Review

Comprehensive Review of Safe Microbial Ingredients for Use in Food

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Posted Date: 21 May 2026

doi: 10.20944/preprints202605.1453.v1

Keywords: GRAS; food cultures; fermentation; safe microbial species

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Comprehensive Review of Safe Microbial Ingredients for Use in Food

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Abstract

This publication identifies microbial species that are internationally recognized as safe for use in food, based on both a long history of human consumption and a consensus of safety established through rigorous scientific evaluation by independent, qualified experts. While the United States (U.S.) Code of Federal Regulations (CFR) references microbial ingredients, it does not provide a published list of safe and suitable microorganisms for use in food, leading to uncertainty about which species are considered Generally Recognized as Safe (GRAS) for their intended use. This review addresses that gap by compiling a species-level list of microbial ingredients that are recognized as safe by a consensus of global experts. It also outlines the criteria for GRAS designation of microorganisms, emphasizing the importance of strain-level safety assessments by manufacturers to confirm the absence of pathogenicity or toxigenicity. Excluding genetically engineered microorganisms (GEMs) and ingredients intended for vulnerable populations, this work aims to bring clarity to the current recognition of safe and suitable microbial species for use in food. It serves as a consensus resource for the safety of these microbial ingredients for use in food.

Keywords: GRAS; food cultures; fermentation; safe microbial species

Introduction

Microbial ingredients have played a crucial role for millennia in the production and safety of food and continue to be an integral part of our food supply. Without microbial ingredients acting as fermentation agents, we would not have bread, beverages, dairy and meat products (cheese, yoghurt, sausage) or in more recent times, plant-based alternatives to dairy and meat. Microorganisms have been utilized throughout history — often unknowingly — to enhance food preservation, flavor, organoleptic qualities, and nutritional value. Today, microbial ingredients are manufactured to meet the precise needs of the food industry, supporting modern day safety standards and consumer tastes.

The selection of unambiguously identified and characterized microbial strains belonging to safe species is crucial for maintaining the integrity of our food systems and protecting consumer health. Strains used in food must undergo thorough evaluations for their safety. This includes assessing their taxonomic identity, metabolic activities, potential for toxin production, and antibiotic/antifungal resistance profile (Pariza et al., 2015).

In the U.S., microbial ingredients are referenced as parts of food standards of identity throughout 9 CFR and 21 CFR. Although some jurisdictions have comprehensive lists of microbial species that are safe for use in food, the U.S. lacks such a list. This disconnect has created confusion among food manufacturers regarding the compliance status of common microbial ingredients, hindering industry innovation. As a result, experts in the field of microbial food safety have conducted significantly redundant evaluation work as evidenced by multiple GRAS notice (GRN)

 doi:10.20944/preprints202605.1453.v1

submissions to the Food and Drug Administration (FDA), e.g., *L. acidophilus* (GRN 171, 357, 463, 502, 865, 871 and 1083) and *L. rhamnosus* (GRN 231, 281, 288, 845, 1013, 1084, 1093, 1130, 1240, and 1264) (U.S. FDA, 2026).

Once a microbial species has been determined to be safe for use in food (and no evidence of toxigenic or pathogenic strains belonging to that species surfaced in an accompanying literature search), it is unlikely that toxigenicity or pathogenicity would be introduced by an additional strain of that species, and this is confirmed by strain level verification for safety-relevant traits (Pariza et al., 2015; EFSA, 2007). To our knowledge, the European Food Safety Authority (EFSA) has rarely, if ever, removed a species from the Qualified Presumption of Safety (QPS) list as part of their periodic review since 2007. Instead of providing transparency and meaningful benefit to general consumer safety, redundant evaluations take away prioritization from higher risk substances. This publication establishes a list of microbial ingredients on a species level that qualified experts accepted to be safe for use in food without the need for further evaluation or limitation. The list is non-exhaustive but provides a sample of species that have been evaluated and benefit from significant consensus on their safety, regardless of specific regulatory requirements.

Substantiation of Safety for Microbial Species

An important implication of this publication is the generation of a conservative list of microbial species that are recognized as safe for use in food based on publicly available data and according to independent and rigorous scientific reviews by globally qualified experts. As an example, this list could be of great interest to the FDA in relation to GRAS. While the substantiation of safety may vary slightly across regions, the responsibility of scientific experts everywhere is to assess whether a microorganism is safe for consumers under the intended conditions of use. Pariza et al. (2015) provides a comprehensive and systematic decision tree that is widely used to evaluate the safety of microbial ingredients, incorporating information at both the species and strain level. This decision tree is a 13-question framework designed to systematically evaluate the safety of microbial cultures for human or animal consumption. It focuses on assessing taxonomic identification, history of safe use, toxigenic potential, and pathogenicity at the species level, often leveraging whole-genome sequencing (WGS) to determine if a strain is safe or requires further testing (Chokesajjawatee et al., 2020; Morovic et al., 2017; Spears et al., 2021). The decision tree has been embraced in multiple GRAS Notices (GRN 736, 758, 760, 810, 956, 1113) (U.S. FDA, 2026). Strain-specific evidence supporting the safety of microbial ingredients includes clear taxonomic identification using the latest genomic sequencing technology. The results are used to determine inter-strain differences by comparing the strain in question to the reference strain (e.g., using American Type Culture Collection's (ATCC) reference strain) or other well-characterized strains within the same species. The comprehensive genotypic and phenotypic characterization of the organism corroborates safety, ensuring a lack of antibiotic resistance genes capable of gene transfer, virulence factors, and toxin production. Microbial safe-strain lineage ancestries, as revealed through phylogenetic trees and genetic lineage tracing, divulge the associated physiological functions. Most microbial ingredients in use today stem from fermented foods or from microbes colonizing a healthy human microbiome. Beyond potential opportunistic pathogenicity in immunocompromised patients, such as is documented for *L. rhamnosus*, their low pathogenic and/or toxigenic potential is well-recognized Karime et al., 2022; Mikucka et al., 2022; Salminen et al. 2004; Yelin et al., 2019). The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) expert consultation reported that "no pathogenic or virulence properties have been found for lactobacilli, bifidobacteria, or lactococci" (FAO/WHO, 2002).

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Lists and Inventories of Safe Microbial Ingredients Around the Globe

Over time, lists of microbial ingredients have been documented by internationally recognized organizations and regulatory authorities in various jurisdictions; these reference lists are summarized in Table 1 with more detail provided here.

EFSA QPS list of microbial species used in food and feed. The QPS approach was developed by the EFSA's Scientific Committee to provide a streamlined approach for the EFSA to assessing the risk of microorganisms intentionally introduced into the food and feed chains (EFSA, 2007; Herman et al., 2019). The list and associated procedures are maintained online (https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps; last accessed 5.18.26) and frequently updated (most recently in EFSA BIOHAZ Panel et al., 2026). The central idea is that species that are QPS do not require a full safety assessment, though manufacturers must ensure that the strain meets all the qualifications imposed for the taxonomic unit. Strain-specific requirements include confirmation of taxonomic identity, the microorganism's lack of antibiotic resistance and confirmation that the organism does not produce toxigenic secondary metabolites (EFSA, 2025). If the status of the species is "unconditionally" safe, there is no need to ascertain lack of pathogenicity at the strain level (European Commission, 2003).

It is important to note that the QPS list is not exhaustive, meaning it does not cover all possible microbial species that could be considered safe or "conditionally safe." The list only includes microorganisms that have been specifically brought to EFSA for assessment and opinion. A lack of inclusion may simply be due to no application for that microorganism having been submitted for evaluation.

In the case of subset of the list presented here (Table 1), all species are unconditionally safe. The listed species are incapable of producing toxins, so no additional testing is needed for strains belonging to these species (EFSA, 2025).

International Dairy Federation's (IDF) inventory of safe microbial food cultures. The International Dairy Federation (IDF) has compiled an extensive list documenting the myriad uses of microbial ingredients, underscoring their global importance in the food sector. The "Inventory of Microbial Food Cultures with Safety Demonstration in Fermented Food Products" was first published in 2002 solely to document microbial ingredients used in dairy (Mogensen, 2002a,b). The 2002 IDF inventory has undergone several updates since then due to additions and taxonomic changes (Bourdichon et al., 2012a,b,c, 2018, 2022). The IDF inventory will continue to be updated as additional taxonomic changes to microbial ingredients are made over time, such as the major taxonomic update to Lactobacilli nomenclature outlined in Zheng et al. in 2020. The safety conclusions for the resultant list have been adapted from the generic Codex description (USP, 2010) of the components of risk assessment, based upon peer-reviewed rationale. The first IDF list (Mogensen et al., 2002) resulted from a collaboration between EFFCA (European Food and Fermentation Culture Association) and IDF. The information needed on microbial species safety for consideration in the IDF list comprises both current taxonomy and available evidence of safe food usage which is not limited to those listed in the current inventory. The incorporation of species on the list requires documentation in two peer-reviewed food microbiology publications. The IDF inventory provides the most exhaustive inventory of safe microbial food cultures specific to various food matrices and provides public documentation of microbial species actively used in food. Although not legally binding, the success of the IDF list is evident from its citation in the Food Chemical Codex (FCC) (USP, 2019).

International Journal of Food Microbiology's (IJFM) list of microorganisms with technological beneficial use. The IJFM List is a widely respected reference that catalogs over 260 microbial species with a documented history of safe use in food (Bourdichon et al., 2012d). It expands upon earlier inventories such as the "2002 IDF inventory" by including a broader range of food matrices and updating microbial taxonomy. While not legally binding, the inventory supports regulatory frameworks by providing evidence of traditional use, helping guide GRAS evaluations and QPS assessments. In the most recent coverage period, it continues to be one of the most read and

 doi:10.20944/preprints202605.1453.v1

cited papers for IJFM, including multiple policy citations (Plum Analytics, 2026). Its readership includes regulators, researchers, and industry professionals.

The IJFM list was supplemented through contributions from national committees of IDF, members of EFFCA, and a comprehensive review of scientific literature (e.g., Hammes et al., 1992, Stackebrandt, 2007 Casaregola et al., 2011). Microorganisms were included based on their documented presence in fermented foods across diverse food categories, evidence of desirable technological function (i.e., fermentation, flavor development, or preservation), and history of safe use – defined as significant human consumption over generations in genetically diverse populations with no known side effects. Microbial species were excluded when there was a lack of documentation of beneficial function, as well as when the species were known contaminants or spoilage organisms, or if they posed toxigenic or pathogenic safety concerns. Only species with clear technological benefits and safety profiles were retained.

Danish Veterinary and Food Administration's (DVFA) list of microbial cultures. The DVFA maintains a registry of safe microbial cultures at the species level approved from 1973 until 2010. DVFA states that in accordance with their updated nomenclature (DVFA, 2016), the DVFA considers a culture safe following the same process as QPS regarding identification, expert review, absence of pathogenic or toxigenic properties and absence of transferable antibiotic resistance as laid out in Table 1. After 2010, notification to the Danish food authorities is voluntary for placement on the list and it remains a valuable public tool for manufacturers on the DVFA's website (DVFA, 2016).

Microbial ingredient safety in Canada (Health Canada Probiotics Monograph). Health Canada's Probiotics Monograph lists safe microbial species based on a robust, evidence-based framework for the safety evaluation of microbial ingredients independent of the regulatory classification . Microbial species listed on the monograph are pre-cleared for a streamlined regulatory authorization process for use as Natural Health Products (Health Canada, 2025). As a regulatory document issued by an authority with international recognition, it reflects the scientific consensus that specific microbial species can be considered safe. Microbial species included in the monograph require strain characterization through genotypic and phenotypic analyses, and must demonstrate the absence of virulence determinants, toxigenic activity, and unexpected/atypical antibiotic resistance (e.g., acquired and/or transferable). Species excluded from the monograph are not deemed unsafe but require additional assessment prior to regulatory approval. One exception is *Streptococcus thermophilus* that is not explicitly listed as pre-cleared, this microbial ingredient is regarded as safe and is a mandatory ingredient in yogurt. The only information Health Canada requires is information relevant to establishing identity.

The scientific and regulatory principles embedded in this monograph are consistent with international standards, such as the EFSA's QPS approach on microorganism safety assessment. Due to many consistencies between this list and others presented in this paper, it reflects broader global consensus on microbial ingredient safety.

Table 1. Summary of Global Reference Lists Documenting Microorganisms with General Recognition of Safety for Use in Food.

Reference List	Authoritative Body	Description	Basis for Safety Determination
Qualified Presumption of Safety (QPS) List of Microorganisms Used in Food and Feed	European Food Safety Authority (EFSA)	EFSA safety assessment tool to evaluate microorganisms for use in food and feed. List is updated every six months to incorporate new assessments (EFSA BIOHAZ Panel, 2025)	Reasonable evidence that species of microorganisms do not raise safety concerns. During QPS assessment, the following are assessed at a species level (EFSA, 2007b): - Taxonomic identity - Body of knowledge (including historical use and scientific literature) - Potential safety concerns, including acquired antimicrobial resistance.
Inventory of Microbial Food Cultures	International Dairy Federation (IDF)	A catalogue of microbial food cultures considered safe for use in fermented	- History of use, supported by peer-reviewed references demonstrating application in food - Occurrence in fermented foods

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Demonstrated to be Safe		foods. It is regularly updated to reflect new scientific findings and taxonomy changes (Bourdichon et al., 2022)	- Evidence of beneficial presence of the microorganism in one or more food categories - Taxonomic identification in accordance with international classification guidelines - Definition of the reference strain, ensuring traceability and reproducibility - Entries are peer-reviewed by three IDF committees (Mogensen et al., 2002).
List of Notified Microbial Cultures Applied in Food	Danish Veterinary and Food Administration (DVFA)	A registry of microbial cultures submitted by companies for approval for use in food. The list was completed in 2010 and was updated for taxonomic changes in 2016 (DVFA, 2016).	Until 2010, Danish regulations required approval for use in food after review of (DVFA, 2026): - Identification by approved analytical methods. - Culture cannot contain harmful and/or large amounts of contaminating organisms. - Absence of pathogenic and toxigenic properties. If organism is capable of producing toxins, must show that concentration is below concern. - Absence of transferable antibiotic resistance.
List of Micro-organisms with Technological Beneficial Use	International Journal of Food Microbiology (IJFM)	A comprehensive list of a wide range of bacteria and fungi with technological beneficial use in food fermentation (Bourdichon et al., 2012d)	Expert evaluation of publicly available information on each species' history of use, genetic information, and safety or toxicological information. Evaluation of history of safe use of a includes evidence whether the presence of the microorganism is beneficial, fortuitous, or undesired. (Bourdichon et al., 2012d)
Probiotics Monograph listing safe microbial species	Health Canada	A monograph of safe bacteria and yeast species precleared by Health Canada for market access without assessment (Health Canada, 2025).	Initiated in 2009 and continuously evaluated to reflect the latest scientific advancements. - Absence of pathogenic properties - Body of scientific knowledge supporting safe use - Genotypic and phenotypic identity - Absence of transferable antimicrobial resistance

List of Safe Microbial Species for Use in Foods

This review aims to outline those microbial species for which scientific consensus is documented publicly as safe for use by the general population in food without limitations. A key inclusion criterion for establishing consensus in this publication was the presence of each microbial species on all five publicly available reference lists. This list is not exhaustive simply because some species may not have been submitted for inclusion in one of the five lists. As scientific opinion continues to evolve, so too will the consensus of safety. As such, each of the five publicly available lists is dynamic and will change over time as the lists and species they contain are subject to reevaluation.

Microbial ingredients used in food production are not explicitly listed in this review because such evaluations have already been published and are accepted in the FCC (USP, 2012). The historical safety of these microbial ingredients has been considered in the context of this publication. While microbial ingredients encompass bacteria, yeasts, and filamentous fungi, this paper focuses exclusively on bacteria and yeast. The consensus list of safe microbial species applies only to their intended use in food for the general population. Applicability of the list is subject to regulatory discretion by the relevant authorities. For example, use for certain vulnerable subpopulations like infants may be subject to separate regulatory frameworks with mandatory premarket notification and safety oversight. This publication excludes GEMs because genetic engineering aspects should be examined at the strain level, as discussed in Hanlon and Sewalt (2021).

Based on their presence on each of the five authoritative lists outlined in Table 1, we derived a composite list of safe microbial species for use in food as presented in Table 2. Because the microbial ingredients included in this composite list are recognized as safe by numerous global authorities and scientifically respected organizations in the public domain, this conserved list can be regarded as a consensus list of safe microbial ingredients for use in food.

For completeness, the composite list in Table 2 contains appropriate synonyms for those species that have undergone taxonomy changes. In addition, fungi are listed with anamorphic and obligate synonyms. Prior to 2013, the asexual form of fungi (anamorphs) had different taxonomic names (IAPT, 2019). Both sexual form (teleomorph) and asexual form are currently listed under the same

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taxonomy. Obligate synonyms are based on the same type specimen rather than changes to taxonomy (U.S. Department of Agriculture, 2022).

As all microbial species included in the composite list have been evaluated as safe for use in food by the scientific community, it is sufficient for microbial ingredient manufacturers to maintain internal documentation to support the safety status of their specific strains, for example GRAS status in the U.S. (see Lists and Inventories of Safe Microbial Ingredients Around the Globe). This safe list of microbial ingredients can also be used as a reference companion to CFR references (e.g., 21 CFR 131.111, 21 CFR 133.113, 9 CFR 424.21) that refer to common microbial ingredients, but do not define them.

Table 2. Microbial Ingredients with Significant Global Scientific Agreement of Safety in Food.

Species name	Synonym
Acidipropionibacterium acidipropionici	*Propionibacterium acidipropionici*
Bacillus subtilis	N/A
Bifidobacterium adolescentis	*Bifidobacterium stercoris*
Bifidobacterium animalis	*Bifidobacterium animalis* subsp. *animalis*, *Bifidobacterium animalis* subsp. *lactis*, *Bifidobacterium lactis*
Bifidobacterium bifidum	N/A
Bifidobacterium breve	N/A
Bifidobacterium longum	*Bifidobacterium longum* subsp. *infantis*
Carnobacterium divergens	*Lactobacillus divergens*
Companilactobacillus alimentarius	*Lactobacillus alimentarius*
Companilactobacillus farciminis	*Lactobacillus farciminis*
Debaryomyces hansenii	Anamorph name: *Candida famata*. Obligate synonyms: *Debaryozyma hansen*ii, *Pichia hansenii*, *Torulaspora hansen*ii, *Debaryomyces hansenii* var. *hansenii*, *Debaryomyces tyrocola* var. *hansenii*
Fructilactobacillus sanfranciscensis	*Lactobacillus sanfranciscensis*
Kluyveromyces lactis	Anamorph name: *Candida spherica*. Obligate synonyms: *Guilliermondella lactis*, *Zygofabospora lactis*, *Zygorenospora lactis*, *Kluyveromyces marxianus* var. *lactis*, *Dekkeromyces lactis*
Kluyveromyces marxianus	Anamorph name: *Candida kefyr*. Obligate synonyms: *Dekkeromyces marxianus*, *Guilliermondella marxiana*, *Zygofabospora marxiana*, *Zygorenospora marxiana*, *Zygosaccharomyces marxianus*
Lacticaseibacillus casei	*Lactobacillus casei*
Lacticaseibacillus paracasei	*Lactobacillus paracasei*
Lacticaseibacillus rhamnosus	*Lactobacillus rhamnosus*, *Lactobacillus casei* subsp. *rhamnosus*
Lactiplantibacillus pentosus	*Lactobacillus pentosus*
Lactiplantibacillus plantarum	*Lactobacillus plantarum*, *Lactobacillus arizonensis*
Lactobacillus acidophilus	*Lactobacillus acidophilus*
Lactobacillus crispatus	N/A
Lactobacillus delbrueckii	*Lactobacillus delbrueckii* subsp. *bulgaricus*
Lactobacillus gasseri	*Lactobacillus gasseri*
Lactobacillus helveticus	*Lactobacillus suntoryeus*
Lactobacillus johnsonii	*Lactobacillus johnsonii*
Lactobacillus reuteri	N/A
Lactococcus lactis	*Lactococcus lactis* subsp *lactis* biovar. *diacetyl*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Lactoccocus lactis* subsp. *lactis diacetylactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*,

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	Lactobacillus xylosus, *Streptococcus cremoris*, *Streptococcus diacetylactis*, *Streptococcus lactis*, *Streptococcus lactis* subsp. *diacetylactis*, *Staphylococcus xylosus*, *Lactobacillus xylosus*, *Streptococcus lactis*
Lactococcus cremoris	N/A
Latilactobacillus curvatus	*Lactobacillus curvatus*
Latilactobacillus sakei	*Lactobacillus sakei*, *Lactobacillus bavaricus*
Leuconostoc citreum	*Leuconostoc amelibiosum*
Leuconostoc mesenteroides	*Leuconostoc dextranicum*
Leuconostoc pseudomesenteroides	N/A
Levilactobacillus brevis	*Lactobacillus brevis*
Ligilactobacillus salivarius	*Lactobacillus salivarius*
Limosilactobacillus fermentum	*Lactobacillus fermentum*
Oenococcus oeni	*Leuconostoc oeni*
Pediococcus acidilactici	N/A
Pediococcus pentosaceus	N/A
Propionibacterium freudenreichii	N/A
Saccharomyces bayanus	N/A
Saccharomyces cerevisiae	Obligate synonyms: *Mycokluyveria cerevisiae*, *Eutorulopsis cerevisiae*, *Eutorula cerevisiae*, *Kloeckera cerevisiae* Exceptional synonym (frequently used): *Saccharomyces boulardii*
Streptococcus thermophilus	*Streptococcus thermophilus* subsp. *salivarius*

GRAS Microbial Food Ingredients

In the U.S., the Federal Food, Drug, and Cosmetic Act stipulates that any substance intentionally added to food is considered a food additive and is subject to premarket review and approval by FDA unless the substance, under its conditions of use, is determined to be GRAS by qualified experts or is otherwise exempt from the definition of a food additive (e.g., color additives).

There is a discrepancy between the way microbial ingredients are handled throughout the CFR as parts of standards of identity, versus the way microbial ingredients are addressed by the GRAS notification process. For example, many dairy applications, such as acidified milk (U.S. FDA, 2025a), permit the inclusion of "characterizing microbial organisms" without specifying the genus, species, or strain. This can also be seen in meat applications as seen in 9 CFR § 424.21 (U.S. Department of Agriculture, 2025) (food ingredients approved for use in the preparation of meat products), which lists "harmless bacteria starters of the acidophilus type, lactic acid starter or culture of *Pediococcus cerevisiae*" for flavor development. Another well-known example can be found in the yogurt standard of identity (21 CFR 131.200) (U.S. FDA, 2025b), which requires "characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus.*" In these cases, terminology referencing microbial ingredients is inconsistent and lacks definition to a characterizing level (e.g., species, strain).

In contrast, the data requirements for GRAS notification of a biological material found in 21 CFR 170.230(a)(2)(i) (U.S. FDA, 2025c) states "[T]he taxonomic source (e.g., genus, species) including, as applicable, data and information at the sub-species level (e.g., variety, strain);" must be included. This has been interpreted by some in industry and the Agency to mean that GRAS notification is on a strain level and that the next isolate strain belonging to the same species would need to be notified againThe inconsistency in clearly defining the compliance of microbial ingredients throughout the CFR and within the GRAS framework has presented confusion among industry, and a lack of transparency for consumers. Microbial ingredients have a long history of safe use in food and have been an integral part of food production throughout history improving food quality and safety. Many microbial ingredient manufacturers have relied on international, robust assessments for their self-GRAS affirmation of individual microbial strains. Therefore, this comprehensive review serves as

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exhaustive documentation that could aid in preventing redundant work to review individual strains belonging to a species that is clearly GRAS. Historically (prior to when GRAS notification was initiated in 1997), the FDA regarded many microbial ingredients as prior sanctioned as "harmless lactic acid bacteria".

For various reasons, many individual strains of microorganisms have gone through the GRAS notification process, often based on inquiries by third parties (e.g., finished food manufacturers who are using microbial ingredients) who require a notified GRAS dossier response for any substance used in the manufacture of their food. The GRAS Notice Inventory (https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory) contains over 1300 GRAS notices, of which 147 are for live microbial ingredients across 40 species. Intended uses range from technical effects in food to use as food ingredients (U.S. FDA, 2018). Approximately half of these GRAS notifications concern species in the *Bifidobacterium* and *Lactobacillus* genera. GRAS notifications performed on individual strains of microbial ingredients due to the inconsistency in interpretation of the scope of a GRAS substance has led to redundant GRAS notifications and, therefore, inefficient use of regulatory resources that detract from the valuation of higher risk substances.

This publication provides a list of species that defines "characterizing microbial cultures" or other terminology used throughout the CFR referencing microbial ingredients, given that international safety requirements are aligned with U.S. GRAS standards to support a reasonable certainty of safety under the intended conditions of use for microbial ingredients used in food as laid out in GRAS regulations per 81 Federal Register 54869 and 81 Federal Register 54960 (U.S. FDA, 2017, 2016). We anticipate this to be a useful tool for regulatory authorities, industry, and consumers by providing a clear indication at the taxonomic level, which species have a general recognition of safety when used in food under the intended conditions of use.

Additional Requirements for GRAS Substances

Method of manufacture. All food ingredients must follow good manufacturing practices (GMP's) in accordance with 21 CFR parts 110 and 117 and as required in 21 CFR 170.230 or equivalent (U.S. FDA, 2025c, 2025d, 2025e). The manufacture of microbial ingredients must follow the same GMP regulations. All compliant microbial ingredient manufacturers follow the same principles to produce microbial ingredients for use in the food industry.

Microbial ingredients are selected for use in foods based on desirable traits for their intended application, including - but not limited to - flavor development, preservation, texture modification, or fortification (Hansen et al., 2019). Once a microbial ingredient is selected for use in food, it is maintained as an individual strain (or culture) in a culture bank.

Seed material is inoculated into a fermentation growth medium that is optimized to meet the nutritional needs of individual microbial ingredients. Growth media are made up of substances safe for human consumption such as carbohydrates, amino acids, vitamins, and minerals. The microbial ingredient is then fermented under growth conditions that are optimized for each strain with respect to temperature, pH, oxygen, and nutrient supply. During fermentation, the microbes are metabolizing nutrients and using them to propagate. Fermentation is stopped when pre-defined parameters are met that optimize cell growth. Live microbial cells are then harvested through standard techniques such as centrifugation or filtration to concentrate the microbes and remove spent fermentation media (Praveen & Brogi, 2025).

Safe and suitable food ingredients or processing aids may be added post-fermentation to ensure viability of microbes during recovery, freezing, lyophilization, and prior to application (CCFA, 2026). Microbial ingredients can be sold as a concentrated liquid, in frozen or freeze-dried product formats. Throughout the process, Quality Control measures are implemented to ensure safety, purity, activity, and consistency of the microbial ingredients.

Nutrients in fermentation media are typically consumed or separated from the finished microbial ingredients and allergens are managed by each manufacturer as part of hazard

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identification and risk management (U.S. FDA, 2015). The same is true for heavy metals. While microbial ingredients will unlikely contribute heavy metals to a finished food product, the raw materials used in the manufacturing process will be assessed by each manufacturer according to risk.

Minimum Contaminant Requirements. Unlike other food ingredients, microbial ingredients require special attention during manufacturing and quality testing because the microbial ingredient is alive, and any microbial contaminant carried with the intended culture is also alive. This is particularly important because microbial ingredients are often added to food post-pasteurization. With that in mind, food safety and sanitation are paramount in the production process of microbial ingredients as is quality testing.

Due to the wide variety of microbial ingredients' uses and use conditions, there are no universal product specifications for microbial ingredients. Authorities generally rely on Hazard Analysis Critical Control Point (HACCP) planning to establish reasonable safety, quality, and purity (lack of impurity). In the absence of regulatory specifications, several industry standards were established in the early 2010's, for example, FCC references International Standardization Organization (ISO) 27205 as the public standard for purity of lactic acid bacterial cultures (USP, 2019). Specifications for microbial ingredients used in fresh dairy applications need to be stricter than for other food applications due to perfect growing conditions in fresh dairy for any microbial contaminants.

There are several other publicly available standards for microbial ingredients used in specific food categories, for example such standards are the EFFCA "Industry Guidelines for Food Cultures used in Meat Fermentation" (EFFCA, 2015) and the "International Oenological Codex (OIV) Lactic Acid Bacteria Standard" (OIV, 2025). Quality specifications in these standards are like ISO 27205, with minor application-specific deviations. For example, OIV has set heavy metal specifications on lactic acid bacteria used in wine because the agricultural commodity (grapes) used in wine production already carries a heavy metal risk to the final product.

In general, heavy metal contamination is of low concern for microbial ingredients as the fermentation process is contained, and hence, a rigorous raw material qualification program will serve as adequate control. Furthermore, as microbial ingredients are added in very small amounts into finished food applications, most authorities (e.g., EFSA, Health Canada) do not set heavy metal specifications on microbial ingredients but rather set limits on the finished food (European Commission, 2023). FDA has become more focused on heavy metal specifications for microbial products (U.S. FDA, 2025c). Specifications must be established based on risk associated with the intended use and population.

Dietary Exposure. A pivotal consideration for the safety of any ingredient is the intended use and whether the dietary exposure is within the margin of safety, sometimes referred to as margin of exposure (Sewalt et al. 2016). For food cultures used as microbial ingredients, this is independent of the inoculation level, as that level only influences the total fermentation time. As discussed in FDA's guidance on manufacturing changes published June 2014 (U.S. FDA, 2014), a self-limiting level of use can help ensure safety which is the case for microbial ingredients as they only grow up to 10^{10} CFU/100 g in consumer foods; dietary exposure to microbial ingredients is unique in that species are typically approved at levels consistent with good manufacturing practices (GMPs) rather than a specified limit for specific food categories (Moreno et al., 2006).

This unique attribute allows for the generic use of microbial ingredients in food and the development of globally referenced positive lists of microbial species with general recognition of safety for use in food (as outlined in Table 1). Contributing to the rationale for not setting dietary intake limits for microbial ingredients are the following: the transient nature of microbial food cultures in the gut; the inability of these microorganisms to bioaccumulate; and the absence of toxigenic concern (Faith et al., 2013; Leeming et al., 2019). Thus, dietary exposure to microbial ingredients, while an important aspect of safety, does not follow some of the conventional assumptions with respect to dietary intake and safe use levels to which other typical food substances are subjected.

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It is known that the adult microbiome is very stable and only shifts with significant dietary changes (Faith et al., 2013). Additionally, lactobacilli and bifidobacteria become undetectable a few days after ceasing consumption, thus indicating that these bacteria do not colonize the gastrointestinal tract (Vandenplas et al., 2007). Microbial ingredients found in Table 2 have been present in the food supply for centuries and associated with human consumption and the gut microbiome. The addition of microbial ingredients is not anticipated to significantly alter the gut microbiota in the general population. There is no potential for cumulative exposure, as microbial ingredients are transient in the gastrointestinal tract and will not proliferate in the foods to which they are applied. Furthermore, the number of applications to which microbial ingredients can be added is limited because of the specific conditions that are needed to retain microbial viability in food.

While the level of inclusion of the microbial ingredients will vary depending on the type of food and application of intended use, a conservative approach with respect to dietary exposure will assume that various microbial ingredients are included in at least 50% of all food consumed. Typical levels in existing microbial ingredients GRAS notifications range from 10^8 to 10^{10} colony forming units (CFU)/serving at the time of manufacturing. The average consumption of a healthy individual is approximately 20 servings of all combined foods per day (Millen et al., 2006). If half of these foods were to contain microbial ingredients, the maximum intake of microbial ingredients in conventional foods is estimated to 10^{11} CFU/day. As microbial ingredients will not proliferate in foods and beverages but instead will decline over the shelf-life of the food, the expected total intake would be significantly less. Despite the U.S. including expected daily intake limit, microbial ingredients, when adequately characterized, are safe to consume at any level and references mentioned in this paper do not include upper limits. Acceptable daily intake limits have not been scientifically established for the species listed in Table 2.

Conclusions

The microbial ingredient species listed in Table 2 of this review paper are widely recognized as safe for use in food. All species on this list are present on all the five individual lists reviewed in this paper and therefore have a consensus by qualified experts as safe for use in food. The five lists are all published and in the public domain, a prerequisite for GRAS determination. Even though two of the lists reviewed have industry input, the species outlined in Table 2 were all independently qualified by three authoritative non-industry agencies. Based on the references presented in this paper, there is consensus of safety on the microbial species found in Table 2 provided that strain-specific traits are determined by the manufacturer. Strains belonging to the listed species all share the same basic safety profile as they all have already been proven, throughout history and scientific assessment, to be safe for human consumption. A reduction in individual strain notifications would benefit authorities by reducing repetitive and labor-intensive reviews of low-risk substances; benefit industry by promoting innovation without delays; and benefit consumers and society at large by allowing food safety authorities to focus on higher-risk substances.

Author Contributions: Arie Carpenter, Miriam Carnovale, Michael De Cicco, Solange Henoud, Svend Laulund, Nassim Naderi, Winnie Ng, Vincent Sewalt: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing. Jayne Davies: Writing - Original Draft.

Acknowledgments: The authors would like to acknowledge the IFAC for their continuous support throughout the writing and submission process, with a special thank you to Berit Dockter for her guidance and contributions. The authors also extend their gratitude to Lane Highbarger, PhD, of Highbarger Consulting, LLC, for his detailed review and thoughtful discussions that enriched the manuscript.

Conflicts of Interest: The authors were employed by their respective companies at the time of writing this publication. No additional funding sources were used to develop this paper, a collaborative industry initiative conducted under the International Food Additives Council's (IFAC) anti-trust guidelines. IFAC is providing the funds for open access to this publication.

 doi:10.20944/preprints202605.1453.v1

Abbreviations

ATCC: American Type Culture Collection; CFR: Code of Federal Regulation; CFU: Colony Forming Unit; GMP: Good Manufacturing Practices; DVFA: Danish Veterinary and Food Administration; EFFCA: European Food and Fermentation Cultures Association; EFSA: European Food Safety Authority; FAO: Food and Agriculture Organization of the United Nations; FCC: Food Chemical Codex; FDA: Food and Drug Administration; GEM: Genetically Engineered Microorganisms; GRAS: Generally Recognized as Safe; GRN: GRAS Notice; HACCP: Hazard Analysis Critical Control Point; IDF: International Dairy Federation; IFAC: International Food Additives Council; IJFM: International Journal of Food Microbiology; ISO: International Standardization Organization; OIV: International Oenological Codex; QPS: Qualified Presumption of Safety; U.S.: United States; WGS: Whole Genome Sequencing; WHO: World Health Organization.

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