

POLICY BRIEF

How to regulate new microbial solutions for plant protection in Europe

Introduction

How prepared are we to regulate the currently available microbial pesticides?

How ready are we to regulate the emerging microbial solutions?

Innovation in the risk assessment of new microbial solutions: what else is needed?

Biopesticides are at the forefront of innovation in plant protection. Microbial pesticides are the most advanced group of biopesticides, both in terms of discovery and risk assessment. Still there are steps that should be made in risk assessment to unravel the full potential of this new era of plant protection products (PPPs). Microbials are currently based on bacterial, fungal and viral components, but new microbial solutions based on bacteriophages, microbial consortia and protists are at the doorstep of the EU. Despite advances in this area, the risk assessment of new microbial solutions is still lagging behind. We describe the current scene of risk assessment for microbial pesticides and highlight the unique features of new microbial solutions that should be considered for the urgently needed reform of risk assessment of these innovative products.

INTRODUCTION

Synthetic pesticides are still a cornerstone of modern agriculture. However, their extensive use has raised concerns about their impact on the environment and human health, despite the stringent regulatory framework that is in place to control their placement in the EU market. Industrial innovators and the European Commission are investing in the discovery and development of innovative biopesticides such as microbials, semiochemicals /pheromones, botanicals (plant extracts and pure plant derived compounds) and other substances of

biological origin (e.g. natural peptides, ds-RNA). Still, the placement in the market of this new era of products has been restrained by Europe's delayed development of relevant regulatory instruments, as the present EU pesticide regulatory framework has been tailored to the needs of synthetic pesticides and does not consider the unique features of biopesticides. In 2022 the European Commission made available for the first time the data requirements for the approval of microbial pesticides, and in October 2023 it provided, through Explanatory notes (in the

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framework of Reg. (EC)No 1107/2009), technical information on how these data requirements can be addressed, or which guidance document or guidelines may apply. In the same context, the European Commission has established the term low-risk substances to put a “safety label” on such products, which is given only after risk assessment is concluded. The criteria for the approval of microbial and non-microbial pesticides as low risk are included in the point 5 of annex II of Regulation EC no 1107/2009. Complementing this, the European Food Safety Authority (EFSA) has proposed the term “low-concern substances”, which includes all substances of biological origin that are potentially of low risk. Microbial pesticides are the most populated and advanced category of biopesticides with currently 71 of them

approved at EU level and 26 applications pending^[1]. Amongst the approved microbials, fungal-based products dominate (40), followed by bacteria (23) and viruses (8). The list of pending microbial PPPs includes the first application of a bacteriophage product, while several other bacteriophage-based plant protection products (PPP) are in the production pipeline. In addition to bacteriophages, other novel microbial PPPs based on protists and synthetic microbial consortia of variable complexity are under development and expected to be submitted for approval. A major challenge we are currently facing is to provide a novel regulatory framework for a fast track, efficient and scientifically sound risk assessment of existing, and most importantly, of future microbial solutions.

HOW PREPARED ARE WE TO REGULATE THE CURRENTLY AVAILABLE MICROBIAL PESTICIDES?

Microbial pesticides are most probably the best regulated group of biopesticides. Regulation 283/2013 Part B^[2] describes the requirements that should be met in order for a microbial product to be placed on the market. Recent guidance documents have addressed the main safety concerns for microbial pesticides like:

- The carriage and transmissibility of antibiotic resistance genes (ARGs) (European Commission 2020, SANTE/2020/12260)^[3]
- The biosynthesis of secondary metabolites of concern (European

Commission 2020, SANTE 2020/12258)^[4]

- The pathogenicity and infectivity potential

Whole Genome Sequencing (WGS) analysis of microbial strains used in PPPs is now recognized as an invaluable tool for early screening and detection of genetic elements of concern and dictates the generation of follow-up data or the rejection of products. Furthermore, advances in sequencing technologies enable the rapid and cost-effective WGS of bacterial and fungal strains, facilitating accurate phylogenetic

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identification even at the strain level. This can avoid unnecessary testing based on established knowledge about the safety of microorganisms belonging to certain species or genera. In this frame EFSA assesses the safety of microorganisms used in different applications (food and feed additives, PPPs) based on the Qualified Presumption of Safety (QPS) approach that covers safety concerns for humans, animals and the environment based on the taxonomic identity of the microorganism, related body of knowledge and potential safety[5]. However, the main question remains “Do regulators have the necessary knowledge and access to tools that will facilitate risk assessment decisions?” The answer to this question is not trivial. Only few member-state regulatory bodies (less than half) have staff with the expertise to delve into WGS data[6]. EFSA made available a guidance document for the assessment of the data obtained from WGS analysis. In addition, EFSA has developed and provided to regulatory bodies the Microorganisms Pipeline (MoPs) tool, a non-open access pipeline that

identifies potential functional traits of concern in microbial genomes like virulence factors, resistance to antimicrobials of clinical relevance for humans and animals and biosynthesis of known toxic secondary metabolites. WGS analysis through MoPs is based on state-of-the-art bioinformatic tools, but relies on a limited number of microbial genomes that are present in the MoPs database, while there is limited information on the potential transferability of ARGs, a key component of the risk assessment of microbial pesticides.

Where do we need to focus on in order to improve our risk assessment of the currently available microbial pesticides (bacterial, fungal and viral PPPs)?

1. Mobilization of more regulatory experts with specialization in microbial ecology, environmental microbiology and bioinformatics
2. Improvement of currently available tools or development of novel open-access pipelines that will use the full breadth of curated sequencing data available worldwide.

HOW READY ARE WE TO REGULATE THE EMERGING MICROBIAL SOLUTIONS?

Before we answer this question we need to define which are those emerging microbial solutions that we will be asked to regulate:

- Bacteriophages (or phages)
- Protists

- Microbial consortia or Synthetic microbial communities

No regulatory documents are available for any of these novel microbial solutions except for bacteriophages

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for which an Organization of Economic Co-operation and Development (OECD) guidance document has recently been published[7]. Risk assessment for these new microbial solutions should be always case by case and in most cases will be qualitative, often using a weight-of-evidence approach. Expert judgment will be needed to determine what should be and what should not be considered a foreseeable risk. For this, good knowledge of the ecology and biology of the particular microbial solution is highly relevant.

Bacteriophages are viruses that specifically infect bacteria. Their main characteristics that will be relevant for risk assessment innovation are summarized below:

- Many phages are highly specific to their bacterial host (sometime even at the strain level), making them a potentially ideal and highly selective tool in crop protection[8].
- The use of mixtures of phages (so-called phage cocktails) with different infection profiles is a scientifically and commercially promising strategy to combat multiple disease-causing bacterial strains and to limit the emergence of resistance.
- Phages that reproduce using only the lytic life cycle, infect and kill the bacterial cell directly. This life cycle is considered preferable and more relevant for crop protection applications than the lysogenic life cycle, in which the phage integrates into the bacterial genome with the risk of transferring virulence genes to the host.

Environmental signals can trigger a switch from lysogeny to lysis.

- There are established methods for quantification including classic microbiological and molecular methods.
- WGS approaches can be used for identification, detection of antibiotic resistance genes and prediction of life cycles and functions.
- Phage survival and infectivity are highly sensitive to environmental parameters and this should be considered in the mode of application.
- Phages are not infectious to eukaryotic cells and are not known to produce secondary metabolites.
- Production of phages is straightforward but requires a bacterial host. The presence of the host should be checked and considered in the risk assessment.
- Phage-dedicated collections are available, allowing deposition, which is a prerequisite for product registration.
- Non-target effects on the microbiome should be tested under different environmental scenarios (e.g. high or realistic pressure on bacterial populations).

Phage PPPs are currently available in the USA market for the control of (a) *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* (b) *Xyllela fastidiosa* (c) *Pectobacterium carotovorum* (*Erwinia carotovora*) and (d) *Xanthomonas citri* pv. *citri* while several others are either pending authorization or are in the pipeline of production for the market[9][10].

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Protists are the most morphologically and phylogenetically diverse group of microorganisms[11]. They encompass a range of lifestyles spanning from saprotrophy (i.e. feeding on dead organic matter) and phototrophy (i.e. using light as primary energy source) to predation, the latter being the most common lifestyle and the one most relevant for crop protection. Most predatory protists feed on bacteria but feeding on fungi is also ubiquitous[12]. Certain characteristics of protists that should be considered relevant for risk assessment innovation are summarized below:

- WGS analysis as a tool in risk assessment is becoming possible due to recent sequencing of 10000 protist genomes[13].
- The mode of action of protists could be direct (via predation) or indirect (via stimulation of microbes with enhanced phytopathogen inhibition capacities, plant growth promoting or plant protecting traits) and should be treated differently in the risk assessment.
- There is little knowledge about the ability of protists to produce toxins, secondary metabolites and to carry ARGs. WGS analysis could facilitate early detection and clarify data requirements.
- Protists also include human and animal pathogens and parasites, while predatory protists could be pathogenic under certain conditions.
- Compared to phages, protist predation on bacteria is rather non-specific, although cases of

strain-specific predation have been postulated and feeding preferences and selective grazing have been shown.

- The mass production of protists might require feed with prey (single or multiple), which may raise concerns about the presence of prey cells as contaminants in the product, while also axenic growth (i.e. without bacterial prey) is possible for certain protists.
- Large-scale isolation and the cultivation from soil remain difficult and another major barrier is the lack of dedicated culture collections that are necessary for product registration.

Currently, there are no protist-based crop protection products on the market in the EU and the USA. However, several recent reports of their activity as controllers of soil-borne bacterial pathogens (e.g. *Ralstonia solanacearum*)[14] are likely to stimulate interest in the development of protist-based PPPs.

Microbial consortia of different complexity could also be a viable and novel microbial solution in the new era of crop protection. They can be characterized by low or high complexity. In the former category we include consortia constructed by combining different well-characterized strains of bacteria or fungi (intra-kingdom SynComs), or bacteria and fungi (trans-kingdom SynComs) that have complementary modes of action or different optima of activity expected to maximize efficiency compared to individual strains.

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In the latter category we could consider naturally isolated enrichment cultures of bacteria (mostly) and/or fungi (rarely) that are stable in composition with often more than 10 distinct members. Regarding microbial consortia an amendment to the Regulation 283/2013 with Regulation 1439/2022[15] considered for the first time in a regulatory context the application of products that are composed of “a qualitatively defined combination of strains as they occur naturally or by manufacture”. In addition, the first considerations of risk assessment for microbial consortia were set out. The risk assessment of synthetic microbial consortia of low complexity seems to be rather straightforward with key notes:

- Data requirements will be defined according to the characteristics of the consortium and the intended use.
- A qualitative definition of the consortium and the range of content (minimum and maximum) of each member should be requested.
- All components should be deposited in culture collections.
- WGS analysis per consortium member is required to define risks (pathogenicity, infectivity, antibiotic resistance, toxin and secondary metabolite biosynthesis).

Less consideration has been given to natural microbial consortia derived from enrichment cultures, that may be characterized by higher complexity, compared to synthetically produced consortia, and may involve other unique features that should be

taken into consideration in risk assessment innovation. Compositionally stable enrichment cultures could be fully defined qualitatively and certain features relevant for risk assessment (virulence factors, ARGs, gene clusters coding for the biosynthesis of secondary metabolites) could be clarified by whole (meta)genome sequencing and bioinformatics.

However, it is often the case that enrichment cultures could not be disentangled fully to their members due to the limited cultivability of individual members or nutritional and metabolic interdependencies (e.g. provision of vitamins and amino acids) [16] which limit axenic cultivation. This is a limitation that contrasts with the requirements for deposition of individual consortium members to culture collections. Specific adjustments to this requirement should be considered to allow deposition and also testing for the whole consortium rather than for individual members. Another important aspect for naturally derived consortia is the mode of action and the definition of the functional role of its individual member. These could be determined by more advanced omic tools (metatranscriptomic, metaproteomic, meta-metabolomic) followed by demanding bioinformatic analysis. It is often the case that only one (or a few) of the members of a microbial consortium have a plant protection relevant mode of action while the rest confer supportive services to the coherence of the consortium adhering to the widespread auxotrophy amongst prokaryotes[17].

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INNOVATION IN THE RISK ASSESSMENT OF NEW MICROBIAL SOLUTIONS: WHAT ELSE IS NEEDED?

Risk assessment of novel microbial solutions requires fast and efficient steps to match the pace of research and industrial innovation. Amongst the upcoming microbial solutions, phages primarily and microbial consortia secondarily are on the doorstep of the EU market. Despite that, we do not have regulatory procedures in place to address their characteristics and meet the requirements for a proper risk assessment scheme. On the other hand, PPPs based on alive protists are not yet, as far as we know, in the production pipeline, but scientific

evidence suggests that they might be possible candidates for development if certain safety limitations at technological or biological level are addressed. Therefore, we urgently need to develop guidelines and recommendations for the risk assessment of those novel microbial solutions that will consider all their specific features and cases deployed above. This will encourage and benchmark innovation in the discovery and development of novel biobased solutions for crop protection.

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TERMINOLOGY

Auxotrophy: the inability of an organism to synthesize de novo a particular organic biomolecule required for growth.

Botanicals: are active substances which carry pesticide activity and they are obtained by processing material of botanical origin. Botanical pesticides could be a mixture of several different plant-derived compounds or purified substances.

Semiochemicals: are substances emitted by plants, animals, and other organisms that evoke a behavioural or physiological response in individuals of the same or other species. Amongst them pheromones are produced by individuals of a species and modify the behaviour of other individuals of the same species.

Metagenome: the sum of the genomes of all microorganisms of a microbial consortium.

Metatranscriptome: the sum of all transcripts of all microorganisms of a microbial consortium produced under a specific growth condition.

Metaproteome: the sum of all proteins of all microorganism of a microbial consortium produced under a specific growth condition.

Metametabolome: the sum of all metabolites of all microorganisms of a microbial consortium produced under a specific growth condition.

Whole genome sequencing: sequencing of the entire genome of an organism including chromosome, plasmids, mitochondria and other organelles.

Synthetic microbial communities (SynComs): carefully chosen microbial strains that are grown together in a single community to produce the desired microbiome function (e.g. plant protection, pollutants degradation, human and animal protection)