TESTBIOTECH Background 08 - 04 - 2024

Input to the public consultation on the "Scientific Opinion on New developments in biotechnology applied to microorganisms"

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Summary

The draft opinion issued by EFSA (2024) correctly identifies several deficiencies in current guidance for the risk assessment of genetically engineered (GE) microorganisms. New genomic techniques (NGTs, new genetic engineering) and synthetic biology will enable companies to develop a broad range of GE microorganisms. Therefore, the existing EFSA guidances should undergo an in-depth evaluation in regard to its ability to meet upcoming challenges.

The EFSA (2024) draft opinion only deals with one specific sector, i. e. products currently being developed for use in agriculture and food production. Therefore, further opinions will be needed, e.g. for applications other than for agriculture and food production such as with non-domesticated species or GE microorganisms intended to change the composition of the microbiome in mammals for purposes other than food and feed, as these may also potentially impact food safety.

Furthermore, it does not include important applications within the food production sector, e.g. microbial pesticides, paratransgenesis and application of viruses or synthetic communities of microbes.

Further consideration is also needed in regard to contamination with GE microorganisms (or specific nucleotides) not meant for food production. It should be noticed that Fraiture et al. (2024) found intact antibiotic resistance genes in almost 48% of the enzyme preparations they tested. Since these genes can be transferred to pathogens or microorganisms in the intestinal microbiome, contamination with DNA from GMMs should fall under the GMO Regulation 1829/2003 and should be taken into account by the final opinion of EFSA.

Other considerations in this respect include the concept of the holobiont, the impact of artificial intelligence (AI), the assessment of cumulative effects or interactions and the introduction of cut-off criteria needed in cases where there are too many uncertainties or unknowns.

In addition, it is important to integrate a requirement for data on the phenotypic characteristics of GE microorganisms at each step of risk assessment.

It should be made clear that all microorganisms derived from EGTs or NGTs must be subjected to mandatory risk assessment, as required by current regulation. These microorganisms should be defined as genetically engineered (GE). There does not appear to be any necessity to change current regulatory framework in order to deal with these microorganisms, as the processes used to generate GE microorganisms may be associated with intended or unintended effects unlikely to occur with conventional mutagenesis or similar.

In addition, other microorganisms derived from non-regulated techniques, which have traits or specific characteristics not previously present in the ecosystems / food production, should also undergo mandatory risk assessment. An additional register and decision-making process should be established for this purpose.

Furthermore, risk management recommendations should include measures and methodologies allowing the overall control of the scale of releases into a given receiving environment. This should further include measures to reduce the number and scale of releases.

1.2 Definition of new developments in biotechnology for the Terms of Reference

The definition should be reconsidered: in many cases, applications involving transgenic organisms (that may be derived from EGTs, e.g. Lovett et al. 2019; Leonard et al. 2020; Katac et al., 2023), cannot generally be regarded as ‘established’ (see also EFSA, 2020). Therefore, not only new technologies, but also the fields of application need to be considered in the context of new developments in biotechnology applied to microorganisms. EFSA did not take this into account in its draft opinion (2024) and the document is, therefore, based on misconceptions.

1.4 General outline of risk assessment for genetically modified microorganisms

General comments:
The title of the opinion should make it very clear that it is directed solely at applications in food production and agriculture. Consistency should be established throughout the text, e.g. if biomass-production is mentioned, this should be qualified.

It should further be mentioned that an additional opinion is necessary to include other applications, such as (i) GE microorganisms used in, e.g. biodegradation, waste treatment and bioremediation; (ii) genetic engineering of microorganisms associated with or targeting non-domesticated species (such as insects); (iii) potential releases of GE microorganisms (including viruses) meant to change, e.g. the characteristics of non-domesticated plants and animals or (iv) applications of GE microorganisms meant to change the microbiome of epithelial cells in mammals with potentially therapeutic effects.

It should be made clear that all microorganisms obtained from EGTs or NGTs must be subject to mandatory risk assessment, as required by current regulation. These microorganisms should be defined as genetically engineered (GE). In addition, other microorganisms derived from non-regulated techniques with traits or specific characteristics not previously present in the ecosystems / food production, should also undergo mandatory risk assessment. An additional register and decision-making process should be established for this purpose.

The opinion should further take the concept of the holobiont into account: many microorganisms are closely associated with other species and are often considered to be ‘hosts’. Therefore, these biological systems cannot be assessed simply by looking at individual bits and pieces in isolation; they all have to be considered as part of larger units known as holobionts or hologenomes. Assessment must take into account that all species in the same habitat interact and influence each other (see, for example, Richardson, 2017; Sanchez-Canizares, 2017). EFSA did not include this concept, and therefore any mention in the draft opinion of possible interactions remains fragmentary.

Molecular characterisation should:
- require the identification of intended and untended genetic changes caused by the specific techniques that may otherwise be unlikely to occur.
- take into account the potential presence of artificial guide RNAs that may trigger activities of the CRISPR mechanisms that naturally occur in the cells.
- include empirical data on potential changes in the biology of the genetically engineered microorganisms, e.g. in regard to gene expression, proliferation, horizontal gene transfer, population dynamics.

Food and feed health risk assessment should:
- include empirical data on the phenotypic characteristics of genetically engineered microorganisms, e.g. in regard to proliferation, horizontal gene transfer, mobility, population dynamics, potential for colonisation, persistence and spread;
- require assessment of cumulative effects and potential interactions with other GE microorganisms present in the food chain;
- consider health risk assessment of GE microorganisms (or specific nucleotides) that may contaminate the food chain, without being intended for consumption (see for example Fraiture et al., 2024).

Environmental risk assessment should:
- include empirical data on the phenotypic characteristics of genetically engineered microorganisms, e.g. in regard to proliferation, horizontal gene transfer, mobility, population dynamics, potential for colonisation, persistence and spread. For example, in 2020, EFSA identified the following risks as being relevant: "*Altered efficacy can be related to levels of exposure by e.g. 1) increased environmental survival and host colonisation, 2) increased invasiveness and, 3) increased competition in naturally evolved microbial communities due to enhanced fitness, thereby displacing beneficial microorganisms or disrupting, 4) altered metabolism, e.g. by changes in substrate utilisation opening new environmental niches; 5) altered lifestyle, e.g. by energy use (aerobic versus anaerobic) opening new environmental niches."*
- specifically require the assessment of potential impacts which changes in symbiotic microbiomes (such as those associated with fungi, lichens, plants, animals, humans, in the soil, above soil or within aquatic systems) may have by taking the concept of the holobiont into account;
- include the assessment of cumulative effects and potential interactions with other GE organisms sharing the same receiving environment;
- consider risk assessment of GE microorganisms (or nucleotides) that may contaminate the fields and /or the food chain, but are not meant for food production (see for example Fraiture et al., 2024);
- define cut-off criteria in order to make adequate decisions if too many unknowns and uncertainties emerge (Then et al., 2020). As EFSA states (EFSA, 2020): "*Even with the complete genetic information of a synthetic microorganism, it is beyond the capacity of any existent bioinformatic analysis to fully predict the capability of a synthetic organism to survive, colonise and interact with other organisms under natural conditions, given the uncountable diversity of potential microhabitats and their temporal variability.*"

Risk management recommendations: Risk management should not only include monitoring, but also measures and methodologies that allow control of the overall scale of releases within a given receiving environment. Risk management tools should be established to allow control of, e.g. the duration of releases, the number of organisms per species, the number of species, the combination of traits (by parallel releases) and, if required, a reduction in the number and scale of releases.

2.4 Selection and description of the case studies

The case studies should be broadened to include:
- GE microorganisms (especially viruses) that may be released in genetically engineered plant populations or other organisms in and around the fields (Eckerstorfer et al., 2024)
- Introduction of whole sets synthetic microbial communities (de Souza et al., 2020; Rubin et al., 2022; Ali et al., 2023).

- GE microorganisms (including viruses) that may be released as plant protecting effectors, or as ‘bio-pesticides’ in and around the fields, or intended to control parasites (Huang et al., 2023; Lang et al., 2023; Lovett & Leger 2017; Zhao et al., 2016; Eckerstorfer et al., 2024)
- GE microorganisms inheriting specific systems meant to change, e.g. the characteristics of plants, animals or other microorganisms, thus involving specific vectors, (see, for example, Reeves et al., 2018), or mobile genetic elements (artificial transposons), or gene drive-like mechanisms (see, for example, patent application WO2016177682 which includes artificial mobile genetic elements).
- GE microorganisms (or nucleotides) that may contaminate the food chain or agricultural production, without these being meant for food production or consumption (see, for example, patent application WO2016177682 which includes antibacterial GE microbes for the protection of surfaces, substrates and fluids or contaminations as found by Fraiture et al., 2024);
- GE microorganisms meant to change the characteristics of a host (like honey bees) via paratransgenesis (Wilke et al., 2025; Leonard et al., 2020) or other specific host-related mechanisms;
- GE microorganisms meant to change the composition of the gut microbiome (Bai et al., 2023) or control bacteria on epithelial cells (WO2017112620, WO2017087909), which may have therapeutic properties, but could also contaminate or otherwise impact and change microbiomes beyond the intended purpose (WO2017087909, describing processes to control mastitis in dairy).

Furthermore, it should be taken into account that some of these applications involve microorganisms developed from EGTs, although their application in the environment cannot be regarded as ‘established’ (see for example Lovett & Leger 2017). Therefore, not only new technologies, but also the fields of application have to be considered in this context.

Further considerations should be given to:
- the impact of (generative) AI on the future development of microorganisms (Helmy et al., 2020; Radivojević T. et al., 2020; Noshay et al., 2023);
- how rapidly the various GE microorganisms are introduced into a shared receiving environment and /or the food chain. This should include their potential interactions and potential cumulative impact on health and the environment (as discussed by Koller et al, 2023);
- GE microorganisms (or nucleotides) that may interfere with food and feed safety, but are not intended for use in food production.

3.1.1 AQ1. What are the new techniques/approaches developed since 2001 (namely, new developments in biotechnology) which could be applied/are applied to microorganisms?

The opinion should be broadened in regard to:
- delivery techniques involving specific vectors, mobile genetic elements (artificial transposons) or gene drive-like mechanisms (see, for example, patent application WO2016177682);
- applications meant to change the characteristics of a host (like honey bees) via GE microorganisms (see, for example, Leonard et al., 2020);
- use of (generative) AI for the development of microorganisms with completely novel characteristics (Helmy et al., 2020; Radivojević T. et al., 2020; Noshay et al., 2023).

3.1.2 AQ2. Are there any novel hazards that these new developments in biotechnology applied to microorganisms could pose to humans, animals and the environment, as compared to established genomic techniques and conventional mutagenesis?

The opinion did not identify any new potential technology-related hazards for humans, animals or the environment caused by NGT applications in microorganisms in comparison to EGTs or conventional mutagenesis. This needs to be corrected as GE may overcome natural mechanisms of genome organisation in microorganisms, thus causing intended or unintended effects that are otherwise unlikely to occur:

Complex factors in the genome organisation of, e.g. bacteria (but also of archeae and viruses), are known to make some genetic changes much more likely than others. These are understood as factors in genome organisation which stabilise bacterial genomes in some genomic regions, whilst other regions undergo changes much more frequently (see Lee et al., 2012; Fang et al., 2008; Martincorena et al., 2012).

For example, essential genes are very often organised in clusters located at greater distances to other regions which have a higher frequency of mutation (Fang et al., 2008; Hosseini & Wagner, 2018). Repair processes which occur more frequently in some genomic regions also seem to play a crucial role in this context (Costantino & Court, 2003; Jee et al., 2016.)

These effects are also relevant to risk assessment: for example, specific gene deletions may cause bacteria to become more adapted, more virulent or pathogenic under specific environmental conditions (see, for example, Albalat & Cañestro, 2016). At the same time, the frequency of gene deletions is not randomly distributed throughout the genome (Hosseini & Wagner, 2018).

The mechanisms behind these occurrences are not fully understood. However, it is a known fact that they do occur, thus making it necessary to use risk assessment to address intended and unintended genetic changes caused by specific techniques (such as NGTs), which are otherwise unlikely to happen. In this context, empirical data on the phenotypic characteristics of the genetically engineered microorganisms must be taken into account, e.g. in regard to proliferation, horizontal gene transfer, mobility, population dynamics, potential for colonisation, persistence and spread.

Furthermore, the technical potential of NGTs means that various microorganisms with completely novel characteristics may be released into the environment or used in food production, both very quickly and in large quantities. Therefore, if EGTs are to be compared to NGTs, the speed of development has to be taken into account for the assessment of cumulative aspects and overall exposure within a given period of time.

3.1.3 AQ3. Are there any novel risks that these new developments in biotechnology applied to microorganisms could pose to humans, animals and the environment, as compared to established genomic techniques and conventional mutagenesis?

EFSA is wrong in assuming that no novel risks and potential hazards of microorganisms derived from NGTs can be identified in comparison to those derived from EGTs or conventional mutagenesis (see comment to point 3.1.2).

3.2.1 AQ1 and AQ2. What kind of GM microorganisms and GM microbial products within the EFSA remit have been identified and can be expected in the next 10 years that were developed using new developments in biotechnology?

This question is misleading as it suggests the opinion covers all identified genetically engineered microorganisms and products derived from new advancements in biotechnology, which are expected to fall within the remit of EFSA and developed within the next 10 years. However, the opinion and the chosen examples are only applicable in the context of agriculture and food production. Therefore, an additional opinion is needed to include other applications of (i) GE microorganisms, e.g. for use in biodegradation, waste treatment and bioremediation (see for example Rafeeq et al., 2023); (ii) genetic engineering of associated microorganisms or targeting non-domesticated species (such as insects; see for example Katac et al., 2023); (iii) potential releases of GE microorganisms (including viruses) meant to change, e.g. the characteristics of non-domesticated plants and animals (see for example Eckerstorfer et al., 2024; Lentzos etal ., 2022) or (iv) applications of GE microorganisms meant to change the microbiome of epithelial cells in mammals, and thus achieve therapeutic effects (Bai et al., 2023; WO2017087909).

In addition, the cases chosen for the food production sector do not cover the full range of relevant applications (see comment to points 2.4 and 3.1.1).

Therefore, the draft opinion is not conclusive in regard to the selected case studies and the assessment based on existing risk assessment guidelines.

3.2.3.1 Comparative approach: use of a comparator

The EFSA guidance (2011) lacks essential criteria needed to perform comparative risk assessment of the phenotype of the GE microorganisms in regard to molecular, microbial and phenotypic characterisation.

The molecular characterization should:
- require the identification of intended and untended genetic changes caused by the specific techniques, which may otherwise be unlikely to occur.
- take into account the potential presence of artificial guide RNAs that may trigger activities of the CRISPR mechanisms that naturally occur in the cells.
- include empirical data on potential changes in the overall biology of the genetically engineered microorganisms, e.g. in regard to proliferation, horizontal gene transfer, mobility, population dynamics, potential for colonisation, persistence and spread.

For example, Case Study 1 would require an assessment of the potential for nitrogen-fixing bacteria to persist and spread in the environment as well as their potential to colonise other plant species (including weeds). Therefore, data would be needed to compare the phenotypic characteristics of the GE bacteria to the wildtype. Consequently, the existing guidance needs to be extensively amended.

In addition, Case Study 2 would require an assessment of changes in the interactions between host and endophyte. In addition, data should be required on the ability of the endophyte to persist and spread in the environment as well as on its potential to colonise other plant species (including weeds). Consequently, this would require the existing guidance to be extensively amended, as no such parameters are required under current EFSA (2011) comparative approach.

3.2.3.2 Microbial characterisation

The microbial characterisation should:
- require the identification of intended and untended genetic changes caused by the specific techniques, which may otherwise be unlikely to occur.
- include empirical data on potential changes in the overall biology of the genetically engineered microorganisms, e.g. in regard to proliferation, mobility, population dynamics, potential for colonisation, persistence and spread.

It should be made clear the that QPS-concept (qualified presumption of safety) cannot be applied at the level of genomic data, as this would require data on potential phenotypic changes in GE microorganisms compared to the wild type.

Therefore, the current guidelines have to be extensively amended.

3.2.3.3 Information relating to the manufacturing process and product specifications

The current guidelines for the use of Category 3 GE microorganisms do not require the assessment of, for example, enzymatic activity in the final products, e.g. bread or beer. Example 10 seems to be the only case to include enzymatic activity resulting from the genetic engineering process. However, enzymatic activity is relevant to many food production processes. The current guidance should be amended to include the assessment of enzymatic activity in the final product if it is derived from GE microorganisms.

3.2.3.4 Compositional analysis

Data on the phenotypic characteristics of the GE microorganisms is needed to perform comparative risk assessment (see comments on point 3.2.3.1 and 3.2.3.2). The compositional analysis is not sufficient to draw any conclusions at this stage of risk assessment. Therefore, the current guidelines have to be extensively amended.

3.2.3.10 Potential environmental impact of GMMs and their products

Environmental risk assessment should:
- include empirical data on the phenotypic characteristics of the genetically engineered microorganisms, e.g. in regard to proliferation, horizontal gene transfer, mobility, population dynamics, potential for colonisation, persistence and spread; for example, in 2020, EFSA identified the following risks as relevant: *"Altered efficacy can be related to levels of exposure by e.g. 1) increased environmental survival and host colonisation, 2) increased invasiveness and, 3) increased competition in naturally evolved microbial communities due to enhanced fitness, thereby displacing beneficial microorganisms or disrupting, 4) altered metabolism, e.g. by changes in substrate utilisation opening new environmental niches; 5) altered lifestyle, e.g. by energy use (aerobic versus anaerobic) opening new environmental niches."*
- specifically require the assessment of potential impacts of changes in symbiotic microbiomes (such as those associated with fungi, lichens, plants, animals, humans, in the soil, above soil or within aquatic systems) by taking the concept of the holobiont into account;
- include the assessment of cumulative effects and potential interactions with other GE organisms sharing the same receiving environments;

- consider risk assessment of GE microorganisms (or specific nucleotides) that may contaminate the fields and /or the food chain, but are not meant for food production (se for example Fraiture et al., 2024);
- define cut-off criteria to enable adequate decisions to be taken in cases where there are too many unknowns and uncertainties (Then et al., 2020). As EFSA states (EFSA, 2020): "*Even with the complete genetic information of a synthetic microorganism, it is beyond the capacity of any existent bioinformatic analysis to fully predict the capability of a synthetic organism to survive, colonise and interact with other organisms under natural conditions, given the uncountable diversity of potential microhabitats and their temporal variability.*"

3.3.2.11 HGT

For NGT-Ms in which sequences have been deleted, EFSA suggests that assessment of HGT would not be considered applicable, as there is no new genetic information which can be transferred to other microorganisms. However, this is not entirely correct since deletions of gene sequences may favour the likelihood of HGT in other gene sequences.

EFSA proposes that for non-living NGT-Ms (Category 3), assessment of HGT would only be needed for acquired ARM gene(s) because of potential environmental selection after transfer. However, this is not correct. As, for example, Case Study 7 shows, the assessment of HGT for Category 3 microorganisms is highly relevant in relation to other newly introduced DNA sequences.

EFSA should also take into account that microorganisms can exchange a wide range of ncRNAs such as recently detected 'obelisks' (Zheludev et al., 2024).

3.3.2.12 PMEM

Risk management should not only include monitoring, but also measures and methodologies that allow control of the overall scale of releases within a given receiving environment. Risk management tools should be established which allow control over, e.g. the duration of releases, the number of organisms per species, the number of species, the combination of traits (by parallel releases) and, where required, the reduction in number and scale of releases.

3.3.3 Future recommendations

EFSA is wrong in stating that the processes to establish new traits in microorganisms would not be relevant to risk assessment (see point 3.1.2). Therefore, it should be made clear that all microorganisms derived from EGTs or NGTs must be subject to mandatory risk assessment, as required by current regulation. These microorganisms should be regarded as genetically engineered (GE).

In addition, other microorganisms derived from non-regulated techniques which have traits or specific characteristics not previously present in ecosystems / food production should undergo mandatory risk assessment. An additional register and decision-making process should be established for this purpose.

In addition, a further opinion is needed to include further applications of (i) GE microorganisms for, e.g. use in biodegradation, waste treatment and bioremediation; (ii) genetic engineering of microorganisms associated with or targeting non-domesticated species (such as insects); (iii) potential releases of GE microorganisms (including viruses) meant to change, e.g. the

characteristics of non-domesticated plants and animals, or (iv) applications of GE microorganisms meant to change the composition of the microbiome in mammals for purposes other than food and feed, but with potential impact on food safety.

References

Albalat R., Cañestro C. (2016) Evolution by gene loss. Nat Rev Genet 17: 379–391 . https://doi.org/10.1038/nrg.2016.39

Ali S., Tyagi A., Mir R.A., Rather I.A., Anwar Y., Mahmoudi H. (2023) Plant beneficial microbiome a boon for improving multiple stress tolerance in plants. Front Plant Sci, 14: 1266182. https://doi.org/10.3389/fpls.2023.1266182

Bai X., Huang Z., Duraj-Thatte A.M., Ebert M.P., Zhang F., Burgermeister E., ... & Zuo T. (2023) Engineering the gut microbiome. Nat Rev Bioeng, 1(9): 665-679. https://doi.org/10.1038/s44222-023-00072-2

Costantino N. & Court D.L. (2003) Enhanced levels of lambda red-mediated recombinants in mismatch repair mutants. Proc Natl Acad Sci USA, 100: 15748-15753. https://doi.org/10.1073/pnas.2434959100

de Souza R.S.C., Armanhi J.S.L., Arruda P. (2020) From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. Front Plant Sci, 11: 1179. https://doi.org/10.3389/fpls.2020.01179

Eckerstorfer M.F., Dolezel M., Miklau M., Greiter A., Heissenberger A., Engelhard M. (2024) Scanning the horizon for environmental applications of genetically modified viruses reveals challenges for their environmental risk assessment. Int J Mol Sci, 25: 1507. https://doi.org/10.3390/ijms25031507

EFSA (2011) Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA J, 9(6): 2193. https://doi.org/10.2903/j.efsa.2011.2193

EFSA (2020) Scientific Opinion on the evaluation of existing guidelines for their adequacy for the microbial characterisation and environmental risk assessment of microorganisms obtained through synthetic biology. EFSA J, 18(10): 6263. https://doi.org/10.2903/j.efsa.2020.6263

EFSA (2024) Scientific Opinion on New developments in biotechnology applied to microorganisms. Draft for public consultation, https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0lTk000000C3VB/pc0848

Fang G., Rocha E., Danchin A. (2008) Persistence drives gene clustering in bacterial genomes. BMC Genomics, 9(4): 1-14. https://doi.org/10.1186/1471-2164-9-4

Fraiture M-A., Gobbo A., Guillitte C., Marchesi U., Verginelli D, De Greve J., D'aes J., Vanneste K., Papazova N., Roosens N.H.C (2024) Pilot market surveillance of GMM contaminations in alpha-amylase food enzyme products: A detection strategy strengthened by a newly developed qPCR method targeting a GM Bacillus licheniformis producing alpha-amylase. Food Chemistry: Molecular Sciences, 8: 100186. https://doi.org/10.1016/j.fochms.2023.100186.

Helmy M., Smith D., Selvarajoo K. (2020) Systems biology approaches integrated with artificial intelligence for optimized metabolic engineering, Metab Eng Commun, 11: e00149. https://doi.org/10.1016/j.mec.2020.e00149

Hosseini S.-R. & Wagner A. (2018) Genomic organization underlying deletional robustness in bacterial metabolic systems. Proc Natl Acad Sci USA, 115(27): 7075–7080. https://doi.org/10.1073/pnas.1717243115

Huang Q., Lariviere P.J., Powell J.E., Moran N.A. (2023) Engineered gut symbiont inhibits microsporidian parasite and improves honey bee survival. Proc Natl Acad Sci USA, 120(25): e2220922120. https://doi.org/10.1073/pnas.2220922120

Jee J., Rasouly A., Shamovsky L., Akivis Y., Steinman, S.R., Mishra, B., Nudler, E. (2016) Rates and mechanisms of bacterial mutagenesis from maximum-depth sequencing. Nature, 534: 693-696. https://doi.org/10.1038/nature18313

Katak R.d.M., Cintra A.M., Burini B.C., Marinotti O., Souza-Neto J.A., Rocha E.M. (2023) Biotechnological potential of microorganisms for mosquito population control and reduction in vector competence. Insects,14: 718. https://doi.org/10.3390/insects14090718

Koller F., Schulz M., Juhas M., Bauer-Panskus A., Then C. (2023) The need for assessment of risks arising from interactions between NGT organisms from an EU perspective. Environ Sci Eur, 35(1), 27. https://doi.org/10.1186/s12302-023-00734-3

Lang H., Wang H., Wang H., Zhong, Z., Xie, X., Zhang, W., ... & Zheng, H. (2023) Engineered symbiotic bacteria interfering Nosema redox system inhibit microsporidia parasitism in honeybees. Nat Commun 14: 2778. https://doi.org/10.1038/s41467-023-38498-2

Lee H., Popodi E., Tang H., Foster P.L. (2012) Rate and molecular spectrum of spontaneous mutations in the bacterium Escherichia coli as determined by whole-genome sequencing. 109(41): E2774-E2783. https://doi.org/10.1073/pnas.121030910

Lentzos F., Rybicki E.P., Engelhard M., Paterson P., Sandholtz W.A., Reeves G. (2022) Eroding norms over release of self-spreading viruses, Science, 375 (6576): 31-33. https://doi.org/10.1126/science.abj5593

Leonard S.P., Powell E., Perutka J., Geng P., Heckmann L.C., Horak R.D., Davies B.W., Ellington A.D., Barrick J.E., Moran N.A. (2020) Engineered symbionts activate honey bee immunity and limit pathogens. Science, 367: 573-576. https://doi.org/10.1126/science.aax9039

Lovett B., Leger R.J.S. (2017) Genetically engineering better fungal biopesticides. Pest Manag Sci, 74(4): 781-789. https://doi.org/10.1002/ps.4734

Lovett B., Bilgo E., Millogo S.A., Ouattarra A.K., Sare, I., Gnambani E.J., Dabire R.K., Diabate A., Leger, R.J.S. (2019) Transgenic Metarhizium rapidly kills mosquitoes in a malaria-endemic region of Burkina Faso. Science, 364(6443): 894-897. https://doi.org/10.1126/science.aaw8737

Martincorena I., Seshasayee A.S.N., Luscombe N.M. (2012) Evidence of non-random mutation rates suggests an evolutionary risk management strategy. Nature, 485(9): 95-98. https://doi.org/10.1038/nature10995

Noshay J.M., Walker T., Alexander W.G. Klingeman D.M., Romero J., Walker A.M., Prates E., Eckert C., Irle S., Kainer D., Jacobson D.A. (2023) Quantum biological insights into CRISPR-Cas9 sgRNA efficiency from explainable-AI driven feature engineering. Nucleic Acids Res, 51(19): 10147–10161. https://doi.org/10.1093/nar/gkad736

Radivojević T., Costello Z., Workman K., Garcia Martin H. (2020) A machine learning Automated Recommendation Tool for synthetic biology. Nat Commun, 11: 4879. https://doi.org/10.1038/s41467-020-18008-4

Rafeeq H., Afsheen N., Rafique S., Arshad A., Intisar M., Hussain A., Bilal M., Iqbal H.M.N. (2023) Genetically engineered microorganisms for environmental remediation. Chemosphere, 310: 136751. https://doi.org/10.1016/j.chemosphere.2022.136751

Reeves R.G., Voeneky S., Caetano-Anolles D., Beck F., Boete C. (2018) Agricultural research, or a new bioweapon system? Science, 362(6410): 35-37. https://doi.org/10.1126/science.aat7664

Richardson L.A. (2017) Evolving as a holobiont. PLoS Biol 15(2): e2002168. https://doi.org/10.1371/journal.pbio.2002168

Ronda C., Chen S.P., Cabral V., Yaung S.J., Wang H.H. (2019) Metagenomic engineering of the mammalian gut microbiome *in situ*. Nat Methods,16(2): 167-170. https://doi.org/10.1038/s41592-018-0301-y

Rubin B.E., Diamond S., Cress B.F., Crits-Christoph A., Lou Y.C., Borges A.L., ... & Doudna J.A. (2022) Species- and site-specific genome editing in complex bacterial communities. Nat Microbiol 7: 34-47. https://doi.org/10.1038/s41564-021-01014-7

Sanchez-Canizares C., Jorrín B., Poole P.S., Tkacz A. (2017) Understanding the holobiont: the interdependence of plants and their microbiome. Curr Opin Microbiol, 38: 188-196. https://doi.org/10.1016/j.mib.2017.07.001

Then C., Kawall K., Valenzuela N. (2020) Spatio-temporal controllability and environmental risk assessment of genetically engineered gene drive organisms from the perspective of EU GMO Regulation. Integr Environ Asses, 16(5): 555-568. https://doi.org/10.1002/ieam.4278

Wilke A.B., Marrelli M.T. (2015) Paratransgenesis: a promising new strategy for mosquito vector control. Parasit Vectors, 8: 342. https://doi.org/10.1186/s13071-015-0959-2

Zhao H, Lovett B, Fang W. (2016) Genetically engineering entomopathogenic fungi. Adv Genet, 94: 137-163. https://doi.org/10.1016/bs.adgen.2015.11.001

Zheludev I.N., Edgar R.C., Lopez-Galiano M.J., de la Peña M., Babaian A., Bhatt A.S., Fire A.Z. (2024) Viroid-like colonists of human microbiomes, bioRxiv 2024.01.20.576352. https://doi.org/10.1101/2024.01.20.576352