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Horizon Scan of Synthetic Biology Developments for Microorganisms with application in the Agri-Food Sector

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Abstract

The European Commission requested the European Food and Safety Authority for an opinion on genetically modified organisms for agri-food uses and developed through synthetic biology (SynBio) and their implications for risk assessment methodologies. In preparing this opinion EFSA has asked RIVM to perform a horizon scan of SynBio developments with application in the agri-food sector. Relevant SynBio cases were identified using a search strategy including scientific publications and grey literature, websites demonstrating commercial activities in synthetic biology, databases of regulatory agencies and iGEM projects. The outcome of this scan supports part of the terms of references of the EC mandate and EFSA in defining relevant case studies to consider during the drafting of the scientific opinion.

Key words: Synthetic Biology; Agri-Food; Microorganism; Commercialization; Deliberate Release

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Summary

Synthetic biology is a rapidly developing research field resulting in new techniques for the design of genetically modified organisms (GMO). In order to get an overview of GMO applications that are in the pipeline for commercialization EFSA requested RIVM to map these developments using a horizon scan. More specifically, this horizon scan focusses on synthetic biology (SynBio) cases describing the use of genetically modified microorganisms (GMM) intended for a deliberate release into the environment and with a possible application as an agri-food product in the near future. This request is to address the Terms of Reference of an EC mandate on this topic published under mandate number M-2018-0205 in the EFSA register of questions.

Relevant SynBio cases were identified using a search strategy including scientific publications and grey literature, websites demonstrating commercial activities in synthetic biology, databases of regulatory agencies and iGEM projects. Inclusion criteria on type of agri-food product and intended use of a GMM, enabled the listing of relevant SynBio cases.

In total eleven SynBio cases were listed as a result from the search strategy. Five cases fully passed all the inclusion criteria. Six SynBio cases are listed separately as they do not fully match all the criteria, or the available information was insufficient to verify that the case matched all the inclusion criteria.

The listed cases represent agri-food products for human and animal consumption, for use as plant protection product, biosensor and fertilizer. The market readiness of the cases as indicated in this report is based on the technical advances of the product and the author's judgement and does not reflect the official status of the cases.

The GMMs as part of the listed SynBio cases were constructed by making use of genetic part libraries (i.e. the use of genetic elements) and genome editing. More advanced techniques like xenobiology or use of minimal cells, artificial cells or protocells are not yet in use for the construction of a GMM with a functionality as agri-food product. These techniques are still in an early phase of development and successful use for constructing a GMM that can survive outside the laboratory will need more time.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Rapidly evolving developments in synthetic biology may require adaptation of existing risk assessment methodologies. Revisiting and improving these methodologies are suggested to ensure continued safety of future products resulting from applications of synthetic biology1. This was concluded by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the Scientific Committee on Health and Environmental Risks (SCHER)2 and the Scientific Committee on Consumer Safety (SCCS).

These scientific committees defined synthetic biology (SynBio) as "the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms". They recognized six categories of SynBio developments: 1) Genetic part libraries and methods; 2) Minimal cells and designer chassis; 3) Protocells and artificial cells; 4) Xenobiology: 5) DNA synthesis and genome editing; and 6) Citizen science (Do- It-Yourself biology).

At the end of 2018, the European Commission requested the European Food and Safety Authority (EFSA) for an opinion on genetically modified organisms for agri-food uses and developed through SynBio and their implications for risk assessment methodologies. This request is published under mandate number M-2018-0205 in the EFSA register of questions3.

In preparing this opinion EFSA has asked RIVM to perform a horizon scan of SynBio developments with application in the agri-food sector under the contract title 'Mapping of microorganisms SynBio developments in the agri-food sector'. The contract was awarded by EFSA to Cécile van der Vlugt under the contract number NP/EFSA/SCER/2018/02 for the duration of 3 months (the period of investigation was from 1 January to 31 March 2019). The overall objective of the contract was to gather relevant information on micro-organisms SynBio developments moving towards practical applications in the next decade, arising from the previously mentioned SynBio categories, and to report this information in a technical report.

The outcome of this scan supports the Terms of References 1 and 2 of the above mandate and support EFSA to define relevant cases to consider during the drafting of opinion on SynBio developments mandated by the European Union. The specific objectives of the contract resulting from the present procurement procedure are as follows:

1) Considering the SynBio categories defined above, to identify and produce a list of microorganisms SynBio developments with potential applications in the agri-food sector4 and their intended use. This task should focus on applications that are in the pipeline for

[1] SCENIHR, SCCS, SCHER (2014) Synthetic Biology I Definition, Opinion, September 2014. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_044.pdf
SCENIHR, SCCS, SCHER (2015) Synthetic Biology II - Risk assessment methodologies and safety aspects, Opinion, May 2015. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_048.pdf
SCENIHR, SCCS, SCHER (2015) Synthetic Biology III – Research priorities, Opinion, December 2015. Available from: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_050.pdf
[2] SCENIHR and SCHER are merged into the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER)
[3] http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?5
[4] With a focus on agri-food/feed products falling within the remit of EFSA. These include: GMOs, food enzymes, novel food, as well as plant protection products.

commercialization (with a focus on deliberate release into the environment for commercial purposes) or in an experimental phase beyond the (theoretical) proof of concept stage (with a focus on deliberate release into the environment for experimental purposes).

2) Based on the list produced in the objective above, to prioritise the microorganisms SynBio developments that are most likely to move to practical applications in the agri-food sector in the next decade. Criteria used to prioritise relevant microorganisms SynBio developments should be made explicit and justified, and be agreed with EFSA.

1.2. Interpretation of the Terms of Reference

To achieve the highest quality of data, within the time and budget constraints of this study, several sources for searching relevant SynBio cases were chosen. For screening the scientific literature, the existing RIVM literature collection on synthetic biology was used. This literature collection started from 2014 and has yielded relevant literature for recent and ongoing RIVM projects on Synthetic Biology and Modern Biotechnology.

Next to this, relevant SynBio reports, websites and iGEM projects were chosen for screening. Websites demonstrating commercial activities and websites of regulatory agencies, were used to identify SynBio cases close to commercialization. It is noted that a full inventory on market readiness of the SynBio developments was not part of this study. The time-period for market readiness as presented in this report is therefore hypothetical and does not reflect the official status of the cases.

This horizon scan does not include the use of genetically modified (GM) plants and GM animals, nor GMMs used in agri-food products in which the GMMs are absent in the commercial product.

Based on the EFSA remit inclusion criteria for listing relevant SynBio cases were determined in close collaboration with EFSA (see 2.2.2 and 2.2.3). Likewise, the criteria for prioritization and the design of the output table (as presented in Appendix A) were determined.

2. Data and Methodologies

2.1. Data presentation

SynBio cases listed during the search strategy are presented in the table SynBio Developments for Microorganisms, in Appendix A. The lay out of this table was determined in close collaboration with EFSA.

In this table, the specifications of each listed SynBio case is presented as follows:

- Column 1 presents the Record ID;
- Column 2, 3 and 4 give details on the phase of development of the SynBio case and the setting (laboratory, greenhouse, etc.) of its GMM use in that stage. These details follow the requisites of inclusion criterion 3. The columns are coherent: from the dropdown list in column 2, one of the following stages is selected: "experimental stage" for those cases that reflect a proof of concept stage, or "placing on the market" for cases that are in the pipeline for commercialization or commercially available outside Europe.
  If "experimental stage" is selected, then the setting of the GMM use in its experimental stage was selected from the dropdown list in column 3. If "placing on the market" is selected, then the setting of the GMM use for placing on the market was selected from the dropdown list in column 4;

- Column 5 gives an estimate of year of commercial release in the EU. In this column a selection can be made from a dropdown list representing <5 years, < 10 years, or > 10 years while having in mind the criteria for prioritization (see section 2.2.3);
- Column 6 and 7 give information on the biological entity of the GMM: in column 6 the applicable taxon is selected from the dropdown list with the taxa listed in inclusion criterion 1. In column 7 the species and / or strain name of the GMM is given;
- Column 8, 9, 10 refer to the applied technique and the degree of modification used to construct the GMM. In column 8 the dropdown list shows those techniques that are recognized as synthetic biology as mentioned in section 1.1. In column 9 the origin of the inserted genes is presented and in column 10 the number of modified genes;
- Column 11 and 12 describe the GMM phenotype and its intended use by the consumer;
- Column 13 presents a dropdown list of the products falling within the remit of EFSA (see inclusion criterion 2). The type of GMM product is selected from this list;
- Column 14 contains a dropdown list from which the geographic region where the GMM is developed and/or already in use, is selected;
- Column 15 gives reference to the scientific publication, database or website describing the GMM and resulting from the search strategy.
- Column 16 is available for additional remarks.

All data presented in the table were retrieved from the references listed in column 15 of this table.

The Table consists of two datasheets. One datasheet is called "Deliberate Release" and lists the SynBio cases that passed all the inclusion criteria. The second sheet "Questionable cases" contains SynBio cases that do not fully match the inclusion criteria, or the available information was insufficient to make sure that the case matched the inclusion criteria. An argumentation for placing SynBio cases on this sheet is given in the Table (column 16).

2.2. Methodologies

2.2.1. Sources

Following the objectives for this horizon scan, different sources that report developments in synthetic biology and use of microorganisms were used. This section lists all sources that were used for searching relevant SynBio cases.

RIVM literature collection

The existing RIVM literature collection on synthetic biology originates from weekly searches of the citation database Scopus and the media monitoring tool Howards Home by use of the search string "synthetic biology" (more precisely synthetic biolog*). This literature collection has proven to be very useful over the past years for RIVM to monitor technical advances in synthetic biology and applications in microorganisms as well as in higher organisms.

For this horizon scan the literature collection covering the period 2014–2018 was used. Considering that weekly searches in Scopus resulted in, on average 25 hits and in Howards Home 15 hits, approximately 10.000 items became available during the period 2014-2018.

The selection of relevant items (pdf's) from the weekly searches was performed manually and items were stored in folders with dedicated keywords. Items from the Howard Home search were regarded

as indicative for the news value of a scientific development, and were rarely saved in the folders of the RIVM literature collection.

The RIVM literature collection 2014-2018 contains 1398 publications on synthetic biology. From this collection those folders were selected that are relevant for the mandate e.g. that cover the SynBio categories as defined by the European Scientific Committees (see section 1.1). The match between these folders and the SynBio categories is shown in Table 1. In addition, folders were selected that cover 'other techniques' (like protein engineering and protein design) and specific taxa of microorganisms (bacteriophages and micro-algae). The selected folders contain a total of 733 publications.

Folders in the RIVM literature collection that were not relevant for this horizon scan were those folders having keywords related to GM plants, medicine, cell free systems, biosecurity, bionanotechnology and risk governance. All 733 publications in the selected RIVM folders were part of the search strategy.

Table 1: The categories of SynBio techniques (as presented in section 1.1 of this report) and the corresponding, as well as additional RIVM literature folders that were screened in this horizon scan. Numbers represent the number of individual pdf's in the indicated folders covering the period 2014 to 2018.

Category of SynBio techniques	RIVM folders with the following keywords were screened	Number of individual pdf's screened
Genetic part libraries	Metabolic pathway, metabolics, gene regulation, biosensor	212
Minimal cells and designer chassis	Minimal cell, synthetic genome, synthetic chromosome, microbial consortia	54
Protocells and artificial cells	Protocell	33
Xenobiology	Orthogonality, xenobiology, biocontainment	59
Genome editing	CRISPR, genome editing	243
	Protein engineering, protein design	53
	Bacteriophages, algae	79
		Total 733

Reports related to Synthetic Biology

Reports on synthetic biology were screened for references describing SynBio cases possibly relevant for this horizon scan. If a reference seemed relevant, the publication was screened in more detail. A list of the screened reports is available as Appendix B.

Websites

Websites demonstrating commercial activities in synthetic biology that were screened for relevant Synbio cases were:

- Labiotech, digital medium covering the European Biotech Industry. https://labiotech.eu/ News - Category Food. Accessed February 26, 2019.
- SynbiCITE, Innovation and Knowledge Centre for Synthetic Biology in the UK. http://www.synbicite.com/news-events/news/ Accessed March 1, 2019.
- SynBioBeta, innovation network, weekly updates on the latest news in synthetic biology. https://synbiobeta.com/category/agriculture/;https://synbiobeta.com/category/food-and-nutrition/ Accessed February 21, 2019.
- Woodrow Wilson Institute - Synthetic Biology products and applications inventory. Last update in 2015. http://www.synbioproject.org/cpi/ Accessed March 1, 2019.
- Bio-start - an annual competition (a one-off in 2017) designed to commercialize the engineering of biology. http://www.bio-start.uk/ Accessed March 4, 2019.
- iGEM projects that have resulted in start-ups. https://igem.org/Startups Accessed March 5, 2019.

Databases of regulatory agencies

The following databases of regulatory agencies were screened for SynBio cases:

- USDA:https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/sa_permits/status-update/release-permits. Permits since 2014 searched with keywords 'viroid, virus, bacteriophage, micro-organism, bacterium, fungus, microalga'. Accessed February 14, 2019
- Australia: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1 Table of applications and authorizations for Dealings involving Intentional Release and the Table Status of license applications for release of GMOs into the environment. Accessed February 14, 2019.
- Canada: https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products.html Accessed February 14, 2019.

Websites of EPA, FDA and from Japan and China were not available in a way that screening for relevant SynBio cases was possible. This was due to the absence of a searchable database or use of language.

iGEM projects

iGEM projects assigned to the tracks "Environment" and "Food" in the years 2014, 2016 and 2017 were screened for relevant SynBio cases. From a total of 798 projects presented during these three years, the track "Environment" covered 140 projects, the track "Food" 46 projects.

Selected journals

Being aware that the use of the search string 'synthetic biology' could miss those publications that lack this string as a keyword, journals dedicated to synthetic biology were screened as well. Titles of publications on the content pages of the journals *ACS Synthetic Biology*, *PLOS ONE Synthetic Biology* and *Trends in Biotechnology* were screened manually. If a title mentioned any possible indication for a GMM having an application as an agri-food product, the abstract was read. The Journal *Algal Research* was screened for relevant algal SynBio cases. Content pages of the years 2014 up to and including 2018 were screened for all four journals.

2.2.2. Inclusion criteria

In order to list relevant cases related to synthetic biology and microorganisms for application in the agri-food sector according to the EFSA remit, the following inclusion criteria were defined in close collaboration with EFSA.

Inclusion criterion 1: Products consisting or containing viable genetically modified organisms, as defined in Directive 2001/18, therefore able to replicate or transfer genetic material. The GMMs covered include Archaea, bacteria and Eukaria, as defined in the EFSA guidance 20115. Eukaria include filamentous fungi, yeast, protists and microscopic algae. For the purpose of this horizon scan and the mandate also viroïds, viruses and bacteriophages are included.

Inclusion criterion 2: Products related to 'agri-food' uses meaning agri /food /feed products falling within the remit of EFSA. Such GMMs can for example be used for:

- Human or animal consumption, e.g. food/feed products, probiotics, microbiome engineering;
- Silage agents;
- Starter cultures, fermentation agents and biocontrol in food;
- Pesticide (plant protection products (PPP) and biocides) use, e.g. PPP providing resistance or biocontrol agents;
- Biosensors, e.g. used in food contact materials;
- Fertilizers (micro-organisms that promote plant growth, providing and mobilizing nutrients or growth factors, in rhizosphere or plant tissue);
- Biocontrol agent as feed additive, e.g. modification of micro-organisms to reduce disease of honeybees;
- Miscellaneous or borderline cases. This category is used for cases that not fully fulfill the product criterion or it is doubtful that they do. Those cases are left to the consideration of the EFSA working group on synthetic biology.

Inclusion criterion 3: Product deliberately released into the environment for experimental or commercial reasons. The following terminology is used in the systematic listing of SynBio cases (see section 3). The product should be meant for deliberate release or its deliberate release is connected to its use, comprising

- experimental stages: setting of the GMM in its experimental stage cover laboratory, greenhouse, farm, mesocosm, field trial or food pilot plant. All listed cases must be past the scientific proof of concept phase that merely describe the methodology;
- placing on the market. This includes greenhouse, farm, pond, field trial, food production plants or it concerns a product that is already on the market in territories outside of Europe.

Inclusion criterion 4: possibility of reaching the EC market during the next decade.

All sources listed in section 2.2.1 were screened for relevant Synbio cases by reading the available information and having in mind the inclusion criteria 1 to 4.

2.2.3. Criteria for prioritization

To be able to give an indication for the time-period a SynBio case could most likely move to a commercial application in the agri-food sector, the technical aspects of product development were considered. The following criteria were defined and agreed in close collaboration with EFSA:

- A time-period of less than 5 years is indicated for a SynBio case that is:
  - technically ready for a commercial activity, or

[5] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2011.2193

- commercially available outside the EU.

- A time-period of less than 10 years is indicated for a SynBio case that needs additional experiments under less confined conditions to proof its usefulness.
- A time-period of more than 10 years is indicated if no estimation is possible based on the previous criteria.

These criteria are solely based on the technical advances of the product development and do not take into account the influence of societal perception on market readiness.

3. Results

The SynBio cases resulting from the search strategy are listed in the Table SynBio Developments for Microorganisms, see Appendix A. The information available from the found publication or website (see Column 15 of the datasheets) was used to collect further details on the GMM's intended use, its molecular characteristics and development phase.

In total, eleven SynBio cases were found. These cases are presented in separate datasheets of the Table SynBio Developments for Microorganisms: five cases are presented on the datasheet "Deliberate Release" listing those cases that passed the inclusion criteria. Six cases are listed on the datasheet "Questionable Cases" as they did not fully match the inclusion criteria, or the available information was insufficient to make sure that the case matched the inclusion criteria. An argumentation for placing SynBio cases on datasheet B is given in column 16 (see Appendix A).

Table 2 gives an overview of all eleven SynBio cases. Cases are named by the GMM species name that is present in the product and ordered according to the agri-food product categories. From this Table it is clear that the SynBio cases cover only a few categories of agri-food products. For the categories silage agent, starter culture, fermentation agent and biocontrol in food/feed, no SynBio cases were found.

Cases describing a GMM with a product use that does not fall in the agri-food product categories, are presented as "borderline" or "miscellaneous". A case is categorized as "borderline" when the product design or intended use could be developed for agri-food use. The category "miscellaneous" is used for cases that are suggested for getting attention of the EFSA.

Table 2: The SynBio cases are presented according to the agri-food product categories as stated in criterion 2 and are indicated by the GMM's species name. Numbers in brackets refer to the Record ID in the Table SynBio Developments for Microorganisms (Appendix A). Questionable cases (Datasheet B) that did not fit an agri-food product category are assigned to the category 'borderline' or 'miscellaneous'.

Category of agri-food product	Cases from datasheet Deliberate Release	Cases from datasheet Questionable Cases
For human consumption	*Bacteroides thetaiotaomicron* as biosensor (5)	*Saccharomyces* hybrid in beer (3); *Saccharomyces* in wine (4)
For animal consumption	*Escherichia coli* and *Salmonella* Typhimurium as "synthetic microbiome" (3)	No cases identified
Silage agent	No cases identified	No cases identified
Starter culture	No cases identified	No cases identified
Fermentation agent	No cases identified	No cases identified
Biocontrol in food	No cases identified	No cases identified
Plant protection product	Citrus tristeza virus (1)	No cases identified

Biosensor	*Bacillus subtilis* sensing meat spoilage (2)	No cases identified
Fertilizer	*Sinorhizobium meliloti* (4)	Pivot Bio Proven selected microorganisms (2)
Feed additive biocontrol agent	No cases identified	No cases identified
Borderline	No cases identified	*Escherichia coli* as therapeutic probiotic for human or animal use (1); *Metarhizium pingshaensei* for control of vectors spreading Malaria (5)
Miscellaneous	No cases identified	*Synechococcus elongates* PCC7492 biocontainment strategy in potentially biofuel producing micro-algae (6)

In the following a short description regarding the genetic modification and the intended use of the SynBio cases is presented. Additional details can be obtained from the references listed in column 15 of the Table SynBio Developments for Microorganisms (Appendix A).

SynBio cases listed in Datasheet A

The first SynBio case (Record ID 1) refers to a GM virus (Citrus tristeza virus) that was developed as a biocontrol agent against citrus greening disease. It expresses a defensin encoding gene from Spinach and is currently being tested under field conditions in the US. Though citrus greening disease does not occur in Europe, this case is considered to be indicative for the design of a GM virus as biocontrol agent against plant diseases.

The next Synbio case describes a GM Bacillus subtilis designed as a biosensor for meat spoilage (Record ID 2). The bacterium was modified with a fluorescent protein (GFP) and a host-own promoter that is specifically activated by volatiles released from spoiled meat. A proof of concept of volatile detection is based on fluorescence detection by flow cytometry. Opportunities for further development of this biosensor regarding the use of indicators visible to the naked-eye and application of the biosensor in a sealed sticker to be added to packaged meat, are described in the references (see column 15).

Escherichia coli and Salmonella Typhimurium (Record ID 3) have been genetically modified with either a signaling or responding genetic circuit derived from a natural quorum-sensing mechanism. After administering the signaling and responder strain (called 'synthetic microbiome' by the researchers) to mice, presence of anhydrotetraclin (ATC) in drinking water was visualized in fecal samples by colored colonies after plating. Based on this proof of principle that GM strains are able to communicate in the complex environment of the mammalian gut, the researchers aim to create synthetic microbiomes consisting of GM bacteria with specialized functions (e.g., detecting and curing disease, creating beneficial molecules, improving digestion, etc.) for animal consumption and on the longer term for human consumption as well.

The fourth SynBio case describes the use of the plant-symbiotic bacterium Sinorhizobium meliloti. The GM strain resulted from the insertion of the megaplasmid pSymA which is essential for symbiosis with the legume host and for bacterial fitness in the rhizosphere. The transfer of the plasmid between two S.meliloti strains was performed by conjugation, a natural process of genetic recombination and included

the use of recombinant plasmids. As a result of the additional plasmid the S. meliloti strain showed a cultivar-specific improvement of the symbiotic relation compared to the parental strains. Intended use of this bacterium is in agriculture and the case is categorized as fertilizer according to the agri-food product list.

The last SynBio case of Datasheet A describes the development of CRISPR interference technology and the use of genetic elements for Bacteroides thetaiotaomicron, a common human gut bacterium. In the current stage of development the GM strain is successfully tested in mice to detect certain stimuli in the gut. As B. thetaiotaomicron is known as a commensal of the human gut its future development as biosensor for detecting biomarkers was suggested by the researchers. Due to its functioning after oral uptake and digestion, this case is listed as a possible agri-food product for human consumption.

SynBio cases listed in Datasheet B

The first SynBio case refers to GM Escherichia coli Nissle 1917 (Record ID 1). Deletion and upregulation of some host genes resulted in a strain that, after consumption, detoxifies ammonia in the gut by converting it to L-arginine. The use of this GMM was described as a therapeutic probiotic for human use and it is already tested in a phase one clinical trial. Though medical use of GMMs had been excluded from this horizon scan, this case was listed as the researchers mentioned a possible use as feed additive i.e. for animal consumption. Based on this uncertainty in future development of the product, the case is listed as "borderline" (see column 15).

The second SynBio case describes a plant fertilizer consisting of a selection of microorganisms. Information on the bacteria species present in this product or the possible modification of their genes was not available in the listed references from commercial websites. It could not be concluded whether this product contains GMMs or not. The case is listed as it is illustrative for the agri-food category of fertilizers, that gains a lot of attention in the literature.

The next two cases are both describing the genetic modification of Saccharomyces strains for beer or wine production (Record ID 3 and 4). The beer strain has a mutation in the FDC1 gene for reducing the production of phenolic off-flavor compounds resulting in improvement of the beer taste. The wine strain was genetically modified to express a metabolic pathway for the production of raspberry ketone. Its use results in a raspberry flavor in wine. The type of agri-food use for both GMMs is indicated as for human consumption. Presence of the GMM in the final product is however questionable as there was no information available from the references (column 15) regarding the setting of the industrial fermentation process (contained or confined) and inactivation of the yeast in the final product.

The fifth SynBio case is the fungal species Metarhizium pingshaensei which is naturally pathogenic to mosquitoes. Its killing ability is enhanced by the introduction and expression of a spider neurotoxin which kills mosquitos faster and at lower spore doses than the non-modified pathogen. This SynBio case is listed as "borderline" as the GMM is a biocontrol agent against malaria-carrying mosquitoes and not for biocontrol of plant pathogens or pests. The case is however illustrative for the development of biocontrol agents for vector-borne plant diseases.

The last case refers to GM Synechococcus elongatus PCC7492, a cyanobacterium for which a biocontainment strategy was designed by the deletion of host genes and addition of genes from Pseudomonas stutzeri and Anabaena. This modification allows the utilization of phosphite as a phosphorous source and prevents the capacity to use phosphate. Phosphite in nature is scarce and thus the organisms cannot grow in absence of phosphite. Therefore, survival outside a containment or open

pond is strongly limiting for this GMM. The application of this GMM was described as a potentially biofuel producer, but not yet as an agri-food product. Therefore, this case is listed as miscellaneous.

Observations from the listed SynBio cases

Considering the category of molecular technique used (see column 8 of the Table SynBio Developments for Microorganisms in Appendix A), the category "genetic part libraries" is indicated for eight SynBio cases. In these cases the respective GMM was constructed by using a combination of genetic elements resulting in e.g. a genetic circuit or metabolic pathway. Only one SynBio case (Record ID 3, Datasheet B) describes a GM Saccharomyces strain for which the technique of "genome editing" is indicated for mutating the FDC1 gene.

For the remaining two cases none of the listed SynBio techniques was appropriate: a GM Sinorhizobium meliloti strain (Record ID 4, Datasheet A) was constructed by natural conjugation (including a recombinant plasmid) to improve the plant symbiotic relation. This technique could not be assigned to the SynBio techniques listed in column 8 of the Datasheet. In the second case, (Record ID 2, Datasheet B) the organism's identity was unsure due to a lack of information from the company regarding the bacterial identity and the genetic modification. Obviously, no technique could be assigned to this case.

The categories of SynBio techniques "use of minimal cells and designer chassis, protocells and artificial cells or xenobiology" did not apply to any of the listed SynBio cases.

Furthermore, the indication for the phase of development (column 2) reveals that all listed SynBio cases are still in an experimental phase of development, except for one. The plant fertilizer consisting of microorganisms of unknown identity and with unknown modification (as this was not specified by the company; Record ID 2, Datasheet B) is already placed on the US market.

Four cases that are in an experimental stage of development, are estimated to be ready for commercialization within five years (Record ID 2, Datasheet A; Record ID 3, 4, 5, Datasheet B). This time-period was estimated by making use of prioritization criteria (see section 2.2.2) and expert judgement. Prioritization is only related to the technical readiness and description of a proof of concept. The influence of societal perception on market readiness is not taken into account.

For the remaining six cases the available information did not present clear data suggesting a market readiness of the agri-food product. Additional experimental data beyond the laboratory level, e.g. from field trials or interaction studies with humans or animals, would be required to prove efficacy under environmental conditions. Development into a possible commercial product will need more than five years.

4. Conclusions

This horizon scan was undertaken to gather information on SynBio developments for microorganisms in the agri-food sector. The objective was to list SynBio cases describing GM microorganisms, as part of an agri-food product and that are intended for deliberate release into the environment for commercial or experimental purposes.

To this end a search strategy was set up consisting of several literature and website sources covering scientific and commercial developments. The RIVM literature collection and the selected journals (PLOS ONE Synthetic Biology and ACS Synthetic Biology) were focused on applications of synthetic biology in

a research phase. For cases closer to the market, websites demonstrating commercial activities and databases of regulatory agencies were used.

Relevant publications were selected from literature and websites with having in mind "microorganism, the agri-food product categories and an intended deliberate release of the GMM" according to the inclusion criteria. The search resulted in a list of eleven SynBio cases. The listed SynBio cases comprise GMMs that are still in an experimental phase, mostly in the laboratory. Demonstration of the intended functionality of the GMM in the laboratory is considered as a proof of concept for listing as SynBio case. It is clear that additional experiments will be required to show a proof of concept under environmental circumstances. No GMMs are listed that are currently in the pipeline for commercial purposes in Europe.

During the screening a significant number of publications was found describing the use of GMMs as therapeutic probiotic for human or animal use. Details revealed that the probiotics were mainly designed for medical use, not for agri-food use. Unless no further use as novel food or food/feed additive was mentioned, these GMMs were not listed.

A lot of research interest in the development of agri-food products is also demonstrated by the many iGEM projects (39 out of 186) describing GMMs with an intended use as plant protection products, biosensors, fertilizers and products for human or animal consumption. Only one iGEM project is listed (Record ID 2, Datasheet A), which is not surprising as iGEM projects are short-term projects and need more experimental data to demonstrate a proof of concept stage.

An additional objective of the horizon scan was to indicate a time-period for market readiness of the listed SynBio cases. This prioritization as indicated in the Table SynBio Developments for Microorganisms (Appendix A, column 5) is only based on the technical readiness and description of a proof of concept. It does not reflect the official status of the cases.

It can be argued whether the listed SynBio cases comprise GMMs constructed by techniques of synthetic biology. Most listed cases involve the insertion of one or a few genes in a viral, bacterial or fungal genome which is a straightforward technique of genetic modification. The GMM's intended use might be regarded as a new development, the applied technique of genetic modification, i.e. the use of genetic elements from one organism into another organism, is not new.

Advanced techniques of synthetic biology, like xenobiology or use of minimal cells, artificial cells and protocells, are still in an early and fundamental research phase. Successful use of these techniques for constructing a GMM that can survive outside the laboratory will need more time. Agri-food products developed through these applications were not found by this study.

Abbreviations

ATC	Anhydrotetraclin
EC	European Commission
EFSA	European Food and Safety Authority
GFP	Green Fluorescent Protein
GM	Genetically Modified
GMM	Genetically Modified Microorganisms
GMO	Genetically Modified Organisms
PPP	Plant Protection Products
RIVM	National Institute for Public Health and the Environment
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SynBio	Synthetic Biology

Appendix A – SynBio Developments for Microorganisms

Datasheet A Deliberate Release Cases

Record ID	Phase of Development	Setting of the GMM use (experimental stage)	Setting of the GMM use (on the market)	Estimated year of commercial release in the EU (Expert Judgment)	GMM - taxon	GMM - species and/or strain name	Molecular technique used (Expert Judgment)	Donor organism or artificial design of inserts	Degree of molecular modification	Description of the new trait characteristics	Commercial use	Type of agri/food/feed use falling within the remit of EFSA (Expert Judgment)	Geographic Region	Reference	Remarks
1	Experimental stage	Field trial	_	<10 years	Virus	Citrus tristeza virus	Genetic part libraries	Defensin gene from Spinach	1	Virus delivers defensin protein in citrus trees to resist/combat citrus greening disease	Biocontrol agent	Plant protection product	US	https://www.aphis.usda.gov/brs/fedregister/BRS_20170410.pdf; https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/brs-news-and-information/2018_brs_news/ctv_deis	Citrus greening disease does not occur in the EU. The case is indicative for the design of a gm virus expressing defensin, as a biocontrol agent against plant diseases.
2	Experimental stage	Laboratory	_	<5 years	Bacteria	*Bacillus subtilis*	Genetic part libraries	GFP, promoter sequences from B. subtilis	1	Detection of meat spoilage by sensing the volatiles	The biosensor is intended for use in a sealed sticker, that is added to packaged meat	Biosensor	Europe	https://pubs.acs.org/doi/10.1021/sb5000252; http://2012.igem.org/Team:Groningen	This is a 2012 iGEM project published in 2014.
3	Experimental stage	Laboratory	_	<10 years	Bacteria	*Escherichia coli, Salmonella Typhimurium*	Genetic part libraries	A signaler, responder and memory circuit consisting of genes from bacterial origin (luxI, cro, lacZ,	3	The 'synthetic microbiome' consisting of signaler and responder were administered to mice. Presence of a stimulus in drinking water	It is envisaged to create a synthetic microbiome consisting of engineered bacteria, but that also communicates with others gut bacteria to	For animal consumption	US	https://www.sciencedaily.com/releases/2018/08/180831090941.htm	The researchers aim to create on the longer term synthetic microbiomes for human consumption as well.

								promoter sequences)		was visiualized by colouring of fecal samples.	ensure that they are all balanced for optimal health.				
4	Experimental stage	Laboratory	_	<10 years	Bacteria	*Sinorhizobium meliloti*	Other technique	Insertion of a replicon from another strain of the same species	Gain of 482 genes, loss of 354 genes	Cultivar-specific improvement of symbiotic relation	Application in precision agriculture	Fertilizer	Europe	https://pubs.acs.org/doi/10.1021/acssynbio.8b00158	The hybrid strain results from natural conjugation and recombinant DNA techniques.
5	Experimental stage	Laboratory	_	<10 years	Bacteria	*Bacteroides thetaiotaomicron*	Genetic part libraries	Several sets of genetic circuits	Bacterial genes, CRISPRi technology	Common human gut bacterium programmed to sense, memorize and respond to signals in the gut	Use as a sensor for disease markers or biomarkers is envisaged. Here a general functionality is shown in gut of mice	For human consumption	US	https://www.sciencedirect.com/science/article/pii/S240547121500006X; https://www.pnas.org/content/111/13/4838	The second link describes a similar strategy while using Escherichia coli.

–: no information available

Datasheet B Questionable Cases

Record ID	Phase of Development	Setting of the GMM use (experimental stage)	Setting of the GMM use (on the market)	Estimated year of commercial release in the EU (Expert Judgment)	GMM - taxon	GMM - species and/or strain name	Molecular technique used (Expert Judgment)	Donor organism or artificial design of inserts	Degree of molecular modification	Description of the new trait characteristics	Commercial use	Type of agri/food/feed use falling within the remit of EFSA (Expert Judgment)	Geographic Region	Reference	Remarks
1	Experimental stage	Field trial	_	<10 years	Bacteria	Escherichia coli Nissle 1917, SYNB1020	Genetic part libraries	Deletion and upregulation of host genes	2	Detoxification of ammonia in blood. Data show improvement hyperammonemia and survival in mice. Dose-dependent exposure is	GMM wil be administered as probiotic	Borderline	US	https://stm.sciencemag.org/content/11/475/eaau7975	The authors mention the use of this GMM as feed additive /for animal use. The publication however describes application for human health, being a therapeutic, which doesnot fall in the EFSA remit. Remark to column C:

										shown in healthy humans.					with field trial it is meant here a phase one clinical trial.
2	Placing on the market	–	On the market outside EU	<5 years	–	*Pivot Bio PROVEN™, selected soil microbes attach to plant roots*	–	–	–	Enhanced ability to release nitrogen to the plant roots - it is unclear whether this is achieved by genetic modification	Nitrogen fertilizer for application in corn	Fertilizer	US	https://synbiobeta.com/why-pivot-bios-new-field-data-is-a-boon-for-sustainable-agriculture; https://synbiobeta.com/pivot-bio-proven-outperforms-traditional-fertilizer; https://synbiobeta.com/pivot-bio-re-awakening-the-agricultural-microbiome/	The identification of bacteria is not presented by the company.
3	Experimental stage	Laboratory	–	<5 years	Yeast	*Saccharomyces cerevisiae x Saccharomyces eubayanus hybrid*	Genome editing	Gene of host origin	1	Mutation in FDC1 gene improves the taste of beer	Industrial application, production of lager beer	For human consumption	Europe	https://doi.org/10.1371/journal.pone.0209124	Questionable due to unknown industrial fermentation process and absence/presence of living GMM in final product.
4	Experimental stage	Laboratory	–	<5 years	Yeast	*Saccharomyces cerevisiae*	Genetic part libraries	Plant derived metabolic pathway	4	Raspberry ketone (flavour) production by wine yeast: proof of principle under wine-making conditions	Industrial application, wine production	For human consumption	Australia	https://www.ncbi.nlm.nih.gov/pubmed/26944880	Questionable due to unknown industrial fermentation process and absence/presence of living gmm in final product
5	Experimental stage	Greenhouse	–	<5 years	Filamentous fungi	*Metarhizium pingshaense i*	Genetic part libraries	Toxin encoding genes, promoter specifically expressing genes in blood of insect	A few genes originating from spiders and scropions	The fungus is mosquitoe specific, its natural killing ability is enhanced by introduction of toxin encoding genes	Fungal spores are sprayed in the field	Borderline	–	https://www.nature.com/articles/s41598-017-03399-0	This GMM does not meet inclusion criterion 2 (it is a biocontrol agent, but not for food/feed application). It is listed as it might be an example of a future plant protection product (against vector-borne plant disease).
6	Experimental stage	Laboratory	–	<10 years	Microscopic algae	*Synechococcus elongatus PCC7492*	Genetic part libraries	Phosphite assimilating and transporter genes (Pseudomo	2 genes inserted, 2 genes	Algal growth dependency on phosphite which is scarce in nature	Biocontainment strategy for outdoor cultivation	Miscellaneous	Japan	https://pubs.acs.org/doi/10.1021/acssynbio.8b00199	This SynBio case has no agri/food use yet.

								nas stutzeri, Anabaena)	knock ed-out						

Appendix B – List of Reports

Reports related to synthetic Biology that were screened for this horizon scan:

Synthetic Biology in Australia: an outlook to 2030. Report for the Australian Council of Learned Academies. P. Gray, S. Meek, P. Griffiths, J. Trapani, I. Small, C. Vickers, C. Waldby, R. Wood. 2018. Available from: https://acola.org.au/wp/wp-content/uploads/HS3_SynBiology_WEB_180819.pdf

Synthetic Biology. Second Interim Report of the German Central Committee on Biological Safety. 2018. Available at: https://www.zkbs-online.de/ZKBS/SharedDocs/Downloads/02_Allgemeine_Stellungnahmen_englisch/01_general_subjects/2nd%20report%20Synthetic%20Biology%20(2018).html?nn=11794948

Dealing with Risks of Biotechnology: Understanding the Potential of Safe-by-Design. Z. Robaey. 2018. Available at: https://www.scribd.com/document/397357715/Dealing-with-Risks-of-Biotechnology-Understanding-the-Potential-of-Safe-by-Design

Assessment of human health and environmental risks of new developments in modern biotechnology. Policy report. P.A.M. Hogervorst, H.C.M. van den Akker, D.C.M. Glandorf, P. Klaassen, C.J.B. van der Vlugt, J. Westra. RIVM Letter report 2018-0089. DOI 10.21945/RIVM-2018-0089. Available from: https://biotechnologie.rivm.nl/sites/default/files/2018-07/2018-0089%20Assessment%20of%20human%20health%20and%20environmental%20risks%20of%20new%20developments%20in%20modern%20biotechnology.pdf

National Academies of Sciences Engineering and Medicine. Preparing for Future Products of Biotechnology. Washington, DC: The National Academies Press; 2017. Available at: http://www.nap.edu/24605.

Future introductions of genetically modified microbial biocontrol agents in the EU - Are current EU legislation and risk assessment fit for purpose? RIVM Letter report 2016-0057. Scheepmaker JWA, Hogervorst PAM, Glandorf DCM. Available from: https://www.rivm.nl/publicaties/future-introductions-of-genetically-modified-microbial-biocontrol-agents-in-eu-are

Commission on Genetic Modification (COGEM), Health Council of the Netherlands. Trend Analysis Biotechnology 2016, A regulatory disconnect. Bilthoven: COGEM; 2016. CGM/160614-01. Available from: https://www.cogem.net/index.cfm/en/publications/publication/trend-analysis-biotechnology-2016.

Synthetic Biology. Technical Series No. 82. Secretariat of the Convention on Biological Diversity. 2015. Available from: https://www.cbd.int/doc/publications/cbd-ts-82-en.pdf

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Consumer Safety (SCCS). 2014. Synthetic Biology I Definition, Opinion. Available from: https://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_044.pdf

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Consumer Safety (SCCS). 2015. Synthetic Biology II - Risk assessment methodologies and safety aspects, Opinion. Available at: https://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_048.pdf

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Consumer Safety (SCCS). 2015. Final Opinion on Synthetic Biology III - Risks to the environment and biodiversity related to synthetic biology and research priorities in the field of synthetic biology. Available at:

https://ec.europa.eu/health/sites/health/files/scientific_committees/emerging/docs/scenihr_o_050.pdf

Bergmans H, Vennekens W. Analysis of new developments in white (industrial) biotechnology. Ameco; 2016. Available at: https://biotechnologie.rivm.nl/sites/default/files/2017-11/Biotechnology-and-Safety-Call-2_call-Analysis-of-new-developments-in-white%20biotechnology_April%202016_RIVM_Ameco.pdf