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Horizon scanning on microorganisms and their products obtained by new developments in biotechnology

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Abstract

Background: The aim of this horizon scanning is to map applications of new genomic techniques (NGTs) developed after Directive 2001/18/EC to obtain genetically modified microorganisms (GMMs) of categories 3 and 4, with an application to the agri-food and feed sectors; as well as understanding their relevant safety and risk assessment aspects.

Methods: The review comprised systematic comprehensive searches for the identification of relevant applications: i) structured electronic searches in Medline, EMBASE, and Web of Science, and ii) searches in on-line resources, including websites of companies, regulatory agencies, patents, and registries.

Results: we identified 35 GMMs meeting the eligibility criteria. An evidence table (available in a separate file) offers a detailed description of their characteristics. Most of the GMMs were developed or commercialised by institutions in China or USA (14 and 10 cases, respectively). Of the 35 GMMs identified, 11 were bacteria, 22 yeasts, one fungal endophyte, and one microalga. As for use, 30 GMMs were used as (or as a source of) food or food additives, three as (or as a source of) feed or feed additives, and two for agricultural purposes. Eight GMMs are already commercialized, 9 are published in patent applications, and 18 are under development. When considering the purpose of the new traits introduced, 10 GMMs modify flavours in food; 10 increase the bioproduction of compounds; seven improve food profile/composition; two boost immunity/reduce toxicity in feed additives; five optimize food production processes, and one increases nitrogen-fixation as fertiliser. Only three identified GMMs have been subjected to an authorisation process by national or international authorities, and risk assessment studies are scarcely available. The findings of this horizon scan illustrate the growing worldwide adoption of NGTs in producing GMMs for application in the food and feed sectors.

Keywords: scoping literature review; biotechnology; genetically modified microorganisms; CRISPR

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Disclaimer: The purpose of this literature search is not to be a systematic review; it is an assessment of what is already known about a policy or practice issue applying methods of systematic search and synthesis.

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Summary

This report provides a horizon scanning on microorganisms and their products obtained by new developments in biotechnology, restricted to genetically modified microorganisms (GMMs) of categories 3 and 4 as per EFSA's definitions (EFSA GMO Panel, 2011), with types of use to be released into the environment or placed on the market as or in food and feed. For this study, new genomic techniques (NGTs) are defined as 'techniques that can alter the genetic material of an organism, developed after the publication of EU Directive 2001/18/EC'.

The project aimed to identify and describe the GMMs and their products, as well as their traits and uses; to list the NGTs and modifications used to obtain those GMMs; to list the subgroup of identified microorganisms subjected to authorization procedures by international or national authorities; to identify the available risk assessment reports should they exist; and to describe the risk assessment approaches taken by risk assessors (e.g., issued safety opinions) and potentially available guidance for the risk assessment (e.g., issued guidance documents for risk assessment).

The horizon scanning applied methods of systematic synthesis of the literature. Comprehensive electronic literature searches were conducted on MEDLINE (via PubMed), EMBASE (embase.com), and the Web of Science (Clarivate Analytics), restricted from 2001 to January 2023. These searches retrieved 17,461 unique bibliographic references which were screened to select relevant publications and documents based on specific eligibility criteria.

In addition, complementary on-line searches were conducted on various resources including companies, biotechnology regulators and food safety agencies, the European Patent Office (EPO), and other relevant resources, whose websites were systematically and thoroughly examined. The relevant documents identified in these on-line searches were screened and assessed. Narrative and tabulated synthesis of data were conducted for the GMMs identified in these comprehensive search processes.

As a result of this research process, a comprehensive list of 35 GMMs and their respective products was obtained. An evidence table (available in a separate file) offers a detailed description of their characteristics.

Most of the GMMs were developed or commercialized by institutions in China or USA (14 and 10 cases, respectively). Concerning the microorganisms' taxon, out of the 35 GMMs identified, 11 were bacteria, 22 yeasts (*Saccharomyces cerevisiae*), one fungal symbiont (endophyte), and one microalga. In relation to the reported use of the GMMs, 30 were used as (or as a source of) food or food additives, three as (or as a source of) feed or feed additives, and two for agricultural purposes, to be released in the environment. In relation to the development stage of the identified GMMs, eight GMM have already been commercialized, whereas 9 are published in patent applications, and 18 are under development. Concerning the purpose of

the new traits introduced in the GMMs, 10 modify flavours in food; 10 increase bioproduction of compounds; seven improve food profile/composition; two boost immunity/reduce toxicity in feed additives; five optimize food production processes, and one increases nitrogen-fixation in fertilisers.

The strategies followed to modify the microorganism were to knock out genes encoding non-desirable characteristics or to add genes encoding desirable characteristics. The most used NGTs was CRISPR based (in most instances the CRISPR-Cas system being utilized with a DNA template, specifically SDN-2 and SDN-3).

Only three GMMs and their products identified have been subjected to authorisation processes by international authorities, and risk assessment studies are scarcely available.

Concerning risk assessment guidelines for GMMs, it is worth noting that international standards on the safety of GMMs (including guidelines by Codex Alimentarius 2003, and the OECD compilation of biosafety consensus documents on transgenic organisms 2017), have been widely applied since their publication. It is however unclear whether the risk assessment approaches targeting GMOs developed with traditional genetic modification techniques remain adequate to target GMOs developed with NGTs.

There is significant activity in the development of GMMs products by both private and public/academic entities. Analysis of the data reveals that private companies have a larger number of commercial applications. On the other hand, public/academic organizations play a dominant role in research and development, resulting in a diverse pipeline of organisms and traits being developed. The findings of this horizon scan illustrate the growing adoption of NGTs in producing GMMs for application in the food and feed sectors.

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1. Introduction

In 2011, EFSA issued a Guidance on the risk assessment of genetically modified microorganisms (GMMs) and their products intended for food and feed use (EFSA GMO Panel, 2011). In this guidance, EFSA developed the principles of the risk assessment of GMMs, categorizing them into four categories depending on the type of product and the degree of purification:

- Category 1: Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed (e.g., amino acids, vitamins).
- Category 2: Complex products in which both GMMs and newly introduced genes are no longer present (e.g., cell extracts, most enzyme preparations).
- Category 3: Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present (e.g., heat-inactivated starter cultures, biomasses single-cell protein preparations, and cell extracts).
- Category 4: Products consisting of or containing GMMs capable of multiplication or of transferring genes (e.g., live starter cultures for fermented foods and feed).

In 2020, in the frame of the Commission mandate on synthetic biology, EFSA commissioned a horizon scan of synthetic biology developments for microorganisms with applications in the agri-food sector (van der Vlugt, 2020). Cases were listed with the genetic modification technique used, the status towards commercialization, and their use application.

This work supported the EFSA Opinions on synthetic biology applications in microorganisms addressing risk assessment aspects:

• The opinion on molecular characterization and environmental risk assessment was published in 2020 (EFSA Scientific Committee et al., 2020).

• The opinion on food and feed risk assessment was published in 2022 (EFSA Scientific Committee, 2022).

The genetic modification techniques already in use before the publication of EU Directive 2001/18/EC (2021), such as directed mutagenesis or gene guns, are often considered "established techniques". In contrast, the techniques able to alter the genetic material of an organism developed after 2001 are commonly named New Genomic Techniques (NGTs). A defined characteristic of these NGTs is their target-specificity. That means that they create changes at a specific target sequence. NGTs can be classified into four groups (Broothaerts et al., 2021):

(1) techniques that generate a double-strand break in the DNA, including 'site-directed nuclease' (SDN) techniques based on clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nucleases (TALEN) and zinc-finger nucleases (ZFN). The cellular repair pathways for DNA double-strand breaks include non-homologous end joining (NHEJ) and homology-directed repair (HDR), being the homologous recombination extremely accurate leading to precise repair of the damaged locus using DNA sequences of homologous broken ends;

(2) techniques that utilize either a single-strand DNA (ssDNA) break or no break at all in the genome, including oligonucleotide-directed mutagenesis, base editing, and prime editing;

(3) epigenetic techniques such as RNA-directed DNA methylation and CRISPR interference (CRISPRi); and,

(4) techniques that act directly on RNA (RNA base editing).

The study 'Current and Future Market Applications of New Genomic Techniques' (European Commission et al., 2021) covered the use of NGTs in plants, animals, and microorganisms, in a broad variety of potential market applications, including in the agri-food, medicinal and industrial sector. The report gathered information from Member States and EU-level stakeholders via a targeted consultation. This report indicated that NGTs have the potential for microorganism strain improvement and are already being used by several companies. They are becoming standard tools, along with established techniques such as classical mutagenesis, homologous recombination, and self-cloning. At the same time, the report underlined that collecting data on NGT-derived microorganisms was challenging in terms of identifying data sources. The survey of private companies underpinning the analysis resulted in a relatively small number of companies participating, and generally the answers disclosed little detail on NGT-derived microorganisms (e.g., microorganism strain, the specific technique used, or specific trait obtained) because of confidentiality reasons.

The study noted that knowledge (including safety data) is mainly available for genome editing in plants, making it difficult to conclude animals or microorganisms. It, therefore, recognized the need to generate further knowledge on current and future market applications of NGTs in animals or microorganisms.

Based on and building on the above, we aimed to map new developments in biotechnology applied to microorganisms and get an updated understanding of their developmental status, their envisaged use, and relevant risk assessment considerations.

The specific objectives of the present work include:

1. Identify microorganisms and their products obtained by new development in biotechnology described since 2001 including their traits and uses.
2. List the techniques and modifications used, including an explanation of relevant terminology.
3. Identify microorganisms and their products developed since 2001 subject to authorization procedures by international authorities as well as the available risk assessment should they already exist.
4. Information on risk assessment approaches taken by risk assessors (e.g., issued safety opinions) and potentially available guidance for the risk assessment (e.g., issued guidance documents for risk assessment).

2. Data and Methodologies

2.1 Study design

We conducted a scoping literature review by applying methods of systematic search and synthesis.

2.2 Methodology

2.2.1 Search process

We conducted a two-step search for literature. First, we conducted searches of relevant publications and grey literature, to compose a list of GMMs (categories 3 and 4) created after 2001. Second, we conducted additional (unstructured) searches to complete the evidence table for each identified GMM.

We designed a comprehensive electronic search based on current standards for conducting systematic reviews (Lefebvre et al., 2019). We reported the complete searches in the technical report according to the PRISMA-S statement (Rethlefsen et al., 2021). The search process described below was implemented based on the results of each step, to identify a reasonably sized collection of GMM cases that is diverse and informative of the application of the techniques considered.

2.2.1.1 Initial mapping

Initially, we mapped the references indexed in MEDLINE under the controlled vocabulary term "Microorganisms, Genetically-Modified" [Mesh] with a yield of 737 hits (November 28th, 2022). One field expert screened the references to identify relevant studies for this horizon scanning.

2.2.1.2 Electronic search design

We used these relevant references to design an electronic strategy to search the databases and platforms of interest: MEDLINE (via PubMed), EMBASE (embase.com), and Web of Science (Clarivate Analytics).

To develop the search strategy for MEDLINE, we checked how the relevant references obtained from the initial mapping have been indexed in MEDLINE to define the terms from the controlled vocabulary. Then we checked relevant terms from the title or abstract to define text terms that were truncated when appropriate and tagged to restrict the search to specific fields of the reference (e.g., [tiab] in PubMed to search text terms in the title or abstracts). Once the terms were defined, we combined them to link together those with the same scope using the Boolean OR (e.g., microorganism*[tiab] OR bacillus[tiab]) and the different concepts with the Boolean AND (e.g., microorganism*[tiab] AND engineer*[tiab]). The search strategies for MEDLINE, EMBASE, and Web of Science are available in Appendix C. We restricted the searches from 2001 to January 2023.

2.2.1.3 Searching for other resources

- Tracking relevant studies

In addition to the search of bibliographic databases and platforms, to identify additional studies, we tracked back and forward references of relevant studies. We searched key relevant references in the Web of Science and Scopus and obtained the references of the citations to these index references. The eligible references identified with this procedure were added to the pool of references obtained by the bibliographic searches.

- Tracking non-indexed studies and documents

Other sources of information were consulted to retrieve relevant studies and documents that are not indexed in bibliographic databases. The following processes were followed:

1. Search process for companies: the websites of several companies related to the development of microorganisms were accessed. The general purpose of the company was assessed (e.g., does the company deal with optimized/engineered microorganisms?). The website sections of projects or publications were searched. A general search was conducted with the terms 'GMM', 'genetically modified microorganism', 'genetically modified', 'microalgae', 'yeast', 'bacteria', 'virus', 'fungus', 'fungi', 'engineered', 'gene edited', 'bioengineered'. Any potentially relevant document was retrieved and assessed. All potentially eligible GMMs, regardless of category, were listed with links. GMMs of categories 1 and 2 were listed as excluded. GMMs of categories 3 and 4 were listed as potentially included. The list of companies was

updated as potential GMMs were identified in other sources (e.g., patent agencies, regulatory agencies).

2. Search process for global and national biotechnology regulators and food safety agencies: the websites of the agencies listed in Appendix D were accessed. The website sections of projects or publications were searched. A general search was conducted with the terms 'GMM', 'genetically modified microorganism', 'genetically modified', 'microalgae', 'yeast', 'bacteria', 'virus', 'fungus', 'fungi', 'engineered', 'gene edited', 'bioengineered'. The first 10 potentially most relevant documents identified with each search were retrieved and assessed. All potentially eligible GMMs, regardless of category, were listed with links. GMMs of categories 1 and 2 were listed as excluded. GMMs of categories 3 and 4 were listed as potentially included. Opportunistic identification of guidelines and risk assessments was conducted.

3. Search process for patents: The European Patent Office database (Espacenet) website was accessed. An advanced search was conducted with the following conditions: 1) IPC classification = c12N (corresponding to MICROORGANISMS OR ENZYMES; COMPOSITIONS THEREOF; PROPAGATING, PRESERVING, OR MAINTAINING MICROORGANISMS; MUTATION OR GENETIC ENGINEERING; CULTURE MEDIA), A23C (corresponding to DAIRY PRODUCTS, e.g. MILK, BUTTER OR CHEESE; MILK OR CHEESE SUBSTITUTES; MAKING THEREOF) and A23K (corresponding to FODDER); and 2) text = ' microorganism' / 'microalgae' /'yeast'/ 'bacteria'/ 'virus'/ 'fungus'/'fungi' 'engineered'/ 'gene edited'/ 'bioengineered' + text = 'agricultural' / 'food' / 'feed'. The list of entries retrieved by the search was assessed by reading the abstract to exclude uses out of the scope of the project (e.g., biofuel); by checking the claims section of the patent, to identify processes involving GMM; by checking the documents cited in the patent, if relevant. All potentially eligible GMMs, regardless of category, were listed with links. GMMs of categories 1 and 2 were listed as excluded. GMMs of categories 3 and 4 were listed as potentially included.

4. Search process for registries or existing datasets: the Biosafety Clearing-House Living modified organisms register (https://bch.cbd.int/en/registries) was accessed, and general searches were conducted for the terms 'GMM' and 'genetically modified microorganism'. All potentially relevant GMMs and/or documents were retrieved and assessed. All potentially eligible GMMs, regardless of category, would have been listed with links. GMMs of categories 1 and 2 would be listed as excluded. GMMs of categories 3 and 4 would be listed as potentially included.

2.2.2 Selection of literature

Results of the bibliographic searches were uploaded into the web-based systematic review software Distiller SR (DistillerSR. Version 2.35. DistillerSR Inc.; 2022. Accessed November

2022-June 2023. https://www.distillersr.com/). The screening was done first at the title and abstract level (Level 1), followed by full-text screening (Level 2) of the studies selected (included) at Level 1. The full texts were retrieved in pdf format and uploaded into DistillerSR to proceed with the screening at the full-text level. For both Level 1 and Level 2 screening, we applied the eligibility criteria described in Box 1.

An initial calibration process was conducted with two researchers screening 10 references independently and reaching a consensus in their assessments. Researchers discussed and clarified the selection criteria through discussion with the senior expert. The rest of the screening was conducted by a single reviewer.

Box 1: Inclusion and exclusion criteria.

Biotechnology	In	Genomic techniques developed after 2001 (when Directive 2001/18 was published).
	Out	Established techniques of genetic modification (e.g., classical mutagenesis and self-cloning).
Microorganisms	In	Based on EFSA GMO Panel (2011): • Category 3: Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present (e.g., heat-inactivated starter cultures, biomasses single-cell protein preparations, and cell extracts). • Category 4: Products consisting of or containing GMMs capable of multiplication or of transferring genes (e.g., live starter cultures for fermented foods and feed).
	Out	Purified fermentation products (in which the microorganism's DNA is no longer present). This applies to: • Category 1: Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed (e.g., amino acids, vitamins).

		• Category 2: Complex products in which both GMMs and newly introduced genes are no longer present (e.g., cell extracts, most enzyme preparations).
Uses	In	Microorganisms and products of Category 4 to be released into the environment (e.g., biopesticides, fertilisers) or placed on the market as or in food and feed, and products of Category 3 to be placed on the market as or in food and feed. The microorganism cases of interest must have a level of development with the potential to reach the market (e.g., in the next decade).
	Out	Use of new biotechnologies for fundamental research purposes, such as technology development (e.g., new/improved genome editing tools), gene discovery research, engineered therapeutic bacteria for medical purposes (modified bacteria and carefully formulated microbial communities altering gut microbiome), engineered bacteria to produce compounds for non-food or non-feed applications (e.g., packaging, biofuels).

The selection of references from bibliographic searches was restricted to documents in Spanish or English. Potentially relevant documents identified in the searches of global and national biotechnology regulators, food safety agencies, or patents agencies were automatically translated to assess their relevance.

A bibliographic database was generated in DistillerSR with the bibliographic references obtained from electronic searches and other sources. Each reference was classified as included or excluded by the Level 1 and Level 2 screening.

2.2.3 Data extraction and narrative synthesis

We extracted data about the microorganisms identified in the searches and presented the results in tables in this report and an Excel evidence table provided separately. Data from publications and complementary sources were extracted by a single reviewer.

Results are presented narratively. When appropriate, tables are included to allow a better understanding of the topic covered by the literature review.

The reported GMM cases are used in the agri-food and industrial sector, to be released into the environment (e.g., biopesticides, fertilisers) or for incorporation in food and feed products. The use of each of the included GMMs has been assigned at least one of the following labels:

- **Agricultural**: GMMs obtained through NGTs to be released in the field for agricultural purposes (e.g., plant protection, fertilisation, etc.).
- **Feed**: GMMs obtained through NGTs primarily used as components in animal feed production or in the production of feed-related compounds.
- **Food**: GMMs obtained through NGTs used in human food products, or the development of such products (e.g., involved in the fermentation process).

This study targets GMMs resulting in products that are already being marketed or show market potential for the next decade. As such, each GMM case has been classified into the following phase of development categories:

- **Commercial**: GMMs obtained from NGTs currently marketed in at least one country worldwide.
- **Published in patent applications**: GMMs obtained from NGTs which are patented or part of patent applications.
- **Development**: GMMs obtained from NGTs at the proof-of-concept stage (i.e., testing gene targets for traits of commercial interest).

2.2.4 Database of GMMs developed with NGT

A database of GMMs developed through NGTs was created in an Excel file with the following headings, under which the specified information was added for each case:

Record ID	An identification code assigned for the study
Source of information	Indication of the source from which the information originated (literature, company websites, patents registry, etc.)
Description	General description of the GMM
Technique	The NGT used, in general terms: a CRISPR platform, TALEN, ZFN, etc.
Technique details	More details on the specific NGT employed
SDN type (1, 2, 3)	The type of SDN technique used, where applicable (see Appendix B).

Microorganism taxon	Bacteria, filamentous fungi, yeast, microalgae, bacteriophages
Microorganism species	e.g., *Escherichia coli*
Specific strain	e.g., K-12 strain, if available
Commercial use	Description of the product used by the consumer
Commercial name	If available
Category	Category of the GMM according to GMO Panel (2011)
Company/institution	Name of the product developer /producer /marketer
Country	Country of the product developer /producer /marketer
Trait category	Broad categories of traits
New trait description	Description of the specific trait for which the NGT is employed
Designation/target gene	If available, the name of the gene(s) targeted by the NGT
Phase of Development	Any information about the state of development of the GMM
Authorisation procedures	Indication if the GMM is subject to authorisation procedures by national or international authorities
Risk assessment description	Risk assessment approaches retrieved (e.g., issued safety opinions)
Risk assessment guidance	Identified guidance for the risk assessment (e.g., issued guidance documents for risk assessment)
Patent application ID	For GMM published in patent applications, the patent application identification code and link
Patent link	URL
DOI	Specific link or reference to the web page or article in which the information is reported
Website	Specific link to the GMM entry in the company or developer's website, if applicable
Remarks	Any additional observation, if relevant

3. Results

3.1. Results from the searches and selection of cases

The electronic structured searches in Medline, EMBASE, and Web of Science yielded a total of 17,446 unique references (see Table 1), which were screened at Levels 1 and 2. The on-line searches tracking non-indexed studies and documents, and the unstructured searches (forward and backtracking of key references and Internet searches), identified in total 123 potentially eligible records, which were screened at Level 2. The study selection process is outlined in Figure 1.

The primary focus of this report is the comprehensive identification and description of GMMs. To ensure accuracy and reliability, extensive efforts were made to cross-check information and data obtained from multiple sources to confirm their association with previously identified GMMs. Following the inclusion of a GMM that meets the eligibility criteria, any missing information was sought through online research. However, it should be noted that the data retrieved was often inconsistent, lacked details, or was incomplete (e.g., incomplete information on the microorganism strain, the specific technique applied, or the specific trait obtained). These limitations were due to incomplete reporting, confidentiality reasons, or other inherent characteristics of the data sources. The inconsistencies in the reported information across sources limited the ability to uniquely identify the GMMs or to adequately describe them, and some overlap between cases is possible.

Table 1: Results from the searches.

	Source	Number of hits
Electronic structured searches	MEDLINE	15,268
	EMBASE	17,751
	Web of Science	3,093
	Total	34,316
	Duplicates	16,870
	Total unique	17,446
Other searches	**Source**	**Potentially eligible records**
	Companies	14
	Regulatory agencies	59
	Patents	24
	Registries	None
	Other sources (forward and backtracking of key references, Internet unstructured searches)	26

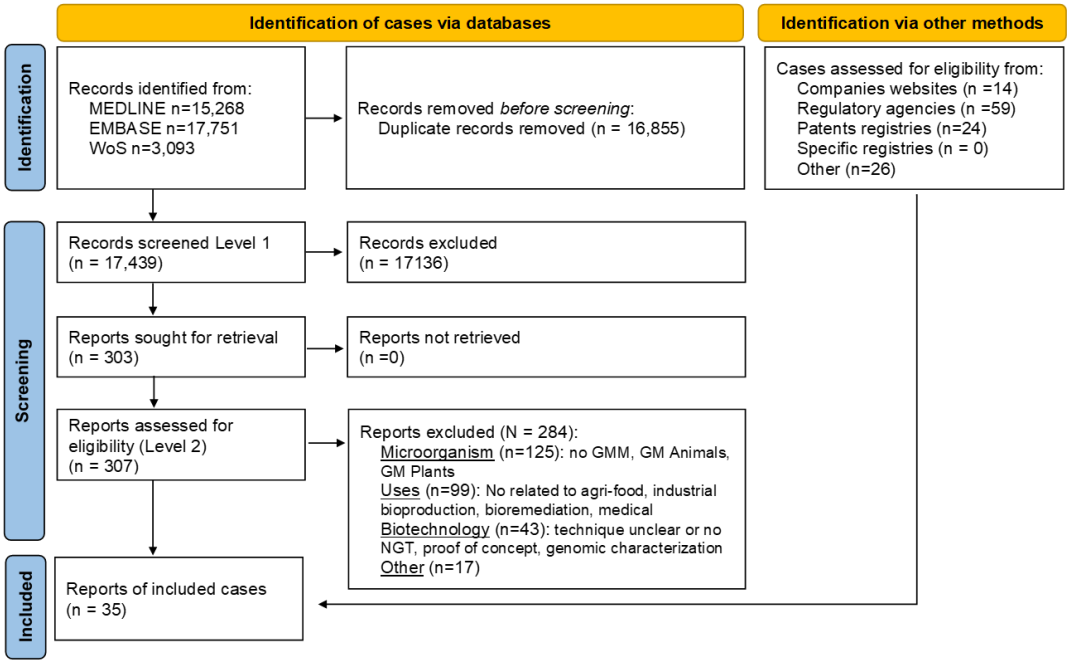

Figure 1: Flow chart of identification and selection of cases.

3.2 Microorganisms and their products obtained by new developments in biotechnology

3.2.1 Selected cases of microorganisms obtained by new developments in biotechnology, including their traits and uses

The horizon scanning conducted in this report has allowed the identification of 35 cases verifying the eligibility criteria. The summary information from the cases identified is presented in Tables 2 to 4, and a selection of representative uses of GMMs is described in Table 5, corresponding to GMMs of different types, categories, and phases of development. Finally, the complete information for the individual cases is presented in Appendix E.

Table 2: Summary of cases identified.

Total number of included cases	35
Category of NGT applied	CRISPR-based (27 cases), combination of Cas9 and homologous recombination (8 cases)

SDN types used (See Appendix B)	1, 2,3
Microorganism type	bacteria (11), yeast (22), fungal endophyte (1), microalgae (1)
Category of GMM	category 4 (20), category 3 (15)
Use reported	food or food additives (30), feed or feed additives (3), agricultural (2)
Phase of development	commercialized (8), pre-commercialization (9), under development (18)
Number of patented cases	patented (6), patent application (5)

In most instances, the development or commercialisation of the GMM corresponds to institutions based in China and the USA (14 and 10 cases, respectively). Additionally, there were 2 cases from Europe (Belgium, and the UK), and 9 cases from elsewhere (Australia, Japan, Korea, and Canada). Table 3 presents the species of microorganisms to which the GMMs belong. While the majority of GMMs analysed in this report refer to a single species, it is worth noting that some patent applications claim the NGTs to be potentially applied to engineer multiple species. In those instances, we have reported the most representative microorganism species in Table 3, while the additional microorganism species indicated in the patent application are only listed in Appendix E.

Table 3: Summary of microorganism taxon and species in included GMM cases.

Microorganism taxon	**Microorganism species**
Bacteria	*Escherichia coli, Bacillus subtilis, Klebsiella variicola, Lactobacillus casei, Lactococcus lactis, Leuconostoc citreum, Pediococcus acidilactici, Clostridium butyricum*
Yeast	*Saccharomyces cerevisiae, Saccharomyces cerevisiae var boulardii.*
Fungus	*Epichloë coenophiala* (formerly *Neotyphodium coenophialum*)
Microalgae	*Nannocloropsis oceanica*

Table 4: Summary of trait categories identified in the included GMM cases.

Trait category	Cases	Cases ID
Modify flavour in food	*10*	*GMM 16, GMM 17, GMM 19, GMM 20, GMM 24, GMM 25, GMM 27, GMM 28, GMM 34, GMM 35*
Increase bioproduction of compounds	*10*	*GMM 05, GMM 06, GMM 07, GMM 08, GMM 09, GMM 12, GMM 13, GMM 14, GMM 23, GMM 30*
Improve food composition	*7*	*GMM 04, GMM 15, GMM 22, GMM 26, GMM 39, GMM 31, GMM 32*
Boost immunity/reduce toxicity in feed additives	*2*	*GMM 02, GMM 03,*
Optimize food production processes	*5*	*GMM 10, GMM 11, GMM 18, GMM 21, GMM 33*
Fertiliser	*1*	*GMM 01*

Table 5: Illustrative uses of GMMs developed with NGT.

Use	Case Category	Microorganism taxon (Bacteria, yeast, fungus)	Application:	Stage of development Name/Company
Agriculture, feed	GMM-03 Category 4	Fungal symbiont (endophyte)	To reduce ergot alkaloids that are toxic to livestock	Pre-commercial, patented University of Kentucky (USA)
Fertilizer	GMM-01 Category 4	Bacteria	To reduce the need for chemical fertilizer while increasing yields	Commercialized Proven40®/ Pivot Bio (USA)
Food ingredients	GMM-05 Category 4	Bacteria, Yeast	To increase the production of riboflavin, b-carotene	Development Kaist Institutes (Korea)
Food (industrial bioproduction)	GMM-07 Category 3	Bacteria	To produce a high yield of L-tryptophan for food, feed, or	Pre-commercial, patented UNIV JIANGNAN (China)

			medicine applications	
Food (prebiotic)	GMM-23 Category 4	Yeast	To produce neoagarooligosaccharides	Development National Research Foundation of Korea (NRF) (Korea)
Food (probiotic)	GMM-13 Category 4	Bacteria	To increase butyrate production and decrease ethanol production	Development Institute of Animal Science, Chinese Academy of Agricultural Sciences (China)
Food (dairy probiotic)	GMM-29 Category 4	Bacteria	To enhance the accumulation of nicotinamide mononucleotide in the probiotic lactic acid bacteria, which will significantly affect the probiotic effects of the dairy products	Development National Natural Science Foundation of China (China)
Food (rice wine)	GMM-11 Category 3	Yeast	To eliminate endogenous protein competitively bound to rapamycin (for reducing urea or EC in the fermentation of rice wine)	Pre-commercial, patented. UNIV JIANGNAN (China)
Food enzymes (fermentation starter)	GMM-04 Category 4	Yeasts	To reduce acute and chronic toxicity in alcohol-based drinks	Development Catholic University of Korea (Korea)
Food additive (fermentation starter in beer)	GMM-16 Category 4	Yeast	To enhance thiol biotransformation to produce beer with strong sensory notes of guava, passionfruit, and grapefruit	Commercial Berkeley Brewing Science, Inc. (USA)
Food (yeast-fermented foods)	GMM-26 Category 4	Yeast	To improve the quality, safety,	Development

			and flavour of fermented foods	Woese Institute for Genomic Biology (China)
Food (natural sweeteners in candies and canned foods)	GMM-30 Category 3	Yeast	To increase the production of Glycyrrhetic acid 3-O-mono-β-D-glucuronide	Development National Natural Science Foundation of China (China)
Food	GMM-22 Category 3	Microalgae	To express genes encoding proteins allowing for reduced cholesterol content.	Pre-commercial, patent application University of Hainan (China)
Feed additive	GMM-02 Category 4	Bacteria	To remove undesirable microorganisms in feed	Commercialized, patented BiomElix Guided Biotics® / FOLIUM Science (UK)

3.2.2 New genomic techniques and modifications used, including an explanation of relevant terminology.

Since Directive 2001/18/EC on the deliberate release into the environment of GMOs, NGTs have emerged to modify the genetic material of an organism. To change a particular trait of a microorganism for industrial use, researchers typically utilize an arsenal of techniques, including established and new genomic techniques. NGTs may significantly reduce the time required to obtain the desirable trait, such as when several different genes must be knocked out. In general, few microorganisms have been optimized as biofactories (chassis) and have a long history of safe use. These microorganisms, which have already been altered or modified for various commercial purposes, are frequently used to add additional qualities.

Based on the scoping literature search, which encompassed relevant publications and grey literature, applications of NGTs falling under group 1, as defined by Broothaerts et al. (2021) (see Introduction), were identified. These applications primarily involved CRISPR-based methods and homologous recombination. The main purpose of these techniques is to knock out genes encoding non-desirable characteristics or introduce genes encoding desirable traits. CRISPR-edited microorganisms emerged as the predominant type of GMMs being developed, with the potential for market release within the next decade and beyond. This approach has been primarily employed to enhance the production or modify the regulation of specific chemicals. Applications of other relevant NGTs such as phage-based approaches, oligonucleotide-directed mutagenesis, meganucleases, Zinc finger nucleases (ZFNs), and TALENs (transcription activator-like effector nucleases) were not identified within the scope of this study. Table 7 presents an illustrative selection of applications of NGTs to generate GMMs.

Table 7: New genomic techniques used to generate selected GMMs.

SDN type (See Appendix B)	NGT	Examples of representative cases	Details about technique	Traits	Risk assessment
2	CRISPR/Cas9	GMM-03 *Epichloë coenophiala* (formerly *Neotyphodium coenophialum*)	Eliminate clusters of genes located in subterminal regions of chromosomes, then eliminate the marker gene and vector backbone used in the transformation procedure	Eliminate the production of compounds (ergot alkaloids) that are toxic to livestock	NA
3	CRISPR/Cas9	GMM-04 (*Saccharomyces cerevisiae*)	Through combination with sgRNA, the Cas9 recognizes 20 bp containing PAM sequence and causes target-specific cleavage. After double-strand DNA breakage, homologous recombination-based repair is performed by Donor DNA	Starter culture to reduce the ethyl carbamate formation in an alcoholic beverage	NA
3	CRISPRi	GMM-05 (*Leuconostoc citreum*)	Two strategies: (i) regulating the knockdown of the branched folate synthesis pathway and a loss of the capability of riboflavin to convert into a flavin mononucleotide using CRISPR interference, and (ii) overexpression of the *rib* operon	Improving riboflavin production	NA

3	CRISPR/Cpf1 (Cas12a)	GMM-06 (*Bacillus subtilis*)	A *Bacillus subtilis* d-alanine racemase gene dal was knocked out through a CRISPR/Cpf1 genome editing technology	Food-grade expression of beta-mannase (for preparation of mannose oligosaccharides)	NA
2, 3	Plasmid based microbial remodelling	GMM-01 (*Klebsiella variicola*)	Genome-edited strains were cured of all plasmids used to carry out genome editing by repeated subculturing followed by sequence verification of the desired edits	Nitrogen-producing soil bacteria	Conducted the registration of microbial pesticide products with the U.S. EPA

3.2.3 Microorganisms and their products subjected to authorisation procedures by international authorities as well as the available risk assessment should they already exist.

Within the GMMs selected for analysis in this scientific publication, only a limited number of microorganisms and their associated products have undergone pre-market authorisation by national or international authorities. Furthermore, the bibliographic and on-line searches (on companies, regulatory agencies, and patents websites) retrieved very little information on risk assessment conducted on these GMMs, showing that available risk assessment studies are scarce about GMMs obtained by NGTs.

One commercial product identified is GMM-01, Pivot Bio PROVEN40®, which is a biological fertilizer containing the bacteria *Klebsiella variicola*. Risk assessment of this product was conducted in support of the registration of microbial pesticidal products with the U.S. Environmental Protection Agency (US EPA). A series of five standard biosafety studies were carried out at a third-party contract research organization, adhering to the principles of Good Laboratory Practice (GLP). These studies were conducted following the test guidelines provided by the US EPA Office of Prevention, Pesticides, and Toxic Substances (OPPTS) to assess the potential pathogenicity and acute toxicity of the product. In addition to the standard tests recommended by the EPA, an additional test, OPPTS 885.3150, evaluating acute pulmonary toxicity/pathogenicity in rats, was also conducted (Wen et al., 2021).

Another product under official assessment is GMM-02, BiomElix Guided Biotic®. According to the information from the producer webpage, the regulatory approval process of this product for the Brazilian market has been initiated by CNTBio, with the first stage of safety assessment

approving the technology as a Non-Genetically Modified Organism for use in 2023 (Comissão Técnica Nacional De Biosegurança, 2020).

Several engineered brewing yeasts, namely GMM-16, GMM-17, GMM-18, GMM-19, GMM-20, and GMM-21, are currently commercialized in the USA by Berkeley Brewing Science (BBS). However, limited publicly available information is accessible regarding the specific risk assessment procedures conducted on these GMMs. For instance, the Generally Regarded as Safe (GRAS) notification submitted by BBS for GMM-19 employs a weight-of-evidence approach, utilizing publicly available data and information, to assess the safety of *S. cerevisiae* strain yBBS002 (GMM-19) through scientific procedures (U.S. Food & Drug, 2019); no risk assessment was identified for the other GMMs commercialized by BBS.

3.2.4 Risk assessment approaches taken by risk assessors and available guidance for risk assessment.

International standards for the safety of GMOs, including GMMs, have been published decades ago and are widely applied since then (Codex Alimentarius, 2003; OECD, 2017). The Codex Alimentarius established guidelines for the evaluation of food safety concerning products derived from recombinant-DNA microorganisms. On the other hand, the OECD compilation of biosafety consensus documents focuses on transgenic organisms and addresses the key aspects deemed relevant by OECD member countries for the risk and safety assessment of GMOs. An environmental safety/risk assessment of GMOs typically considers the characteristics of the host organism, the introduced traits, and the environment into which the organism will be introduced (OECD, 2017).

In the EU, the risk assessment of GMMs that undergo authorisation follows specific regulations, which can be summarized as follows (Aguilera, Gomes, and Olaru, 2013; EFSA Scientific Committee et al., 2020; EFSA Scientific Committee et al., 2022):

1. Microbial and molecular characterisation: aimed to identify the GMM and its parental organism and to identify and characterize related hazards (e.g., antimicrobial resistance, virulence, pathogenicity, toxin production).
2. The safety of the genetic modification: focused on the intended and predicted unintended effects of the genetic modification and potential additional hazards derived from the GMM.
3. The environmental risk assessment (ERA): targeted to assess potential adverse effects on humans, animals and the environment resulting from the deliberate release of the GMM into the environment. ERA is further complemented with post-market environmental monitoring.
4. Safety for humans and animals, including intended and unintended effects.

The Norwegian Scientific Committee for Food and Environment explored whether risk assessment methodologies for conventional GMOs are adequate for risk assessment of organisms developed using NGTs (Bodin et al., 2021). The latest advancements in genome-editing techniques offer the possibility for the creation of a wide range of organisms, varying from those with minor genetic modifications to those with larger genomic regions being inserted or deleted. Existing health and environmental risk assessment protocols, specifically designed for GMOs (based on their genotype), can be effectively applied to genome-edited organisms. In their report, the Norwegian committee concluded that the EFSA guidance on risk assessment of genetically modified plants, animals and microorganisms provides a functional framework for risk assessment of genome-edited organisms that have incorporated genes or substantial DNA fragments, although considerations may need to be added in the guidance regarding specific properties of genome-edited organisms; also, the existing guidelines may not be entirely suitable for genome-edited organisms with minor insertions, deletions, or single mutations. Therefore, it is crucial to incorporate considerations specifically addressing these minor mutations (Bodin et al., 2021).

Additionally, a study commissioned by the Food Standards Agency of the United Kingdom conducted a comparison of regulatory approaches for novel food and GMOs in the EU and selected non-EU countries. The findings of the study highlighted that regulations in both the EU and Australia prioritize the process by which a product is derived. In contrast, Argentina, Canada, and the US may not classify a product as a GMO if it is determined to be substantially equivalent to a product developed through conventional methods. The authors concluded that this more flexible approach enables the incorporation of new techniques, as the emphasis is placed on the final product rather than the specific process employed in its development (Campden BRI (Chipping Campden) Ltd, 2021).

Health Canada's guidelines for the Safety Assessment of Novel Foods derived from microorganisms contemplate GMMs obtained through NGTs by stating that "in cases where a microorganism has been modified using modern genetic techniques, such as recombinant nucleic acid technology, the safety assessment will consider detailed characterization data of a novel food at the molecular level" (Health Canada 2022). Other national regulations for the risk assessment of GMMs or foods derived from GMMs are not tailored to the specificities of NGTs (e.g., Argentina (National Agrifood Health and Quality Service, 2002), or Japan (Food Safety Commission, 2008)).

4. Conclusion

This report provides a horizon scanning of new developments in biotechnology applied to microorganisms, identifying cases of GMMs of categories 3 and 4, either in the market or with market potential, with uses in the agri-food and feed sectors. During the screening process, a substantial number of publications and patent applications were found, providing comprehensive insights into the utilization of GMMs for various purposes. These applications

encompass the development of GMMs as agricultural products for release into the environment, as probiotics in food or feed, and for the contained production of a diverse range of enzymes and food/feed ingredients.

Another aim of the horizon scan was to compile a comprehensive list of techniques and modifications employed in the development of the identified microorganisms. Among the NGTs that induce double-strand breaks in the DNA, CRISPR-based techniques emerge as the predominant approach. In most instances, the CRISPR-Cas system is utilized with a DNA template, specifically employing SDN-2 and SDN-3 approaches.

There is significant activity in the development of GMMs obtained through NGTs by both private and public/academic entities. Public/academic organizations play a prominent role in research and development, leading to a diverse pipeline of organisms and traits. Upon closer analysis, the results of this horizon scan illustrate the increasing adoption of NGTs for generating GMMs intended for use in the food and feed sectors. It is noteworthy that the majority of the identified GMMs originated from the US or China, while only a limited number of the identified GMMs were developed within the EU.

Some caveats need to be made when interpreting the results of this horizon scanning, given the methodology applied. Firstly, the information obtained from electronic bibliographic databases and online searches in public domains is primarily derived from scientific literature or publicly available online sources. These results were not checked for accuracy with the involved stakeholders (e.g., no surveys or contacts with companies were conducted). In the second place, the quality and comprehensiveness of the information varies depending on the sources consulted. This variability can limit the ability to uniquely identify and accurately describe individual cases of GMMs. Also, this horizon scanning study presents a cross-sectional picture of the available information at a particular point in time. It does not aim to provide a chronological development of each GMM (e.g., whether a GMM was originally developed by a specific institution, and later developed by a different institution) or capture any recent developments that have not been publicly disclosed.

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6. Appendices

Appendix A – Abbreviations

Cas	CRISPR-associated protein
CNTBio	National Technical Commission on Biosafety of Brazil
CRISPR	Clustered regularly interspaced short palindromic repeats system
DA	US Food and Drug Administration
EFSA	European Food and Safety Agency
EU	European Union
FMN	Flavin mononucleotide
GLP	Good Laboratory Practice
GMM	Genetically Modified Microorganism
GMO	Genetically Modified Organism
LAB	Lactic acid bacteria
NGT	New Genomic Technique
NMN	Nicotinamide mononucleotide
OECD	Organization for Economic Co-operation and Development
OPPTS	US EPA Office of Prevention, Pesticides, and Toxic Substances
POF	Phenolic off-flavour
SDN	Site-Directed Nuclease
SOD	Superoxide dismutase

Appendix B – Glossary

CRISPR	Clusters of regularly interspaced short palindromic repeats, a component of bacterial immunity used to recognise and protect against viruses. It is commonly used as a shorthand for the CRISPR/Cas9 system.
Epigenetics	The molecular mechanisms (e.g., DNA methylation) controlling the expression of a genetically encoded trait. DNA methylation is reversible, and, although it can be inherited between generations, whether it is retained will depend on the environment.
Genome editing	The process of DNA editing utilizes techniques such as CRISPR, ZNF, and TALEN to precisely target genetic modifications to specific locations within a genome. Typically, these techniques are employed to alter single nucleotides or procreate due to short insertions or deletions (indels) with high precision.
Mutagenesis	Creation of mutation(s) in an organism without insertion of foreign genetic material.
New Genomic Technique	Techniques capable of altering the genetic material of organisms, which have emerged or been developed after the implementation of GMO legislation in 2001
Novel foods	Foods that don't have a 'significant history of consumption' by humans in the US before 15 May 1997. 'Novel Food' can be newly developed, innovative food, food produced using new technologies and production processes, as well as food that is or has been traditionally eaten outside of the EU.
SDN-1	A targeted mutagenesis technique using SDNs to introduce small mutations in a specific location of the genome. In SDN-1, no DNA template is provided, so the type of mutation is random.
SDN-2	A targeted mutagenesis technique using SDNs to introduce small mutations in a specific location of the genome. In SDN-2, a DNA template is used to obtain a desired mutation.
SDN-3	A targeted mutagenesis technique using SDNs to introduce foreign genetic material in a specific location of the genome. Depending on the sexual compatibility between the donor and host organisms, the process is cisgenesis or transgenesis.
Site-directed mutagenesis	Techniques of mutagenesis that induce mutation(s) in selected target locations of the genome without insertion of genetic material.

Appendix C – Search strategies

C.1 Structured search strategy implemented in MEDLINE (PubMed)

#1	"genome edit*"[tiab]	12,647
#2	"gene edit*"[tiab]	9,051
#3	"genome manipulat*"[ti]	38
#4	"genome techn*"[ti]	11
#5	"genetically edit*"[tiab]	75
#6	"site directed"[ti]	5,461
#7	"synthetic gene"[ti]	383
#8	"synthetic genome"[ti]	25
#9	"synthetic biology"[ti]	2,029
#10	recombineer*[ti]	270
#11	"bio engineer*"[tiab]	471
#12	"bioengineer*"[tiab]	10,987
#13	CRISPR*[tiab]	34,649
#14	"ZFN"[tiab]	655
#15	"ZNFs"[tiab]	106
#16	"oligonucleotide-directed mutagenesis"[tiab]	672
#17	"genome modificat*"[ti]	111
#18	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17	61,622

#19	"Bacteria"[Mesh]	1,502,587
#20	"Fungi"[Mesh]	429,596
#21	"Viruses"[Mesh]	992,703
#22	"Rhodophyta"[Mesh]	4,298
#23	"Euglenida"[Mesh]	2,441
#24	"Cryptophyta"[Mesh]	338
#25	"micro organism*"[tiab]	11,228
#26	"microorganism*"[tiab]	140,015
#27	bacteria*[tiab]	840,219
#28	virus*[tiab]	849,578
#29	alga*[tiab]	56,91
#30	yeast*[tiab]	207,502
#31	cyanobacteria*[tiab]	24,266
#32	protist[tiab]	2,768
#33	phage[tiab]	51,044
#34	archaea[tiab]	17,351
#35	"filamentous fung*"[tiab]	10,548
#36	#19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35	3,569,183
#37	#18 AND #36	17,439
#38	#37 AND (published from 2001)	15,268

C.2. Structured search strategy implemented in EMBASE (accessed via embase.com)

#38. #37 AND (published from 2001) 15,955

#37. #18 AND #36 17,751

#36. #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 3,382,696

#35. 'microorganism*':ti,ab 120,887

#34. 'filamentous fung*':ti,ab 8,956

#33. archaea:ti,ab 12,450

#32. phage:ti,ab 39,721

#31. protist:ti,ab 1,594

#30. cyanobacteria*:ti,ab 16,703

#29. yeast*:ti,ab 177,888

#28. alga*:ti,ab 39,097

#27. virus*:ti,ab 779,965

#26. bacteria*:ti,ab 757,130

#25. 'micro organism*':ti,ab 10,436

#24. 'cryptophyta'/exp 460

#23. 'euglenida'/exp 1,417

#22. 'rhodophyta'/exp 3,865

#21. 'viruses'/exp 1,062,706

#20. 'fungi'/exp 458,061

#19. 'bacteria'/exp 1,509,249

#18. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 65,019

#17. 'genome modificat*':ti 92

#16. 'oligonucleotide-directed mutagenesis':ti,ab AND 495

[embase]/lim

#15. 'znfs':ti,ab 99

#14. 'zfn':ti,ab 939

#13. crispr*:ti,ab 39,272

#12. 'bioengineer*':ti,ab 11,009

#11. 'bio engineer*':ti,ab 560

#10. recombineer*:ti 226

#9. 'synthetic biology':ti 1,436

#8. 'synthetic genome':ti 23

#7. 'synthetic gene':ti 301

#6. 'site directed':ti 4,924

#5. 'genetically edit*':ti,ab 117

#4. 'genome techn*':ti 16

#3. 'genome manipulat*':ti 33

#2. 'gene edit*':ti,ab 9,099

#1. 'genome edit*':ti,ab 11,418

C.3. Search strategy implemented in WEB OF SCIENCE – (Citation Index Expanded (SCI-EXPANDED), Conference Proceedings Citation Index – Science (CPCI-S), Book Citation Index – Science (BKCI-S), Emerging Sources Citation Index (ESCI), Current Chemical Reactions (CCR-EXPANDED), Index Chemicus (IC))

#1 "TI=(""genetically modified micro-organism*"" OR ""genetically modified microorganism*"") OR AB=(""genetically modified micro-organism*"" OR ""genetically modified microorganism*"") 330

#2 "TI=(""genome edit*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))131

#3 "TI=(""gene edit*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))50

#4 "TI=(""genome manipulat*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))7

#5 "TI=(""genome techn*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))1

#6 "TI=(""genome edit*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))131

#7 "TI=(""site directed"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))193

#8 "TI=(""synthetic gene"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))12

#9 "TI=(""synthetic genome"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))3

#10 "TI=(""synthetic biology"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))140

#11 "TI=(""recombineer*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))30

#12 "TI=(""bio engineer*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))6

#13 "TI=(""bioengineer*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))100

#14 "TI=(""CRISPR*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))559

#15 "TI=(""ZFN"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*"")) 2

#16 "TI=(""ZFNs"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*"")) 0

#17 "TI=(""oligonucleotide-directed mutagenesis"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))7

#18 "TI=(""genome modificat*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))1

#19 "AB=(""genome edit*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))274

#20 "AB=(""gene edit*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))121

#21 "AB=(""genetically edit*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))1

#22 "AB=(""bio engineer*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))15

#23 "AB=(""bioengineer*"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))173

#24 "AB=(CRISPR* NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*"")) 1627

#25 "AB=(ZFN NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*"")) 8

#26 "AB=(ZFNs NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*"")) 11

#27 "AB=(""oligonucleotide-directed mutagenesis"" NEAR/5 (""micro organism*"" OR ""microorganism*"" OR bacteria* OR virus* OR alga* OR yeast* OR cyanobacteria* OR protist OR phage OR archaea OR ""filamentous fung*""))7

#28 "#27 OR #26 OR #25 OR #24 OR #23 OR #22 OR #21 OR #20 OR #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #13341

#29 #28 AND (published from 2001) 3,093

Appendix D - List of Global and National Biotechnology Regulatory databases/Food and feed safety agencies

Agency	URL
Africa: African Agricultural Technology Foundation (AATF)	https://www.aatf-africa.org/
Argentina: MAGYP - Ministry of Agriculture, Livestock and Fisheries	https://www.argentina.gob.ar/agricultura/bioeconomia/biotecnologia/conabia
Australia: OGTR – Office of the Gene Technology Regulator	https://www.ogtr.gov.au/
Brazil:	
- Ministry of Agriculture, Livestock and Food Supply	http://www.abc.gov.br/training/informacoes/InstituicaoMAPA_en.aspx
- Brazilian National Technical Biosafety Commission (CTNBio)	http://ctnbio.mctic.gov.br/inicio
Canada	
- Health Canada	https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods.html#a2
- CFIA - Canadian Food Inspection Agency	https://inspection.canada.ca/eng/1297964599443/1297965645317
China: China National Center For Food Safety Risk Assessment	https://en.cfsa.net.cn/
Costa Rica: CTNBio-SFE, Ministry of Agriculture	https://www.sfe.go.cr/SitePages/Inicio.aspx
Germany:	
- BMEL - Federal Ministry of Food and Agriculture	https://www.bmel.de/EN/Home/home_node.html
- Federal Office of Consumer Protection and Food Safety (BVL)	https://www.bvl.bund.de/EN/Home/home_node.html
- German Federal Institute for Risk Assessment	https://www.bfr.bund.de/en/home.html
India: Ministry of Science and Technology – Department of Biotechnology (DBT)	https://dbtindia.gov.in/
Japan:	
- Ministry of Health, Labor and Welfare (MHLW) - Office of Health Policy on Newly Developed Foods	https://www.mhlw.go.jp/english/topics/food/

- Institute of Agrobiological Sciences, National Agriculture and Food Research Organization (NARO)	https://www.naro.go.jp/english/about-naro/index.html
Kenya: NBA – National Biosafety Authority	https://www.biosafetykenya.go.ke/
New Zealand: Environment Protection Authority	https://epa.govt.nz/
Nigeria: NBMA - National Biosafety Management Agency	https://nbma.gov.ng/
Philippines: Department of Agriculture	https://www.da.gov.ph/
Russia: Federal service for veterinary and phytosanitary surveillance of the Russian Federation	https://fsvps.gov.ru/ru
Spain:	
- Spanish National Commission on Biosafety and Inter-ministerial Council for GMOs	https://www.miteco.gob.es/en/calidad-y-evaluacion-ambiental/temas/biotecnologia/organismos-modificados-geneticamente-omg-/comision-naciona-bioseguridad.html http://www.cnb.csic.es/index.php/en
- Spanish Agency for Food Safety and Nutrition (AESAN)	https://www.aesan.gob.es/en/AECOSAN/web/home/aecosan_inicio.htm
United Kingdom:	
- DEFRA – Department for Environment, Food and Rural Affairs	https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs
- FSA - Food Standards Agency	https://www.food.gov.uk/
United States:	
- APHIS – Animal and Plant Health Inspection Service, U.S. Department of Agriculture	https://www.aphis.usda.gov/aphis/home/
- U.S. EPA – Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division (OPP/BPPD)	https://www.epa.gov/pesticide contacts
- U.S. FDA – Food and Drug Administration	https://www.fda.gov/
- Agricultural Research Service (ARS)	https://www.ars.usda.gov/

Appendix E - Description of included GMM cases

Case Category	Description	Use	Taxon and species	New strain name	Technique	Target gene	Name/ Company (Country)	Stage / Patent application ID /Authorisation	Reference
GMM-01 Category 4	Nitrogen-producing soil bacteria able to fertilise cereal crops and used as a supplement to nitrogen fertilisers. The active ingredient of the liquid fertilizer PROVEN®, which associates with corn roots and fixes nitrogen	Agriculture, Fertiliser	Bacteria *Klebsiella variicola* Strain KV137	KV137-1036 Δ*nifL*::P*inf C*	Plasmid based guided microbial remodelling.	genes *nif L* and *nif G*, which encode enzymes involved in nitrogen fixation and proteins that regulate nitrogen fixation.	Proven40® / Pivot Bio (USA)	Commercial Patent application WO2021221690 A1	(Wen et al., 2021; Bloch et al., 2020)
GMM-02 Category 4	Engineered bacteria as a feed additive	Feed	Bacteria	strain X	CRISPR/Cas	*E. coli proA* gene; and	BiomElix Guided Biotics® /	Commercial	No referenc

	that selectively removes unwanted bacteria (*Salmonella*) from animal guts		*Escherichia coli* Strain K12 (MG1655) (Other potential applications: *Bacteroides, Bifidobacterium, Clostridium, Escherichia, Lactobacillus, Lactococcus)*			*E. coli proB* gene	FOLIUM Science (UK)	Patented US10463049B2	e identified
GMM-03 Category 4	Fungal symbiont (endophyte) that cannot produce compounds (ergot alkaloids) that are toxic to livestock	Agricultural, feed	Fungal symbiont (endophyte) *Epichloë coenophiala* (formerly *Neotyphodium coenophialum*) Strain e19	EAS1-knockoff strain e7480 (=ATCC PTA-126679)	CRISPR/Cas 9	Ergot alkaloid biosynthesis (EAS) clusters: 196-kb EAS1 cluster and the 75-kb EAS2 cluster	University of Kentucky (USA)	Pre-Commercial Patented US11021760B2	(Florea, Jaromczyk, and Schardl, 2021)

			(ATCC) 90664) *Epichloë hybrida* Lp1 (=ATCC TSD-66) (Other potential applications: *Epichloë festucae*×*Epichloë typhina hybrid*)						
GMM-04 Category 4	Starter yeast culture that reduces chronic toxicity of alcoholic beverages, through reduction of ethanol-derived compounds concentration	Food (fermentation starter)	Yeast *Saccharomyces cerevisiae* Strain GRL6	dCAR1&GZF3	CRISPR/Cas 9	*CAR1* gene encoding for the arginase enzyme responsible for the formation of urea, and the *GZF3* gene encoding the	Catholic University of Korea (Korea)	Development	(Jung et al., 2023)

	during batch fermentation in a yeast growth medium (Makgeolli, the Korean traditional rice wine) containing urea and arginine					transcription factor controlling expression levels of several genes (*DUR1, 2*, and *DUR3*) related to urea absorption and degradation			
GMM-05 Category 4	Modified probiotic bacteria to increase production of riboflavin in industrial biosynthesis by reducing the expression of the *folE* gene. In addition, a	Food (probiotic), medical application	Bacteria *Leuconostoc citreum* Strain CB2567	JW001	CRISPR interference (CRISPRi)	*folE* and *ribF* genes. *folE* gene encodes GTP cyclohydrolase I, which can minimize the flux of GTP toward the folate synthesis	Kaist Institutes (Korea)	Development	(Son et al., 2020)

	ribF gene was chosen as the second target for downregulation, to reduce the loss of riboflavin due to its conversion into FMN					pathway. The *ribF* gene encodes the riboflavin kinase responsible for the bioconversion of riboflavin to FMN.			
GMM-06 Category 3	Engineered food-grade bacteria able to produce β-mannanase (for preparation of mannose oligosaccharides) to be used in microbial fermentation	Food, feed, medicine	Bacteria *Bacillus subtilis Strain* DB104 and FGYM102	Not provided	CRISPR/Cpf1 (Cas12a)	d-alanine racemase gene (*dal* gene) and *Bacillus licheniformis* β-mannanase mutant coding gene	UNIV HEBEI SCIENCE & TECH (China)	Pre-Commercial Patent application CN114703163A	No reference identified
GMM-07 Category 3	Recombinant bacteria strain producing a high yield of	Food	Bacteria	CICC10303-TRYP	CRISPR/Cas9	shikimate kinase encoding gene aroK	UNIV JIANGNAN (China)	Pre-commercial	No reference identified

	the aromatic amino acid L-tryptophan in industrial fermentation as a microbial cell factory		*Escherichia coli* Strain CICC10303			promoter, the benzoate dehydrogenase encoding gene *pheA*; the tryptophan transporter encoding gene *mtr (knocked out)*		Patented CN109486737B	
GMM-08 Category 3	Bacteria strain producing a high yield of 3'-sialyllactose, from glycerol and lactose, optimize the supply of precursors through pathway enhancement, and screen high-efficiency α2,3-sialyltransferas	Food (probiotic), medicine, chemical industry	Bacteria *Escherichia coli* Strain BL21(DE3)	Not reported	CRISPR/Cas9	Gene *lacZ* encoding β-galactosidase, gene *nanA* encoding N-acetylneuraminic acid lyase, gene encoding N-acetylneuraminic acid transporter gene *nanT*, gene *nanK* encoding N-	UNIV JIANGNAN (China)	Pre-commercial Patent application CN114874966A	No reference identified

	e, for production by fermentation					cetylmannosamine kinase			
GMM-09 Category 3	Bacteria with increased capacity to produce L-threonine for industrial bioproduction	Food, feed, pharmaceutical, nutraceutical	Bacteria *Escherichia coli* Strain CICC20905	CICC20905-THR	CRISPR/Cas9	The homoserine kinase-encoding gene, the threonine dehydrogenase-encoding gene *tdcB*, the lysine synthesis pathway dihydrodipicolinate synthetase DHDPS encoding gene *dapA*.	UNIV JIANGNAN (China)	Pre-commercial Patented CN109554322B	No reference identified
GMM-10 Category 3	Yeast that reduces urea accumulation in the fermentation process of rice wine yeast	Food (rice wine)	Yeast *Saccharomyces cerevisiae* Strain JNZ01 (MATa, Δ*ura3*,	JNZ9 (MATa, Δ*ura3*, Δ*trp1*). TOR1S1972, Δ*fpr1*, DAL80::FKBP12,	CRISPR/Cas9; Fusion PCR	*DAL80* (global regulator of the inhibition of nitrogen metabolism)	UNIV JIANGNAN (China)	Pre-commercial Patented CN109504699B	No reference identified

			Δ*trp1*, TOR1S1972R and Δ*fpr1*)	pRS426-TEF-Rps1B-FRB-URA3)		, *FKBP12*, *FPR1*, *TOR1*			
GMM-11 Category 3	Yeast that reduces urea accumulation in the fermentation process of rice wine yeast	Food (rice wine)	Yeast *Saccharomyces cerevisiae* Strain XZ-11(MATa, Δ*ura3* and Δ*trp1*)	JNZ01(MATa, Δ*ura3*, Δ*trp1*, TOR1 S1972R and Δ*fpr1*)	CRISPR/Cas9; Fusion PCR	*FPR1* gene is knocked out and the *TOR1* gene is point-mutated.	UNIV JIANGNAN (China)	Pre-commercial Patented CN109517747B	No reference identified
GMM-12 Category 3	Yeast-engineered strain with the ability to produce a precursor of vitamin D3 (7-dehydrocholesterol)	Feed, medicine, and chemical industry	Yeast *Saccharomyces cerevisiae* Strain CENPK2-1D	7-DHC-3	CRISPR/Cas, Fusion PCR	ergot alkaloid biosynthesis (EAS) genes	UNIV JIANGNAN (China)	Pre-Commercial Patent application CN114703077A	No reference identified
GMM-13	Bacteria that increase	Food (probiotic)	Bacteria	Not provided	Heterologous Type II	*spo0A* gene (the master	Institute of Animal	Development	(Zhou et al., 2021)

Category 4	butyrate production and decrease ethanol production with probiotic purposes		*Clostridium butyricum* Strain CGMCC0313-1 RH-2, MIYARI-588		CRISPR/Cas9 system and endogenous Type I-B CRISPR/Cas9 system	regulator for sporulation), *aldH* gene encoding the enzyme aldehyde dehydrogenase (involved in ethanol production), the *adhE* gene (bifunctional aldehyde/alcohol dehydrogenase) involved in alcohol production	Science, Chinese Academy of Agricultural Sciences (China)		

GMM-14 Category 4	Lactic acid bacterium (*P. acidilactici*) strain with enhanced cell growth and lactic acid production, with application in synthetic biology and bioindustry	Feed (fermented food)	Bacteria *Pediococcus acidilactici* Strain LA412	LA412::ldh	CRISPR/Cas9	*pyrE* (native locus in LA412) encoding the orotate phosphoribosyl transferase	Huazhong Agricultural University (China)	Development	(Liu et al., 2021)
GMM-15 Category 4	Yeast with an increased β-carotene (vitamin precursor) and drug (violacein) production in the mammalian gut	Food (Probiotic)	Yeast *Saccharomyces boulardii* Strain ATCC-MYA796, ΔURA3, ΔTRP1 and ΔHIS3	Not reported	CRISPR/Cas assisted homologous recombination, DNA homologous recombination without DSB (dsDNA integration)	Different genes in the β-carotene and violacein biosynthetic pathways	North Carolina State University (USA)	Development	(Durmusoglu et al., 2021)
GMM-16	Yeast that enhances thiol	Food additive (fermentation	Yeast	London Tropics	CRISPR/Cas9	Not reported	Berkeley yeast	Commercial	(Denby et al., 2018)

Category 4	biotransformation to produce beer with strong sensory notes of guava, passionfruit, and grapefruit	starter in beer)	*Saccharomyces cerevisiae* Strain London Ale, GY Vermont IPA, Hornindal Kveik, and Augustiner Lager				company (USA)		
GMM-17 Category 4	Yeast that produces alpha acetolactate decarboxylase enzyme for preventing Diacetyl Formation	Food (fermentation starter in beer)	Yeast *Saccharomyces cerevisiae* Strain Andechs Lager, Chico Ale, London Ale. GigaYeast Vermont IPA, Augustiner Lager, Weihenstephan Lager	Diacetyl-Free Strains	CRISPR/Cas9	N/A	Berkeley yeast company (USA)	Commercial	(Denby et al., 2018)
GMM-18 Category 4	Yeast that produces both lactic acid and ethanol during fermentation,	Food (fermentation starter in beer)	Yeast *Saccharomyces cerevisiae*	Galactic strains	CRISPR/Cas9	Not reported	Berkeley yeast company (USA)	Commercial	(Denby et al., 2018)

	which enables quick souring in the fermenter without a kettle-pasteurization step		Strain Chico Ale						
GMM-19 Category 4	Yeast that biosynthesises linalool, geraniol, and citronellol	Food (fermentation starter in beer)	Yeast *Saccharomyces cerevisiae* Strain California Ale Yeast	yBBS002	CRISPR/Cas9	The modified strain contains the *S. cerevisiae* HMG-CoA reductase gene (*HMG1*), the *S. cerevisiae* FPP synthase gene (*ERG20*), the *Mentha citrata* linalool synthase gene, and	Berkeley Brewing Science, Inc. (USA)	Commercial	(Denby et al., 2018)

						the *Ocimum basilicum* geraniol synthase gene.			
GMM-20 Category 4	Yeast that produces the terpenes linalool, geraniol, and citronellol during fermentation, with floral and citrus flavour determinants, decreasing cold-side hop additors or avoiding dry-hopping avoiding dry-hopping	Food (fermentation starter in beer)	Yeast *Saccharomyces cerevisiae* Strain Chico Ale	Superbloom strains	CRISPR/Cas9	Not reported	Berkeley yeast company (USA)	Commercial	(Denby et al., 2018)
GMM-21 Category 4	Yeast that completes fermentation in several days	Food (fermentation starter in beer)	Yeast	No Longer Diastatic (NLD) strain	CRISPR/Cas9	Not reported	Berkeley yeast company (USA)	Commercial	(Denby et al., 2018)

	and maintains gravity stable by elimination of gene *STA1*, avoiding over-carbonated beer and exploding bottles		*Saccharomyces cerevisiae* Strain Sacch Trois						
GMM-22 Category 3, 4	Microalga *Nannochloropsis* strain with Low-cholesterol and high-24-methylene-cholesterol, which can be used in industry to produce functional food and aquatic bait with a high polyunsaturated fatty acid, high 24-methylene	Food (probiotic)	Microalgae *Nannocloropsis oceanica* Strain not reported	Nannochloropsis with knockout DWF1 gene	CRISPR/Cas9	DWF1 (sterol C-24(28) isomerase reductase)	UNIV HAINAN (China)	Pre-Commercial Patent application WO2023060761 A1	No reference identified

	cholesterol and low cholesterol								
GMM–23 Category 4	Probiotic yeast that produces neoagarooligos accharides as a microbial cell factory	Food (prebiotic)	Yeast *Saccharomyces cerevisiae var. Boulardii* Strain ATCC MYA-796	BpGH16A	CRISPR/Cas9	*BpGH16A* and *SED1* knock in, *BpGH16A* coding for endo-type β-agarase	National Research Foundation of Korea (NRF) (Korea)	Development	(Jin et al., 2021)
GMM–24 Category 4	Hybrid beer yeast that reduces phenolic off-flavours in beer	Food (fermentation starter for beer)	Yeast *Saccharomyces cerevisiae* Strains SA003 and BE011, *S. eubayanus* strains WL2022 (NPCC1286) and WL024 (NPCC1292)	BE002, BE014, BE020 and BE074	CRISPR/Cas9	*FDC1* coding for Ferulic acid decarboxylase 1	Vlaams Instituut voor Biotechnologie (VIB) (Belgium)	Development	(Mertens et al., 2019)
GMM–25 Category 4	Yeast that produces a non-foam-forming sake,	Food (fermentation starter in sake)	Yeast	K7GE21, K7GE31, and K7GE41.	CRISPR/Cas9	*AWA1*, *CAR1*, *MDE1*, and *FAS2*;	National Research Institute of	Development	(Chadani et al., 2021)

	without producing carcinogens or an unpleasant odour, sake with a sweet ginjo aroma, a strain with reduced urea (the precursor of ethyl carbamate), and increased product of a large amount of ethyl caproate		*Saccharomyces cerevisiae* Strains foaming isolate K7 and genome edited, non-foam-forming isolate K7GE01			awa1Δ/awa 1Δ possesses a non-foam-forming characteristic; car1Δ/car1 Δ, causes a defect in the urea cycle; mde1Δ/md e1Δ sake without unpleasant odour; FAS2 (G1250S)/ FAS2 (G1250S) with a strong ginjo aroma	Brewing (Japan)		
GMM–26	Yeast with enhanced DUR3	Food (fermentation	Yeast	NaDUR1,2-c, NαDUR1,2-c,	CRISPR/Cas9	*CAR1, DUR1,2,*	National Natural	Development	(Wu et al., 2020)

Category 4	expression level, that reduces urea and ethyl carbamate during fermentation	starter in rice wine)	*Saccharomyces cerevisiae* Strain N85	N85DUR1,2-c, NaDUR1,2/DUR3-c, NɑDUR1,2/DUR3-c, N85DUR1,2/DUR3-c		and *DUR3* for enhancement of urea utilization and minimizing EC level in Chinese rice wine	Science Foundation of China (China)		
GMM–27 Category 4	Yeast with enhanced carbon dioxide production in bread that reduces acrylamide formation in potato chips, increases savoury flavours in rice wine, increases the levels of the amino acids which provide a savoury taste, improves the	Food (yeast-fermented foods)	Yeast *Saccharomyces cerevisiae* Strain N1	N1ΔGZF3:PGPD-ASP3	CRISPR/Cas9	*RGT2* and *SNF3* coding for glucose sensors, *ASP3* coding for asparaginase, *URE2* coding for a transcriptional factor of nitrogen catabolite repression	Woese Institute for Genomic Biology (USA)	Development	(Lee et al., 2022)

	flavour of fermented foods (bread, rice wine)								
GMM–28 Category 4	Yeast with increased glycerol levels compared to the parental strain and strikingly higher acetate ester levels akin to the strains that individually overexpresses GPD1 and ATF1, and reduced acetic acid in the final wine	Food (wine, fermentation)	Yeast *Saccharomyces cerevisiae* Strain AWRI1631 (mating type a - MATa)	*AWRI[ATF1GPD1]*	CRISPR/Cas9	*GPD1* encoding for glycerol-3-phosphate dehydrogenase 1, and *ATF1* encoding for alcohol acetyltransferase 1	Department of Molecular Sciences, Macquarie University (Australia)	Development	(Van Wyk et al., 2020)
GMM–29 Category 3	Bacteria that enhance the accumulation of nicotinamide mononucleotide (NMN) in the	Food (dairy)	Bacteria *Lactococcus lactis*	NZ9000ΔnadR, NMN01, NMN02, pKLH32, pLL29,	CRISPR/Cas9	Lactococcus lactis NZ9000, NZ9000ΔnadR, NMN01,	National Natural Science Foundation of China (China)	Development	(Kong et al., 2023)

	probiotic lactic acid bacteria will significantly affect the probiotic effects of the dairy products		Strain *NZ9000*	pHH01, pHH03		pKLH32, pLL29, pHH01, pHH03			
GMM–30 Category 3	Yeast that provides an alternative approach to produce glycosylated triterpenoids, increasing the production of Glycyrrhetic acid 3-O-mono-β-D-glucuronide (GAMG), resulted in the higher conversion rate of glycyrrhetic acid (GA) to GAMG	Food (natural sweeteners in candies and canned foods)	Yeast *Saccharomyces cerevisiae* Strain *BY4741-C-04*	CI04-1-1, CI04-3, CI04-4, GA1, GA8, GA9, GA10, GA11, GA12, GA13, GA14, GA15	CRISPR/Cas9	*PGM1, PGM2, UGP1,* and *AtUDH* were overexpressed to enhance UDP-GlcA supply. *EGH1* was deleted to prevent GAMG degradation	National Natural Science Foundation of China (China)	Development	(Huang et al., 2021)

GMM–31 Category 3	Bacteria for the production of high value-added metabolites, such as acetoin	Food (dairy fermented product)	Bacteria *Lactobacillus casei* strain BL23	BL24, BL25, BL26, BL27	homologous recombination, single-plasmid genome editing	Lactate dehydrogenase gene (*ldh* and *hicD3*), three genes (*pflB, ldh,* and *pdhC*) related to acetoin production	National Natural Science Foundation of China (China)	Development	(Xin et al., 2018)
GMM–32 Category 3	Yeast that reduces urea production in wine production due to the elimination of the arginine permease encoded by CAN1	Food (fermentation starter in wine)	Yeast *Saccharomyces cerevisiae* Strains AWRI796 and Lalvin EC1118	*CAN1* mutants	CRISPR/Cas9	*CAN1* deletion which encodes arginine permease	YeSVitE consortium (Canada)	Development	(Vigentini et al., 2017)
GMM-33 Category 3	Yeast that is slow-growing and can be used in a single co-inoculation with a non-	Food (fermentation starter in wine)	Yeast *Saccharomyces cerevisiae*	*EC1118 SER1-232* (G > C; G78R) homozygous allele	CRISPR/Cas9	*SER1* encoding 3-phosphoserine	Australian Research Council Training Centre for Innovative	Development	(Lang et al., 2022)

	Saccharomyces yeast, optimizing wine fermentation processes		Strain Lalvin EC1118			aminotransferase	Wine Production (Australia)		
GMM-34 Category 3	Yeast with homozygous awa1Δ/awa1Δ deletion alleles responsible for nonfoam formation in sake	Food (fermentation starter in sake)	Yeast *Saccharomyces cerevisiae* Strains *K6, K7, K7S09, K7W16, K9*, and *K10*	A nonfoam-forming isolate *K701*	CRISPR/Cas9	*AWA1* encoding glycosylphosphatidylinositol (GPI)-anchored cell wall protein	National Research Institute of Brewing (Japan)	Development	(Ohnuki et al., 2019)
GMM-35 Category 3	Yeast used to enhance the desirable organoleptic characteristics of wine. Release β-ionone through the heterologous expression of both the enzyme	Food (fermentation starter in wine)	Yeast *Saccharomyces cerevisiae* strain BY4741	*CV, OV, OP, MQ01, MQ02, MQ03, MQ04*	CRISPR/Cas9	*crtYB* gene encoding bifunctional phytoene synthase and lycopene cyclase, and *crtE* (encoding geranylgeranyl diphosphat	Australian Research Council Centre of Excellence in Synthetic Biology (Australia)	Development	(Timmins et al., 2023)

	carotenoid cleavage dioxygenase 1 (CCD1) and its substrate, β-carotene					e synthase) and *crtI* (encoding phytoene desaturase) genes			