※TRADE SECRET: All information on Section 1.3.1

<Annex 06> COA of culture media and cryoprotectant ingredients

1.3.2 Manufacturing process

※TRADE SECRET: All information on Section 1.3.2
1.3.3 NDI specifications

※TRADE SECRET: All information on Section 1.3.3
Nutrient Component Specification and Results

1.3.4 Methods of analysis

※TRADE SECRET: All information on Section 1.3.4
1.3.5 Analysis of potentially toxic processes

※TRADE SECRET: All information on Section 1.3.5
(b) (4)
1.3.6 Shelf-life and conditions of storage

※TRADE SECRET: All information on Section 1.3.6

We confirmed that *Weissella confusa* WIKIM51 (Wilac D001) count was maintained according to the stability test.

<Annex 10> Stability test results
2. DIETARY SUPPLEMENT MANUFACTURE

Not applicable
3. HISTORY OF USE OR OTHER EVIDENCE OF SAFETY

3.1 History of Use

3.1.1 Description of the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

※TRADE SECRET: All information on Section 3.1.1

*Weissella* spp. is widely found in animals (milk, skin and feces), humans (saliva, breast milk, vaginal and feces), vegetables, plants and fermented foods, and etc. Several studies have reported the effects of *Weissella* strains isolated from kimchi for improving obesity and health functional effect such as exopolysaccharide (EPS) production\(^1\)\(^-\)\(^5\). In various thesis, *Weissella confusa* was isolated from food, fermented food, and the feces of a healthy person, and this strain is tested on the lactic acid bacteria characteristics. You can see thesis on [Table 04].

As such, *Weissella confusa* is a species easily found in food and fermented food, and people already have been taking this strain through food for a long time. In addition, it has been recognized for its value as a Lactic Acid Bacteria (LAB).

<table>
<thead>
<tr>
<th>Summary and reference</th>
<th>Source of <em>W. confusa</em></th>
<th>Annex</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) (4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.1.2 Describe identity information verifying the relationship between the historically consumed material and the NDI or dietary supplement containing the NDI

※TRADE SECRET: All information on Section 3.1.2

<Annex 13> Test for properties of the lactic acid bacteria
3.1.4 Adverse events associated with historically consumed material

※TRADE SECRET: All information on Section 3.1.4

Weissella confusa WIKIM51 (Wilac D001) has not been sold history yet as dietary supplement. However, it was found that it is safe for human consumption through various toxicity and safety tests. You can see this result on Section 4.

In addition, there were no significant side effects in DB search to confirm the safety of Weissella confusa. You can see this result on Section 4.1.1.

These findings indicate that Weissella confusa is a common ingredient in vegetable food products fermented with lactic acid. Weissella confusa is commonly found in various fermented food products consumed in many countries across the world, eliminating any safety concerns given that it has long been consumed via food products.
3.1.5 Alternative rationale for reasonable expectation of safety based on history of use

※TRADE SECRET: All information on Section 3.1.5

3.2 Other Evidence of Safety

※TRADE SECRET: All information on Section 3.2

1) Republic of Korea - Possible ingredient lists in human food\(^2\)

There is no limit for intaking amount.
2) International - Inventory of microbial food cultures with safety demonstration in fermented food products (Bulletin of the IDF N° 495-2018) of the IDF (International Dairy Federation)²-²

Intended use is for sour dough and wine.

<Annex 02> Weissella confusa listed in human food (MFDS & IDF)
4. BASIS FOR CONCLUDING THAT THE NEW DIETARY INGREDIENT WILL REASONABLY BE EXPECTED TO BE SAFE FOR USE IN THE DIETARY SUPPLEMENT

4.1 Intake Safety Assessment

4.1.1 Determination of the No-Observed-Adverse-Effect-Level (NOAEL)

※TRADE SECRET: All information on Section 4.1.1
<Annex 17> 13 weeks repeated oral dose toxicity study
<Annex 21> 4 weeks repeated oral dose range finding study

4.1.2 Determination of the Acceptable Daily Intake (ADI)

※TRADE SECRET: All information on Section 4.1.2

4.1.3 Determination of Estimated Daily Intake (EDI)

※TRADE SECRET: All information on Section 4.1.3
(b) (4)
4.1.4 Intake Amount Assessment

※TRADE SECRET: All information on Section 4.1.4
4.2 Toxicology Studies

※TRADE SECRET: All information on Section 4.2
4.2.1 Single oral dose toxicity test (SD Rat)

※TRADE SECRET: All information on Section 4.2.1
4.2.2 4-weeks repeated oral dose toxicity test (SD Rat)

※TRADE SECRET: All information on Section 4.2.2
4.2.3 13-weeks repeated oral dose toxicity test (SD Rat)

※TRADE SECRET: All information on Section 4.2.3

4.2.4 *In vitro* safety test

4.2.4.1 Antibiotics resistance

※TRADE SECRET: All information on Section 4.2.4.1
(b) (4)
(b) (4)
4.2.4.2 Homolytic activity

※TRADE SECRET: All information on Section 4.2.4.2
4.2.4.3 Toxicity production

※TRADE SECRET: All information on Section 4.2.4.3
(b) (4)
4.2.4.4 Metabolic activity

※TRADE SECRET: All information on Section 4.2.4.4
(b) (4)
4.2.5 Genotoxicity test

※TRADE SECRET: All information on Section 4.2.5

(b) (4)

4.2.5.1 Reverse mutation test

※TRADE SECRET: All information on Section 4.2.5.1

4.2.5.2 Mammalian chromosome aberration test

※TRADE SECRET: All information on Section 4.2.5.2

(b) (4)
4.2.5.3 Micronucleus test in SD rats

※TRADE SECRET: All information on Section 4.2.5.3

4.3 Clinical Studies

※TRADE SECRET: All information on Section 4.3
4.4 Other Safety studies

4.4.1 DB search information on the safety of *Weissella confusa*

※TRADE SECRET: All information on Section 4.4.1

(b) (4)
(b) (4)
4.4.2 Other studies

※TRADE SECRET: All information on Section 4.4.2
NDIN for Weissella confusa WIKIM51 (Wilac D001)
Prepared for Pharmsville Co., Ltd.
5. REFERENCE LIST

※TRADE SECRET: All the reference file

<Annex 01> Reference of Weissella
<Annex 02> Weissella confusa listed in human food (MFDS & IDF)
<Annex 03> Certificate and statement of WIKIM51 (Wilac D001) in South Korea
<Annex 04> Receipt in the case of an original deposit
<Annex 05> Report of 16S rRNA gene sequence and DNA genomic analysis
<Annex 06> COA of culture media and cryoprotectant ingredients
<Annex 07> GMP Certificate and Detailed Manufacturing Process
<Annex 08> COA of Weissella confusa WIKIM51
<Annex 09> Codex general standard for contaminants and toxins in food and feed
<Annex 10> Stability test results
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<Annex 12> Letter of a patent
<Annex 13> Test for properties of the lactic acid bacteria
<Annex 14> Product information including W. confusa
<Annex 15> Product information including Weissella spp.
<Annex 16> Reference of Weissella confusa in kimchi
<Annex 17> 13 weeks repeated oral dose toxicity study
<Annex 18> EDI evaluation data
<Annex 19> An analytical method validation of WIKIM51 dosing formulations
<Annex 20> Single oral dose toxicity study
<Annex 21> 4 weeks repeated oral dose range finding study
<Annex 22> In vitro safety test
<Annex 23> Status and Prospect of Lactic Acid Bacteria with Antibiotic Resistance
<Annex 24> PlasmidFinder Result
<Annex 25> Search results for toxin generating factor of W. confusa
<Annex 26> GRN 953
<Annex 27> Bacterial reverse mutation test
<Annex 28> Mammalian chromosomal aberration test
<Annex 29> Micronucleus test in SD rats
<Annex 30> Clinical study
<Annex 31> Reference of safety information of W. confusa
<Annex 32> Reference of other safety studies of W. confusa
<Annex 33> Risk Assessment data of W. confusa
6. TRADE SECRET

1.1 Description of the Identity of the NDI
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   1.3.2 Manufacturing process
   1.3.3 NDI specifications
   1.3.4 Methods of analysis
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2. History of Use
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   4.2 Determination of the Acceptable Daily Intake (ADI)
   4.3 Determination of Estimated Daily Intake (EDI)
   4.4 Intake Amount Assessment
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   5.1 Single oral dose toxicity test (SD Rat)
   5.2 4-weeks repeated oral dose toxicity test (SD Rat)
   5.3 13-weeks repeated oral dose toxicity test (SD Rat)
   5.4 In vitro safety test
   5.4.1 Antibiotics resistance
   5.4.2 Homolytic activity
   5.4.3 Toxicity production
   5.4.4 Metabolic activity
   5.5 Genotoxicity test
   5.5.1 Reverse mutation test
   5.5.2 Mammalian chromosome aberration test
   5.5.3 Micronucleus test in SD rats
6. Clinical Studies
7. Other Safety studies
   7.1 DB search information on the safety of Weissella confusa
   7.2 Other studies

5. REFERENCE LIST