

May 16, 2025

Shayla West-Barnette, Ph.D.  
Division of Food Ingredients  
U.S. Food and Drug Administration  
Human Foods Program  
5001 Campus Drive  
College Park, MD 20740

Dear Shayla:

The following are the responses from Sunway Biotech to the “Reviewers’ Questions for GRN 1232 (Intended Uses of *Lacticaseibacillus paracasei* subsp. *paracasei* NTU 101)” received on May 5, 2025.

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## Chemistry

**Question 1:**

Please clarify whether all raw materials used in the manufacture of *Lacticaseibacillus paracasei* subsp. *paracasei* NTU 101 are used in accordance with U.S. regulations, are GRAS for the intended use, or are the subject of effective food contact notification. Additionally, please clarify whether any of raw materials are major food allergens or are derived from major food allergens.

**Response 1:**

- (a) Sunway has confirmed that all raw materials used to manufacture *L. paracasei* subsp. *paracasei* NTU 101 are food-grade, comply with U.S. regulations, are listed in 21 CFR Chapter I Subchapter B or the SCOGS database, and have passed internal evaluation to ensure their safety and regulatory compliance. The inner layer of the packaging material is composed of materials recognized under 21 CFR as safe for direct food contact.
- (b) Sunway has confirmed that dairy and soy ingredients are used as raw materials during the fermentation process. These allergens will be declared on the product label and/or in the product information sheets, and communicated to customers. For the finished *L. paracasei* subsp. *paracasei* NTU 101 powder, we conducted allergen testing in accordance with the nine major food allergens specified under the Food Allergen Labeling and Consumer Protection Act (FALCPA) and the Food Allergy Safety, Treatment, Education, and Research Act (FASTER Act). These allergens include milk, eggs, fish, crustacean shellfish, tree nuts (specifically almonds, walnuts, and pecans), peanuts, wheat (with direct gluten testing), soybeans, and sesame. The results indicated the presence of soy and milk allergens.

**Question 2:**

In Table 2 (page 14), the notifier provides specifications and the results from the analyses of five batches.

- (a) Please confirm that the analyzed batches are non-consecutive batches.
- (b) For fermentation derived ingredients, we expect that the specifications will include limits for heavy metals, with at a minimum a limit for lead. Please provide a specification for lead along with results from the analyses of a minimum of three non-consecutive batches. Please also provide an analytical method used to analyze for lead. In view of FDA's Closer to Zero initiative that focuses on reducing dietary exposure to heavy metals, the specification for lead should reflect the results from the batch analyses and be as low as possible.

**Response 2:**

- (a) Sunway has confirmed that the analyzed batches are non-consecutive batches. Each batch of the final powder product is produced at a different time and has been verified to meet the required specifications.
- (b) The following are the heavy metal test results for three non-consecutive batches of *L. paracasei* subsp. *paracasei* NTU 101 powder, along with the description of the analytical methods used.

| Heavy metal <sup>a</sup> | Specification | Lot No. /Date of manufacture<br>(Month/Year) |                       |                       | Method  |
|--------------------------|---------------|--|-----------------------|-----------------------|---|
|                          |               | 23360901<br>Jun, 2023                        | 24411101<br>Nov, 2024 | 25330301<br>Mar, 2025 |   |
| Lead                     | < 0.1 ppm     | 0.01   | < 0.01                | < 0.01                | General Method of Test for Heavy Metals, analysis was performed by ICP-MS.<br>(MOHWH0014.03) <sup>b</sup> |
| Arsenic                  | < 0.25 ppm    | 0.13   | < 0.01                | < 0.01                |   |
| Cadmium                  | < 0.15 ppm    | 0.03   | 0.02                  | 0.02                  |   |
| Mercury                  | < 0.1 ppm     | < 0.01                                       | < 0.01                | < 0.01                |   |

<sup>a</sup> Limit of quantification (LOQ) = 0.01 ppm

<sup>b</sup> MOHW, Ministry of Health and Welfare, Taiwan.

**Question 3:**

Please confirm that all analytical methods used for the batch analyses have been validated for their intended purpose.

**Response 3:**

Sunway has confirmed that all methods for the batch analyses have been validated or verified for their intended purpose. The following is a description of each test method.

| Parameter                           | Method   | Method validation  |
|-------------------------------------|--|--|
| <b>Appearance</b>                   | -  | Based on past manufacturing experience   |
| <b>Lactic acid bacteria (CFU/g)</b> | MOHW Food No. 1021950329<br>Announced: Methods of Test for Food Microorganisms-Test of Lactic Acid Bacteria.<br>(MOHWM0013.01) | This method has been officially announced and implemented by the Ministry of Health and Welfare, Taiwan. |
| <b>Water content (%)</b>            | FD-660 Infrared Moisture Analyzer, KETT Japan  | In accordance with the manufacturer's method documentation.  |
| <b>Water activity (Aw)</b>          | HygroLab, Rotronic Switzerland   | In accordance with the manufacturer's method documentation.  |
| <b>Total plate count</b>            | MOHW Food No. 1121900620<br>Announced: Methods of Test for Food Microorganisms-Test of Aerobic Plate Count.<br>(MOHWM0014.02)  | This method has been officially announced and implemented by the Ministry of Health and Welfare, Taiwan. |
| <b>E. coli</b>                      | Chromocult® Coliform Agar /AOAC 020902(Merck) 、 3M Petrifilm™ E. coli/ Coliform Count Plate                                    | Both methods have been AOAC-certified and were performed in accordance with the suppliers' instructions. |
| <b>Mold and Yeast</b>               | MOHW Food No. 1021950329<br>Announced: Methods of Test for Food Microbiology-Test of Mold and Yeast Count.<br>(MOHWM0008.01)   | This method has been officially announced and implemented by the Ministry of Health and Welfare, Taiwan. |
| <b>Coliform</b>                     | Chromocult® Coliform Agar  | Both methods have been AOAC-certified and  |

|                              |  |  |
|------------------------------|--|--|
|                              | /AOAC 020902 (Merck) 、<br>3M Petrifilm™ E. coli/ Coliform<br>Count Plate | were performed in accordance with the<br>suppliers' instructions.  |
| <b><i>Staphylococcus</i></b> | CHROMagar™ Staph aureus<br>(TPM)   | This method was validated by the supplier,<br>and performed following the supplier's<br>official instructions. |
| <b><i>Salmonella</i></b>     | Singlepath® Salmonella /AOAC<br>060401 (Merck)                           | The method has been AOAC-certified and<br>was performed in accordance with the<br>supplier's instructions.     |
| <b><i>Listeria</i></b>       | CHROMagar™ Listeria (TPM)  | Listed in FDA BAM Media as M40a,<br>CHROMagar Listeria.  |

**Question 4:**

On page 18, the notifier states that the intended food uses of *L. paracasei* subsp. *paracasei* NTU 101 include but are not limited to food categories listed in Table 3. We note that Table 3 lists meat products and alcoholic beverages. To include uses in meat products or other products under the jurisdiction of USDA, the notifier would have to provide data demonstrating the suitability of use of the ingredient in those products. These data were not provided in GRN 001232. Please also note that we do not support adding ingredients that have no technical effect in food to alcoholic beverages. Please exclude alcoholic beverages and foods under the jurisdiction of USDA from the intended use of your ingredient.

**Response 4:**

Sunway has confirmed that the intended use of *L. paracasei* subsp. *paracasei* NTU 101 excludes alcoholic beverages and foods under the jurisdiction of the USDA.

**Question 5:**

Additionally, please clarify if the intended food uses of the ingredient are only in the food categories listed in Table 3 or if the intended food uses would encompass all conventional food except infant formula, products under the jurisdiction of the United States Department of Agriculture (USDA), alcoholic beverages, and foods where standards of identity preclude the use of the ingredient.

**Response 5:**

Sunway has confirmed that the intended food uses of *L. paracasei* subsp. *paracasei* NTU 101 encompass all conventional foods, including but not limited to the food categories listed in Table 3, except for infant formula, products under the jurisdiction of the United States Department of Agriculture (USDA), alcoholic beverages, and foods with established standards of identity that preclude the use of this ingredient.

**Question 6:**

On page 19, the notifier provides dietary exposures to *L. paracasei* subsp. *paracasei* NTU 101 estimated using three different approaches. Please confirm that the estimates are expressed in CFU/person/day. We note that approach #2 is the most conservative and is consistent with our typical approach to estimating dietary exposure for fermentation-derived ingredients. Please confirm that this would be the dietary exposure to your ingredient from the intended uses.

**Response 6:**

- (a) Sunway has confirmed that the estimated values from all three dietary exposure assessment methods are expressed in CFU/person/day.
- (b) Sunway has confirmed that the dietary exposure estimated using approach #2 is consistent with the intended uses under typical consumption conditions. This determination is based on the understanding that yogurt has a relatively high live microorganism count and consumption level among all intended uses and is also a food commonly consumed on a daily basis. Accordingly, yogurt was selected as the representative food for estimating dietary exposure.

**Question 7:**

There are no sample sizes listed for the microbial specifications in Table 2. Please provide the appropriate sample size for each microbial specification.

**Response 7:**

The following are the specifications for each microbial test, with the coliform specification corrected from  $10^2$  CFU/g to 10 CFU/g.

| Parameter             | Specification                     |
|-----------------------|-----------------------------------|
| Lactic acid bacteria  | $\geq 1.0 \times 10^{11}$ (CFU/g) |
| Total plate count     | $< 5.0 \times 10^4$ (CFU/g)       |
| <i>E. coli</i>        | Negative (CFU/50 g)               |
| Mold and Yeast        | $< 10^2$ (CFU/g)                  |
| Coliform              | $< 10$ (CFU/g)                    |
| <i>Staphylococcus</i> | Negative (CFU/50 g)               |
| <i>Salmonella</i>     | Negative (CFU/25 g)               |
| <i>Listeria</i>       | Negative (CFU/g)                  |

**Regulatory****Question 8:**

On page 12, the notifier states that excipients are added to the concentrate to standardize the blends and “confer healing properties” to dry powder. Please explain what you mean by “healing properties”. We note that we do not evaluate health benefits of ingredients under the GRAS Notification Program.

**Response 8:**

Sunway has confirmed that the original statement was incorrect, and the correct meaning of this statement is that the excipients provide desirable handling properties to the powder. Accordingly, the corrected description has been revised to: “Excipients are added to the concentrate to standardize the blends and confer desirable handling properties on the dry powder.”

**Question 9:**

On page 4, the notifier states that the ingredient “qualifies as a Nutrient Supplement as defined in 21 CFR §170.3”. We note that *L. paracasei* subsp. *paracasei* NTU 101 does not meet the definition of a nutrient supplement in §170.3 that is defined as a substance necessary for the body’s nutritional and metabolic processes.

**Response 9:**

Sunway has confirmed that *L. paracasei* subsp. *paracasei* NTU 101 is intended for use as a conventional food ingredient rather than as a nutrient supplement. Based on the definition of 'Nutrient Supplement' in 21 CFR §170.3(o), this classification does not apply to *L. paracasei* subsp. *paracasei* NTU 101. This clarification is provided to avoid any misunderstanding of the intended use.

**Products Under the Jurisdiction of the United States Department of Agriculture (USDA)/Food Safety and Inspection Service (FSIS)****Question 10:**

What is the intended use (why is the substance being added to the product/what is it going to do to the product)?

**Response 10:**

Sunway has confirmed that *L. paracasei* subsp. *paracasei* NTU 101 will be used as an ingredient in all conventional foods as a component of food products, such as dairy products, soy products, juices, non-alcoholic beverages, cereals, confectionery snacks, ice cream, chocolate, and chewing gum. However, its intended uses exclude infant formula, products under the jurisdiction of the United States Department of Agriculture (USDA), alcoholic beverages, and foods with established standards of identity that preclude the use of this ingredient.

**Question 11:**

Just to confirm, is the use for meat and meat sauces for products that fall into the ready to eat (RTE) and not ready to eat (NRTE) product categories? Here is a guide to help (see page 3 and 4).

**Response 11:**

As indicated in the response to Question 4, 5 and 10, Sunway has confirmed that the intended use of this ingredient excludes meat products. In our original concept, the use of *L. paracasei* subsp. *paracasei* NTU 101 in meat products would fall under the ready-to-eat (RTE) category, providing biopreservation functions or serving as a starter culture for fermented meat products, such as fermented sausages and salami. This intended use is currently at the conceptual stage, and there are no development plans in the near future; therefore, this use has been excluded from the intended uses of *L. paracasei* subsp. *paracasei* NTU 101.

We hope this response adequately addresses the Agency's questions regarding the GRN 001232. Should any further clarification be required, we would be pleased to provide it upon request.

Sincerely,



Chunchang Fang, Ph.D.  
U.S. Agent for Sunway  
Cell: +1 818 730 3636

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June 2, 2025

Shayla West-Barnette, Ph.D.  
Division of Food Ingredients  
U.S. Food and Drug Administration  
Human Foods Program  
5001 Campus Drive  
College Park, MD 20740

Dear Dr. West-Barnette:

The following answers are the responses from Sunway Biotech to the “Follow-Up Questions for GRN 1232 (Intended Uses of *Lacticaseibacillus paracasei* subsp. *paracasei* NTU 101)” received on May 21, 2025.

**Question 1:**

In Response 2(b), we note that the specification limits proposed for arsenic (< 0.25 mg/kg) and cadmium (< 0.15 mg/kg) are higher than the limit of that we typically see for fermentation-derived ingredients produced in accordance with good manufacturing practices (i.e., ≤ 0.1 mg/kg). In line with FDA’s Closer to Zero initiative we recommend that you lower the specification limits for arsenic and cadmium to better reflect the results of the batch analyses and to be as low as possible. We also note that the arsenic level in batch 23360901 (0.13 ppm) is significantly higher than that in the other two batches (< 0.01 ppm). Please explain the significant variation in the levels of arsenic between the batches.

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**Response 1:**

- (a) Sunway has revised the specification limits for lead, arsenic, cadmium, and mercury to reflect the results of the batch analyses better. The updated limits are provided in the table below.

| Heavy Metal | New Specification | Previous Specification |
|-------------|-------------------|------------------------|
| Lead        | $\leq 0.1$ ppm    | < 0.1 ppm              |
| Arsenic     | $\leq 0.15$ ppm   | < 0.25 ppm             |
| Cadmium     | $\leq 0.1$ ppm    | < 0.15 ppm             |
| Mercury     | $\leq 0.1$ ppm    | < 0.1 ppm              |

- (b) Sunway has confirmed that the arsenic detected in Batch 23360901 originated from the raw materials used in that production batch. The raw materials have since been adjusted to ensure that the arsenic content in subsequent batches remains below the revised specification limit.

**Question 2:**

In Response 6(b), you state that the dietary exposure estimated using approach #2 (p. 19 of GRN 001232) is consistent with the intended uses of your ingredient under typical consumption conditions. Further, you state that yogurt was selected as the representative food for estimating this dietary exposure. We note that approach #2 is based on the assumption that an individual consumes on average 10 servings of food containing your ingredient per day, not the consumption of yogurt only. Please confirm that the dietary exposure of  $1 \times 10^{11}$  CFU/person/d estimated using approach #2 reflects the dietary exposure to your ingredient from the intended uses.

**Response 2:**

- (a) We apologize for not providing sufficient clarification in our previous response. Sunway has confirmed that the dietary exposure of  $1 \times 10^{11}$  CFU/person/day estimated using approach #2 reflects the overall dietary exposure to *L. paracasei* subsp. *paracasei* NTU 101 from the intended uses.

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Yogurt was selected as the representative food because its expected inclusion level of *L. paracasei* subsp. *paracasei* NTU 101 ( $1 \times 10^{10}$  CFU per serving) is higher than that in other food categories. Therefore, yogurt was used as the basis for estimating the maximum dietary exposure.

Under typical use conditions, the actual per-serving inclusion levels of *L. paracasei* subsp. *paracasei* NTU 101 in other food products are generally lower than that in yogurt. Furthermore, since daily dietary intake typically includes a variety of foods rather than yogurt alone, the actual dietary exposure is not expected to exceed the estimated value. In addition, consistent with the dietary pattern described in GRN 1114 (*B. breve* DSM 33444), it is uncommon for half of the conventional foods consumed daily to contain *L. paracasei* subsp. *paracasei* NTU 101. Therefore, the exposure estimated using approach #2 can be considered a relatively conservative upper-bound value that is unlikely to be exceeded under typical consumption conditions.

- (b) Sunway has identified two typographical errors related to the NOAEL in GRN 001232:

First, in Section 3.2 *Acceptable Daily Intake (ADI)* (page 19), the correct description of the NOAEL should read:

*“Based on findings from a 90-day oral toxicity study in animals, the no-observed-adverse-effect level (NOAEL) for L. paracasei NTU 101 was established at 2000 mg/kg body weight (BW)/day.”*

The underlined portion reflects the correction.

Second, in Table 5 (page 33), under the section titled “*Repeat dose 90-day oral toxicity study in rat*”, the corrected entry in the “*NOAEL Conclusion/Findings*” column should be:

*“2000 mg/kg B.W., equivalent to 1.0 × 10<sup>11</sup> CFU/60 kg B.W. in 120-*

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*fold safety factor.”*

The underlined portion reflects the correction.

These typographical errors do not affect the conclusion that the ADI is  
 $1.0 \times 10^{11}$  CFU/60 kg BW/day.

We appreciate the opportunity to provide additional clarification in response to the Agency's follow-up questions regarding GRN 001232, and we remain available to address any further questions the Agency may have.

Sincerely,



Chunchang Fang, Ph.D.  
U.S. Agent for Sunway  
Cell: +1 818 730 3636