CONFIDENTIAL BUSINESS INFORMATION

MICROBIAL COMMERCIAL ACTIVITY NOTICE (MCAN)

TSACMC17

CONTAINED USE OF GENETICALLY MODIFIED [redacted] STRAINS FOR USE IN PRODUCTION OF [redacted]

FILED BY

Danisco US Inc.
A wholly-owned subsidiary of IFF
(Operating as IFF Health and Biosciences)

925 Page Mill Road
Palo Alto, CA 94304

1. Certificate Statement

Document Control Officer
TSCA Document Processing Center (7407)
Rm. L-100
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

CERTIFICATION

I certify that to the best of my knowledge and belief the company named in this submission intends to manufacture, import, or process for a commercial purpose, other than in small quantities solely for research and development, the microorganisms identified in this submission. All information provided in this submission is complete and truthful as of the date of this submission. I am submitting with this submission all test data in my possession or control and a description of all other data known to or reasonably ascertainable by me as required by 40 CFR §725.160.

The company identified in this notice has remitted the fee specified in 40 CFR §700.45(b).

[redacted] August 20, 2024

Signature of Authorized Official Date

Alice Chen, Ph.D.
Director of Global Regulatory Affairs
Danisco US Inc.
(Operating as IFF Health and Biosciences)
925 Page Mill Road
Palo Alto, CA 94304

2. Executive Summary

IFF Health and Biosciences (Danisco US, Inc.), formerly Genencor International, Inc., has constructed strain

[redacted]

[redacted]

A summary of the construction of [redacted] is provided in Section 5 of this submission. A strain tree detailing the generation of [redacted] is provided in Appendix 3. [redacted]

We have determined that the microorganism is new under Section 5 of the Toxic Substances Control Act. It is of note that the new microorganisms meet the Tier 1 requirements for introduced genetic material as described in 40 C.F.R. §725 Subpart G.

The new [redacted] strain will be used in manufacture of [redacted] The modified [redacted] carries the expressed genes integrated within the genome (i.e., not on a plasmid) and the expression cassettes consisting only of [redacted] All these modifications were performed in such a way that no bacterial vector DNA remains present in any of the strains. No antibiotic resistance markers were inserted into the new microorganisms. The final production strains were characterized by PCR analyses and whole genomic sequencing. A complete description of the genetic modifications of the production strains is provided in this submission in Section 5.3.

The [redacted] be dried [redacted] and the resulting active [redacted] will be supplied to customers' [redacted] plants, where the microorganisms contained in the preparation are expected to be inactivated after the starch conversion to [redacted] is completed. [redacted]

3. Confidential Business Claims

IFF Health and Biosciences is claiming certain strain construction, production process and volume information as confidential business information. Below are the answers to questions provided in 'Points to Consider in the Preparation of TSCA Biotechnology Submissions for Microorganisms,' pp. 52-53.

C. General questions:

1. *For what period of time is a claim of confidentiality being asserted?*

   We request the production process and volume information be kept confidential for the indefinite future. Release of this information will give competitors knowledge about the production process, cost structure and size of the facility. This information will give them unfair advantage on pricing and teach them IFF Health and Biosciences' proprietary production process.

   We also request that the strain construction information be kept confidential for the indefinite future. As IFF Health and Biosciences has provided very detailed descriptions of not only the strain construction, but also strain development strategies that will be employed on an ongoing basis, no finite time deadline can be identified at which CBI status will lapse.

2. *Briefly describe any physical or procedural restrictions within the company or institution relating to use and storage of the information claimed as confidential*.

   The confidential information that relates to manufacturing operational procedures that are part of the company's internal operations documentation is being claimed as confidential. Procedures are written and noted for "internal" use only. There is restricted access to the plant facility limiting information exchange with outside persons. The confidential information that relates to strain construction is maintained as part of the intellectual property of the company.

   All IFF Health and Biosciences employees sign confidentiality agreements. They are informed that this type of information is confidential. Disclosure beyond employees only occurs (1) to companies that are contractually bound to preserve confidential status, and (2) to government agencies under appropriate, narrow circumstances.

3. *Has the information claimed as confidential been disclosed to others outside of the company or institution?*

   Process information is considered proprietary and is not disclosed to others outside the company. If vendors or others need to enter the production areas, it is encouraged to obtain a Non-Disclosure Agreement to safeguard against loss of confidential information. Production strain construction information is similarly considered confidential and is not disclosed to others outside the company, except as needed for regulatory approval purposes.

4. *Does the information claimed as confidential appear, or is it referred to, in any of the following:*

(1) Advertising or promotional materials for the microorganism or the resulting end product

   No. IFF Health and Biosciences is a technology company. Our intellectual property is critical to maintain, and all employees must adhere to a strict internal review process with regard to disclosure of information that is or might be valuable.

(2) Material safety data sheets or other similar material for the microorganism or the resulting end product

   No. Product MSDS disclosure is generally limited to exposure limits, clean-up recommendations and toxicity data. Formulation ingredients may be listed.

(3) Professional or trade publications

No. While IFF Health and Biosciences may publish some details of host strain characteristics, production strain construction is proprietary and not subject to publication.

(4) Any other media available to the public or to your competitors

No. Equipment vendors occasionally ask if we can be mentioned as a user of their equipment – that is normally approved at corporate level. Strain development techniques and strategies are not made public to the public or competitors.

(5) Patents

No. While some strain construction elements claimed to be confidential are the subject of pending patents, the production strain construction details of any particular strain are proprietary and not stipulated in patents held by IFF Health and Biosciences.

(6) Local, State or Federal Agency public files

No, to the best of our ability confidential information is not disclosed in these public files.

5. *Has EPA, another Federal agency, a Federal court, or a State made any confidentiality determination regarding the information claimed as confidential?*

No.

6. *For each type of the information claimed as confidential, describe the harm to the company or institution's competitive position that would result if this information were disclosed.*

There are several types of confidential information:

(1) Production quantities;
(2) Process descriptions and chemicals;
(3) Unit operation descriptions & process flow diagram;
(4) Possible organism release points;
(5) Possible worker exposure;
(6) Production strain construction, including the identity of the newly introduced genetic elements; and
(7) Chemical substance produced by the production strain.

(1) Dissemination of planned production volumes would indicate to competitors the scale of IFF Health and Biosciences' product introduction. Armed with this information, knowledgeable competitors could predict the target market(s) and allow them to build focused strategies to thwart the product introduction. Our company's future is highly dependent on successful introduction of valuable new technologies such as this.
(2) Dissemination of a process description would advance a competitor's process and product development capabilities in general and potentially would also allow them to quickly develop a similar product.
(3) Dissemination of unit operations and particularly of a process flow diagram would also allow a competitor to analyze the process yield and determine the cost of manufacturing. This information would give a competitor an unfair competitive advantage against IFF Health and Biosciences. For example, a competitor armed with IFF Health and Biosciences' production cost information could price their product such that IFF Health and Biosciences would be excluded from the market.

(4) Dissemination of possible organism release points tells a competitor what type of equipment is being used to contain and process the organism and the product. This is valuable information leading to pricing and/or process determinations.
(5) Dissemination of possible worker exposure as in #4 tells a competitor what type of equipment is being used, the number of persons involved in controlling the process and the duration of their potential exposure.
(6) Dissemination of strain construction techniques and strategies for developing a particular production organism tells a competitor which genes in the host organism to manipulate to maximize target chemical production and fit to the intended application. In addition, such dissemination would teach competitors how to manipulate the noticed host gene sequences and inserted sequences in such ways that may overcome IFF Health and Biosciences' competitive advantage in the use of the host organism as a safe and suitable [redacted] production organism. Making this information available to competition will allow them to analyze IFF Health and Biosciences' technical capabilities, production and cost structure and give them an unfair advantage in the marketplace.
(7) Dissemination of the chemical substance produced by the production strain signals to a competitor that we have new strains in this market in this field, which may lead a competitor to increase activity in this field to compete with IFF Health and Biosciences. This would lead to a disadvantage for IFF.

7. *If EPA disclosed to the public the information claimed as confidential, how difficult would it be for the competitor to enter the market for the resulting product?*

Competitors have already entered the market and they have developed many similar products that they sell into a number of markets. If fully disclosed, competitors would have no trouble 'reverse-engineering' our product, process, or parts of our process, and this would allow them a competitive advantage in the specific target market for this product and in any number of other markets. The new product is an enhanced variant of an existing product with complementary side activities. Competitor introduction of a similar product would require specialized genetic technical expertise to construct the production microorganism and development of a specialized process to produce the intended end product. Competitors clearly have the expertise to do this type of work.

Dissemination of our confidential information could very well lead to disastrous effects for IFF Health and Biosciences in our target market.

4. Submitter Identification

Name
Danisco US Inc.
(operating as IFF Health and Biosciences)

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5. Microorganism Identity Information

5.1 Generic Name and Use

The submitter is requesting that the generic name for the microorganism and the intended use to be “genetically modified microorganism for the production of a chemical substance.”

The specific name for the microorganism and intended use, which is considered to be confidential business information, is [redacted]

5.2 Recipient Microorganism and New Microorganism

[redacted] through [redacted] molecular sequencing (Appendix 6). [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]
[redacted]
[redacted]

[redacted]

[redacted]

[redacted]

[redacted] is derived from [redacted]

[redacted]

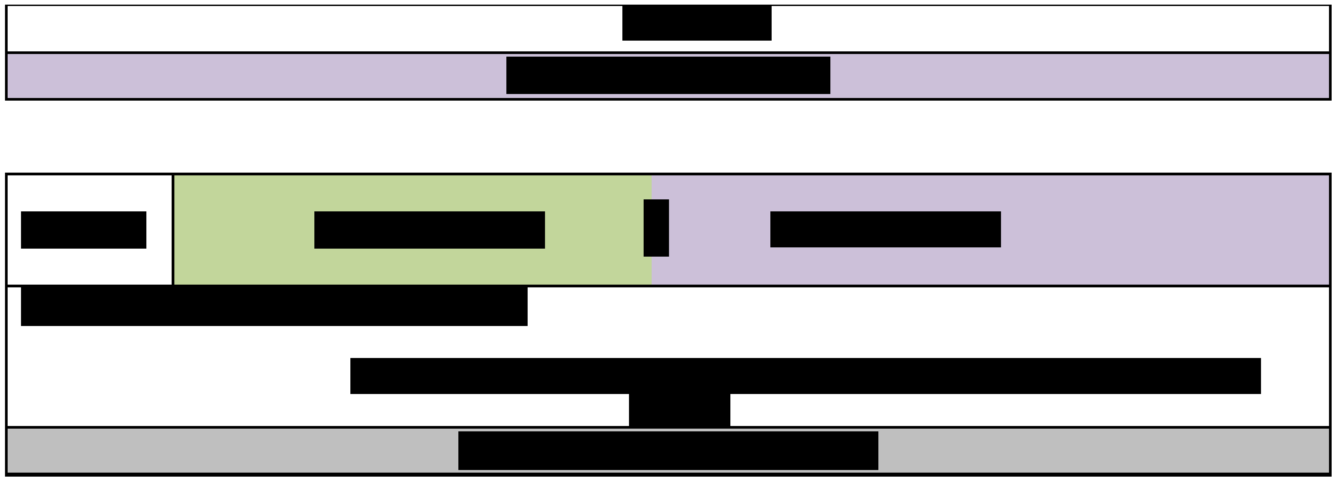
[redacted]

[redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		
				[redacted]	[redacted]	[redacted]

A summary of the construction of [redacted] is provided in Section 5 of this submission. A strain tree detailing the generation of [redacted] is provided in Appendix 3. [redacted] Data to establish the new microorganism taxonomy are provided in Appendix 6.

Construction scheme of [redacted]



All these modifications were performed in such a way that no bacterial vector DNA remains present in the final production strains. No antibiotic resistance markers were inserted into the new microorganism. The final production strains were characterized by PCR analyses and whole genomic sequencing. The taxonomic identity of the donor strain is described under 5.3.1.

5.2.1 Morphological and Physiological Features

[redacted] cultures are typically fast-growing at [redacted] Colonies are usually round. [redacted] These features also apply to the production strains.

[redacted] can be detected by making use of its microbiological phenotype. The strain is capable of [redacted]

[redacted]

5.2.2 Other Data for Identification

Features for unique identification for inventory purposes are described above in 5.2. The new microorganisms can be identified using molecular biological methods like PCR analysis using primers specific for the integrated expression cassettes or the introduced genomic deletions. The PCR identification kit provided in Appendix 1 contains information for the molecular identification of the new microorganisms.

5.3 Genetic Construction

5.3.1 Taxonomy of Donor Microorganisms

[redacted]

5.3.2 Added/Modified Traits

[redacted]

5.3.3 Detailed Description of Construction

Detailed descriptions of the genetic construction of [redacted] are also found in Section 5.2 and in Appendix 2.

[redacted]

5.4 Phenotypic and Ecological Characteristics

5.4.1 Habitat, geographical distribution, and source of the recipient microorganism

[redacted] is ubiquitous in nature. It has been recovered from a variety of sites varying ecological conditions; mostly present on [redacted], and other sources with a high concentration of carbohydrates but is also common in soils. [redacted] is not airborne, but needs a vector (*e.g.*, an insect) to move within and between habitats. It is predominantly associated with environments favoring fermentation (preferring low to neutral pH) and is able to utilize several different carbohydrates depending on the type of metabolism involved [redacted] as well as a wide variety of nitrogen sources [redacted]

5.4.2 Survival and dissemination

[redacted]

[redacted]

The [redacted] strains are not limited by built-in biological barriers; and neither are the final production strains.

[redacted] cultures are typically fast growing at 28-33°C [redacted] At 37°C and above, growth rate is slower. Colonies appear [redacted] Colonies are usually round. A [redacted] is produced by the culture. These features also apply to the production strains.

[redacted]. Once candidate production strain colonies have been isolated these can be identified using molecular biological methods like PCR analysis using primers specific for the integrated expression cassettes or the introduced genomic deletions. Several survival studies on [redacted] comparing wild-type [redacted] and genetically modified [redacted]

The production strains could be accidentally disseminated in the pilot or production plant in areas such as wastewater, surface water, and soil surrounding the production facilities. In case of accidental spill, the production organisms can be inactivated using all known methods to inactivate [redacted], like steam, bleach, etc.

5.4.3 Anticipated Biological Interactions and Transformative Properties

[redacted] (see 10.2.1 below and Appendix 7), and as such does not have a mammalian host nor does it have significant interactions with other organisms, other than [redacted] ones. [redacted] is a very uncommon cause of infection in humans [redacted]

No vector bacterial DNA used in creation of the new microorganism remains in the new microorganism. Since no vector sequences are present in the final strains, the transfer frequency of the integrated expression cassettes is the same as for any other chromosomal sequence, which is low and considered genetically stable.

There are no expected anticipated interactions between [redacted] and the organisms or microorganisms which might be exposed in case of release into the environment. Furthermore, there are no known or predicted effects on plants and animals such as pathogenicity, infectivity, toxicity, virulence, vector of pathogen, allergenicity and colonization from the production strains.

5.4.4 Biogeochemical/Biological Cycling Processes

[redacted] is a [redacted] It is predominantly associated with environments favoring [redacted]

[redacted] A public literature search did not produce any articles describing the involvement of the production strain in biogeochemical processes.

6. Byproducts

[redacted]

7. Production Volume

The new microorganisms will be used in a batch process to manufacture the desired [redacted] product. For each batch, the volume of the broth at harvest will be approximately [redacted]. At a cell density of [redacted] there will be approximately [redacted] and [redacted] of wet cell mass produced per fermentation batch. Using bench scale experiments that model the large-scale production process, we have determined that [redacted] at the end of fermentation is equivalent to approximately [redacted], then the [redacted] is [redacted] to form an [redacted] The dry cell weight of the final [redacted] product is [redacted] The table below approximates the cell mass to be produced in the next three years:

[redacted]

8. Use Information

The new microorganism will be used to manufacture the producing strain at IFF Health and Biosciences-controlled facilities and stored in approved controlled facilities. The product has application .

The

production strain can be added directly to the tank or can be added to a mixing tank and transferred through process piping. The resulting hydrolysate containing all fermentation ingredients is then pumped in its entirety by a closed process to the large main fermentor.

In general, the large main fermentor is prepared in a similar manner as the propagation tank by adding various materials to the liquefact Upon completion of fermentation, this material from the aqueous components (water and solid mash components which includes the producing strain). This aqueous process stream which includes

The subject strains will be used for production on industrial scale; the current industry standard is between As the technology and economy of scale develops, this will likely continue to increase. The current industry operating scale in the U.S. is approximately per year based on 2015 amounts,[1] but larger plants are likely as the industry develops.

At the [redacted] manufacturing plants, the potential for worker exposure to viable [redacted] will occur at the tank addition step similar to additions of current [redacted] producing strains. [redacted]

9. Worker Exposure and Environmental Release

9.1 Submitter-Controlled Sites

9.1.1 Site Information

The stock culture and seed vial lot preparation of the new microorganisms will be conducted at the IFF (Danisco US Inc) manufacturing facility in Rochester, New York. The address is:

Danisco US Inc.
1700 Lexington Avenue
Rochester, NY 14606.

The sterile, sealed, cryo-protected vials will be sent for the manufacture of the [redacted] product to the IFF Health and Biosciences manufacturing facility in [redacted] The address is:

Danisco US Inc.
[redacted]

Or to [redacted] manufacturing facility in [redacted] The address is:

[redacted]

[redacted]

9.1.2 Process Description

Both manufacturing facilities under control of IFF Health and Biosciences are secure facilities that are operated 24 hours a day. People not employed by IFF entering the facility must check-in and be registered. The stock cultures are prepared in a controlled laboratory that is solely for that purpose; access to the lab is limited. The fermentor and processing equipment in [redacted] is located in an area of the plant that is separate from office and laboratory space; access to the production areas is limited.

The fermentor vessels used for the production of the new microorganism are closed stainless steel tanks designed and built according to American Society of Mechanical Engineers (ASME) codes. [redacted] Piping connections such as fill line, draw line, sample port, and inoculation port are highly secure and [redacted]. Positive steam pressure is maintained on the exterior side of each valve in contact with the fermentor to prevent contamination. Compressed air is filtered to remove airborne particles. All procedural steps and engineering standards described above are meant to ensure that a pure culture fermentation is run.

After each fermentation step is completed and the contents of the fermentor have been transferred, the vessel is cleaned in place (CIP) with a [redacted] (sanitation step) and the wash contents of the fermentor are directed to the equalization tank. Bench scale experiments have demonstrated that [redacted] is not viable after [redacted]. The cell will not be viable at [redacted] Other production equipment is also cleaned with a [redacted] in a similar manner. The harvest broth is then [redacted] Prior to use, the equipment in this step is sanitized using various chemical and thermal techniques.

The final [redacted] is stored in cleaned/sanitized stainless-steel tanks and/or totes and maintained under refrigerated conditions. When the process is completed, all equipment is cleaned in place (CIP) with a hot [redacted] and the wash contents are directed to the equalization tank. The [redacted] is then either sold as a [redacted] product, after [redacted] has been added to a final concentration of [redacted] or [redacted]. The final [redacted] product is vacuum packaged into oxygen/air proof bags and sent to a refrigerated warehouse for storage and distribution to the customer. When the process is completed, all equipment (*e.g.,* totes, vessels, filters, extruders, etc.) is cleaned with hot [redacted] and the wash contents are directed to the equalization tank where the contents are [redacted] for inactivation of [redacted] strain. Data provided below [redacted] as the strains are similar and differences are not expected to increase thermal tolerance.

Figure 1. Process Diagram for IFF Health and Biosciences Manufacture of the New Microorganism: Below is a diagram of the major unit operation steps, all of which are described in detail after the diagram.

a. Unit Operation Description

i. Stock Culture Preparation

The following procedures are performed in a bio-safety cabinet by laboratory staff equipped with the typical personal protective equipment (lab coat, safety glasses, latex, or nitrile gloves). Starting with the cell suspension deposited in the IFF Health and Biosciences Culture Collection, flasks are inoculated to grow the microorganism. After appropriate growth, the cells are harvested and mixed with a sterile glycerol stock to reach [redacted] glycerol concentration, dispensed into cryogenic storage vials, and stored in [redacted] freezers. Post storage quality assurance checks include 1) plating to check for the absence of contaminating organisms and for cell survival and purity, 2) comparison of pre-freeze viability with the post-freeze viability to determine the survival rate and 3) testing the vials for productivity. The seed vial lots are prepared in the Culture Collection laboratory according to a Standard Operating Procedure.

ii. Aseptic Inoculation of Production Organism

The seed inoculum is prepared by adding, in a bio-safety cabinet, vials of a stock culture cell suspension of the new microorganism (stored under liquid nitrogen) to a culture bag containing appropriate media. The bag is then placed in an incubator. When appropriate cell mass is achieved, the contents of the bag are aseptically transferred directly into the seed tank by a member of the dedicated fermentation team, equipped with lab coat, latex or nitrile gloves, and safety glasses.

iii. Seed Fermentor

A seed fermentation vessel, controlled at prescribed environmental conditions (temperature, pH control via ammonia addition, pressure, and airflow), is used to generate the inoculum for the production vessel. Transfer to the production fermentor is conducted by dedicated fermentation operators and occurs via steam-sterilized hard piping.

iv. Main Fermentor

The process will use a defined medium as the basis for both seed and production fermentations. [redacted]

The cells transferred from the seed fermentor are allowed to grow until the target population size has been reached. [redacted] The fermentor broth is then transferred to a drop tank. Criteria

are in place for detecting contaminated fermentations. If a run is deemed contaminated, it will not be used.

The fermentors used to grow the production organism are located in a separate area of the manufacturing facility and share an isolated holding tank system. After every run, the main fermentor is washed using a [redacted] as described above. Rinse material from routine tank cleaning is directed to the equalization tank (where the pH is brought to neutral), which flows to the municipal waste treatment facility.

v. Post-fermentation processing

The harvested fermentor broth from the main fermentor is sent to a drop tank [redacted]

The broth is then processed through a series of steps (see below) in order to transform it [redacted] After every batch, the fermentor and drop tank units are run through a [redacted] as described above.

[redacted]

[redacted]

[redacted]

[redacted]

b. Starting Materials and Feedstocks

The new microorganism fermentation is a contained process of a pure culture of the production strain. The ingredients for the seed and main fermentors are as follows:

[redacted]

c. Possible Release Points Identified in Process Diagram

i. Stock Culture Preparation All manipulations are carried out in a bio-safety cabinet to maintain sterility and to protect the laboratory technician and the environment. All disposable items (e.g., gloves, pipets, culture preparation plates, culture spreaders) are placed in a biohazard trashcan, where they are then autoclaved. All glassware (e.g., culture flasks, beakers) is treated with bleach before being washed. Consequently, there is no microorganism release expected at this stage.

ii. **Seed Inoculum Transfer** Once the cell mass in the culture flasks is sufficient, the culture is transferred into a stainless-steel transfer apparatus in a bio-safety cabinet. Empty flasks are treated with bleach and rinsed to the sink (which ends up in the equalization tank before being released to the municipal wastewater treatment system). Dedicated laboratory staff, equipped with lab coats, hard hats, gloves, and safety glasses, transfer the inoculum from the transfer apparatus to the seed fermentor via steam sterilized hard piping. The empty transfer apparatus is [redacted] before being rinsed to the laboratory sink. There are no anticipated releases of the production strains [redacted] at this stage.

iii. **Fermentation**

The fermentors used to grow the production organism are located in a separate area of the manufacturing facility and share an isolated holding tank system. Tanks are routinely run through a [redacted] (CIP) after every run. Rinse material from routine tank cleaning is directed to the equalization tank (where the pH is brought to neutral) that supplies the municipal waste treatment facility.

a) **Fermentation Samples** Routine samples collected from the fermentor are either spot checked on the fermentation floor (*e.g.*, pH determination) or sent to the lab (on site) for routine analysis. All samples are inactivated with either bleach or through autoclaving prior to disposal.

b) **Fermentor Off Gas** Exhaust gas passes [redacted] that reduces the amount of organism released to the environment. The exhaust air is directed [redacted]

iv. **Post Fermentation Processing**

[redacted]

[redacted]

[redacted]

9.1.3 Worker Exposure

a. Stock Culture Preparation

Exposure to the organisms is not expected during stock culture preparation due to the fact that transfers and organism handling occur in a bio-safety cabinet to maintain sterility and to protect the laboratory technician and the environment. Employee exposure to the subject microorganism is also limited at this step by the personal protective equipment worn by the laboratory technician. The stock culture preparation will require approximately [redacted] hours of lab technician time per stock culture batch, and one [redacted]

b. Seed Inoculum Transfer

Exposure to the organism is not expected during seed inoculum transfer due to the fact that transfers use aseptic technique and are conducted in a bio-safety cabinet in a manner that contains the production microorganism. Employee exposure to the subject microorganism is also limited at this step by the personal protective equipment worn by the laboratory technician. [redacted]

After transfer of the inoculum to the seed fermentor, the empty transfer apparatus is given a [redacted] treatment before being rinsed to the sump in the fermentor floor. When the apparatus is broken down for cleaning, it contains only inactivated organisms and [redacted] residue.

c. Fermentation

The activities on the fermentation tank floor that involve potential worker exposure to the new microorganism are described below in two categories – fermentation samples and fermentor off-gas.

i. Fermentation Samples

Process technicians, equipped with uniforms and safety glasses, will occasionally take samples from steam locked sample ports in the fermentor during production runs. The employee exposure to the subject microorganism is limited at this step by the personal protective equipment worn by the process technician and the small sample volumes required. Samples are carried in sealed vials to the dedicated laboratory staff for routine analysis. Exposure to the organism is also limited during this activity by the personal protective equipment that is regularly worn by the dedicated laboratory staff (lab coat, latex or nitrile gloves, and safety glasses). All samples are inactivated with autoclaving prior to disposal. Due to the controls described here, handling of the fermentation samples represents minimal employee exposure.

ii. Fermentor Off-Gas

As described above in 9.1.2.c "Possible Release Points Identified in the Process Diagram," the fermentor exhaust gas passes through a droplet separation system before being released to the atmosphere. Liquid waste collected from the separation system is collected in a surge tank before being discharged to the waste treatment system. The best estimates of the volumetric flow rate of one fermentors vent is [redacted] of air. Small amounts of steam and water condensate are also present in the fermentor off-gas. Exposure to the production strain inside the facility from fermentor off-gas is not expected.

d. Post-Fermentation Processing

[redacted]

e. Drying Process

[redacted] Employees wear the following personal protective equipment during the drying process and cleaning operations to limit exposure to the [redacted], safety glasses, gloves, and uniforms. Respirators with [redacted] are worn during operations with potential for exposures such as cleaning and packaging processes.

Summary of worker exposure information

[redacted]				

9.1.4 Environmental Release and Disposal of Subject Microorganism

Possible release points for and disposal of the new microorganism during manufacture of the [redacted] product are discussed in Section 9.1.2 above. Efficiency of the control technology at the various potential release sites is outlined in the table below.

Control Technology Efficiency at Potential Sites of New Microorganism Release

The three potential sources for release of the new microorganisms from the manufacturing facility are via solid, liquid, or gaseous emissions. During normal operation, no detectable

viable release is expected via solid or liquid waste emissions; a negligible amount of release may occur in the fermentor off-gas and dryer exhaust.

a. Manufacturing Site Proximity to Drinking Water Sources

The [redacted] is located approximately 0.8 miles from the facility.

9.1.5 Transport and Emergency Containment Procedures

In the unlikely event of a large spill of material containing the new microorganism, containment procedures will be followed. Any spill greater than a small leak will be reported to the supervisior and the source of the leak will be addressed by closing associated valves or isolating the leak point. The impacted area will be barricaded off until the material is washed to the internal drain. Wash water will be used to flush the material and the wash water can be heated with steam to ensure thick material is flushed and cleared completely to the drain. The trench drains then feed to an interal sump and surge tank. The surge tank is heated in case the need to inactiveate the spill arises. An incident report is completed for any spills as described above.

Aqueous waste from spills will go to the respective [redacted] Discharge would be indirect to surface waters. The Water Pollution Control facility will be notified if the fermentation spill volume exceeds [redacted] of normal discharge.

9.1.6 Disposal and Inactivation Procedures

The procedures requested here are described above.

9.2 Sites not controlled by the submitter

As the submitter expects that the new microorganism can be sold to more than one customer, we have for this requirement, described a general [redacted] facility. The production plant is designed to efficiently produce [redacted] from [redacted] Generally, the sites are expected to have safety, health and environmental hazard control and mitigation. Microorganisms that are used in these facilities are generally employed under containment conditions to manage worker exposure and environmental release. [redacted] sites generally are known to have Spill Control and Emergency Response programs based on safety, health, and environment (SHE) standards and applicable regulatory requirements (*e.g.*, OSHA 1910.119, EPA Risk Management Plan, State and Federal Spill Prevention and Groundwater Protection programs). The environmental impacts have been reviewed for the strains as part of the MCAN under their intended use conditions and concluded not to be more hazardous than the unmodified recipient strain.

9.2.1 Process description and use operation involving the MCAN strain

The new microorganism will be used for commercial [redacted] manufacture at customer sites, which are not under the control of the submitter. Below is a description of how the new microorganism be handled at potential customer [redacted] plants. All production activities are conducted in a building or tanks and there is no intentional testing outside of

the structure. Buildings enclose most of process except the fermenters are located partially outside of the building enclosure. Access to the building and processing areas is controlled.

Figure 2: Overview of a [redacted]

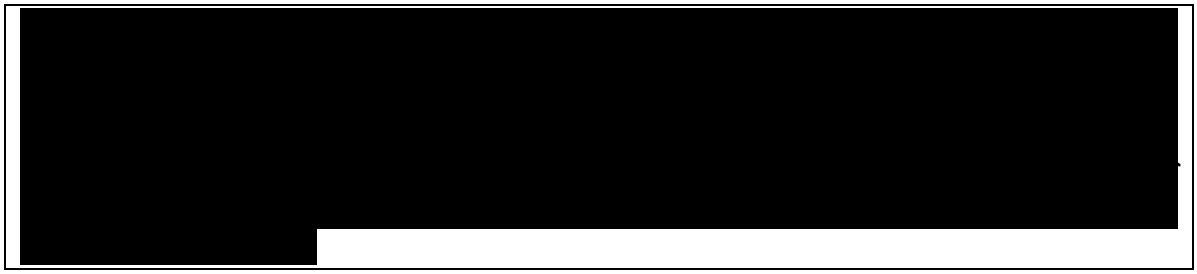


Figure 3: Overview of temperatures associated with [redacted] **production** [redacted]

Figure 4:

After fermentation, the following post-fermentation processes are expected to inactivate the MCAN strain prior to environmental release based on high process temperatures (see Figure 3):

[redacted]

In general, the process containing viable [redacted] is enclosed and the majority of waste streams are recirculated back into the process into the [redacted] and ultimately back into the fermentors. Any viable [redacted] released into waste streams is re-circulated back into the process and inactivated in the distillation column. The temperature of the cook tank and whole stillage tank (after distillation) are [redacted]

[redacted]

Trenches inside the building flow to a sump which is pumped into the beerwell (recycled into the process). All wash downs and spills are collected in the trench. Samples are also poured into the trench. All lines, tanks and processing equipment are cleaned with [redacted]

We have conducted internal heat inactivation studies and determined that our strain is inactivated at [redacted] minute. None of the introduced genetic materials nor endogenous modifications are known in the public literature to increase the thermal tolerance in [redacted] to be the same as the unmodified parental strain. Centrifugation occurs at [redacted] °F (Figure 3) and it is our understanding that this process will take longer than 15 seconds, which is the pasteurization time at the lower temperature of [redacted] °F, we also believe this step will inactivate the MCAN strain. [redacted]

A. Co-product Processing

The co-products are not expected to contain live [redacted] however we have provided some information on this process. Based on our experience, it is our understanding that all products and co-products will be shipped by trucks in pallet containers.

B. Estimated number of Processing or Use Sites

[redacted]

[redacted]

C. Worker Exposure and Control Measures

We believe worker exposure to the viable MCAN strain occurs during adding [redacted] into the fermentor, which generally takes only 1 worker for a period of a few minutes.

[redacted] are through enclosed lines. From the propagator, the broth is transferred through closed lines to the large fermentors. Air from the fermenters exhaust through the CO_2 scrubber [redacted] The scrubber water flows to the cook tank [redacted]

Employees change into uniforms each shift and do not take or wear the uniforms off-site. Uniforms are laundered by a professional service. Employees also wear safety glasses, gloves, and protective shoes. Furthermore, employees receive training by the Technically Qualified Individual on the potential health hazards of the microorganism, work practices, inactivation procedures, equipment and room cleaning and spill clean-up.

D. Environmental Release, Disposal, and Control Measures

Air emissions are generally addressed through the site air permits and managed by the EHS and operations team. For air release, it is our understanding that most [redacted] facilities utilize air capture technology such as water scrubbers. Air from the fermenters exhaust through the CO_2 scrubber [redacted] The scrubber water flows to the cook tank [redacted] It is also our understanding that microbial air release, relative to solid and liquid release, is a minor contribution to environmental release.

[redacted] It is our understanding that many facilities inactivate equipment that has contact with the viable MCAN strain (*e.g.,* autoclaving laboratory equipment). Therefore, solid release is expected to be minimal.

Liquid release is also minimal as many [redacted] facilities reuse their wastewater and treat wastewater prior to discharge into the environment either on site or through a

municipal service. Reuse of wastewater reduces the cost and environmental footprint of [redacted] production, and therefore there are incentives for [redacted] facilities to employ such practices.[1] In general, the process containing viable [redacted] released into waste streams is re-circulated back into the process and inactivated in the distillation column. The temperature of the cook tank and whole stillage tank (after distillation) are [redacted]

Therefore, we also conclude that liquid release from [redacted] production will be minimal.

E. Transport procedures

[redacted]
[redacted]

[redacted]
[redacted]
[redacted]
[redacted]
[redacted]
h
[redacted]

[1] [redacted]

[redacted]

10. Health and Environmental Effects Data

10.1 Test Data on New Microorganism

IFF Health and Biosciences has not conducted tests specifically on the new microorganism, but has conducted a test on a nearly identical strain (industrial strain [redacted] and previously EPA-submitted, [redacted], all of which received a "unlikely to present an unreasonable risk of injury to health or the environment" determination.

The [redacted] strain was tested for pathogenicity and toxicity. The study was initiated by IFF Health and Biosciences, (see Appendix 8). In addition, the non-toxic and non-pathogenic status of [redacted] is well established (see 10.2.1 below). The new [redacted] microorganisms will be contained during use and [redacted]

10.2 Report/Literature Citations for Test Data

10.2.1 Health Effects

[redacted] is a non-pathogenic [redacted] It is not present on the list of pathogens used by the EU [redacted] and major culture collections worldwide. It is classified as Biosafety Level 1 (BSL1) microorganism by the American Type Culture Collection (ATCC) based on assessment of the potential risk using U.S. Department of Public Health guidelines with assistance provided by ATCC scientific advisory committees. BSL1 microorganisms are not known to cause diseases in healthy adult humans. It is listed as being suitable for the construction of Genetically Modified Microorganisms (GMMs) of Risk Group 1 in Germany, The Netherlands, etc. [redacted] has been tested for pathogenicity and toxicity and products derived from various strains of [redacted] have been investigated for numerous toxic endpoints. These included studies published in the literature as well as IFF's studies (Appendix 8). Both the [redacted] and the European Food Safety Authority (EFSA) (2012) have provided an overview of [redacted] deeming its safe as an industrial production organism. [redacted] determine that [redacted] is a safe host for production of substances used in food and food-processing, and that the host is non-pathogenic and non-toxigenic. It is concluded that the new [redacted] microorganisms are nonpathogenic and nontoxigenic.

10.2.2 Ecological Effects

[redacted]

10.2.3 Physical and Chemical Properties

There are no applicable physical and chemical properties data for the subject microorganisms.

10.2.4 Environmental Fate Characteristics

Please see 10.2.2 above.

10.2.5 Human Exposure and/or Environmental Release Data

No additional data are available for these particular microorganisms. However, the risk assessment conducted by the [redacted] provides a general overview of [redacted] and its safety as an industrial production organism [redacted]

10.3 Other Data Concerning Health and Environmental Effects of New Microorganism

10.3.1 Data in Submitter's Possession or Control

A 90-day, oral gavage toxicology study dated February 23, 2016, was performed on modified [redacted] strain [redacted]. This strain was the subject of [redacted] which shares one of the [redacted] Study showed that ingestion of the test article preparation in rats for 90-days resulted in no adverse effects. No test substance-related deaths occurred during the study. No test substance-related ophthalmological neurobehavioral, anatomic or clinical pathology effects were observed. Under the conditions of this study, the no-observed adverse effect level (NOAEL) for strain [redacted] was [redacted] The NOAEL is based on the lack of adverse effects at any concentration tested (Appendix 8).

We have no scientific basis to believe that our introduced genetic material in the subject microorganisms would alter the toxicity profile of the strains. Therefore, the toxicity studies present above serve as appropriate surrogate data for the subject microorganisms.

10.3.2 Data Not in Submitter's Possession or Control

None other than that cited above.

References

[redacted]

[redacted]

[redacted]

18. [redacted]

This document contains TSCA Confidential Business Information

[redacted]
[redacted]