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Essay

From Empirical Microbial Products to Bioinputs 2.0: A Functional and Genomics-Driven Framework in Agricultural Microbiology

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Abstract

Microbial-based products are essential for sustainable agriculture, yet inconsistent performance and limited mechanistic understanding constrain their adoption. While terminology varies globally—from "bioinputs" to "microbial products"—this linguistic diversity reflects a deeper conceptual gap. Historically, the sector has relied on a successful but empirical Bioinputs 1.0 paradigm, based on phenotypic screening and a "black box" approach to efficacy. We propose Bioinputs 2.0 as an evolutionary framework grounded in genomics, functional biology, and advanced formulation. This paradigm integrates microbial ecology, metabolite-driven bioactivity, and systems-level interactions, positioning formulation as an integral design component rather than a secondary step. Transitioning from empirical discovery to knowledge-driven design is necessary to ensure reliable, scalable applications. While particularly evident in biocontrol, this shift provides a stronger basis for interpreting field responses in plant growth-promoting microorganisms. Overall, Bioinputs 2.0 emphasizes integrated, context-dependent biological systems to bridge the gap between laboratory insights and consistent field performance.

Keywords: Bioinputs 2.0; microbial inoculants; plant growth-promoting rhizobacteria; microbial consortia; biocontrol agents; metabolite-driven bioactivity; agricultural microbiology; sustainable agriculture; formulation design

1. A Fragmented Concept: Terminology and Conceptual Gaps

Microbial-based agricultural products have expanded significantly in recent decades, encompassing biocontrol agents, biofertilizers (mainly associated with nutrient supply), and plant growth-promoting microorganisms (PGPR, with broader plant-beneficial functions) [1].

However, their conceptual and technological development remains uneven. In South America, these products are commonly referred to as *bioinputs*, whereas in North America and Europe they are more frequently termed *biologicals* or *microbial inoculants*. Although this distinction may appear purely semantic, it reflects a deeper lack of conceptual consolidation in the field.

More importantly, a shared challenge persists while empirical screening approaches have yielded significant technological successes, many products still rely on observable phenotypes without a detailed understanding of underlying molecular mechanisms or ecological interactions. This "black box" approach contributes to variability in field performance and limits scalability [2]. This issue is further compounded by the lack of standardized field evaluation protocols, which hinders reproducibility and comparability. For example, associative or endophytic nitrogen-fixing

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microorganisms are often credited with high nitrogen inputs, yet robust methods for their quantification under field conditions remain limited [3].

Additionally, inoculation practices are frequently not tailored to the specific microorganism, formulation, or application site, further contributing to inconsistent performance. Together, these gaps highlight the need for a transition toward a Bioinputs 2.0 framework based on functional traits, mechanistic understanding, and genomics-informed criteria, supported by standardized validation strategies.

In this context, Bioinputs 2.0 refers to biological inputs that, building upon microorganisms from the 1.0 model, incorporate rational design supported by genomics and bioinformatics to decode mechanisms of action and reveal functional potential, including functions mediated by metabolites and other biological entities not necessarily linked to cellular viability, evolving toward integrated systems, synthetic consortia, and advanced formulations with greater predictability and scalability.

2. Bioinputs 1.0: The Empirical Paradigm and Its Limits

Bioinputs 1.0 represent a robust and highly successful framework based on the isolation and selection of microorganisms with proven agronomic potential. Their efficacy relies primarily on direct phenotypic performance within biological interaction systems, constituting the undisputed foundation of modern agricultural microbiology. This paradigm has delivered established, large-scale applications across diverse biotechnological functions, proving that empirical selection can yield world-class technological solutions.

The first generation of microbial products—here conceptualized as Bioinputs 1.0—was largely driven by the identification of microorganisms with desirable, observable traits. A paradigmatic gold-standard of this success is *Bacillus thuringiensis* (Bt), whose global impact as a bioinsecticide derives from the production of Cry and Cyt toxins with high specificity against target insects [4]. The reliability of Bt in both sprayable formulations and transgenic crops stands as a testament to the power of the 1.0 model in providing consistent pest control through a "black box" approach, where the phenotypic output validates the technology.

However, the very success of this model has brought its inherent limitations into sharper focus, creating what we define as an "evolutionary threshold." While the biodegradability of these toxins is environmentally advantageous, it often results in reduced persistence under fluctuating field conditions. Furthermore, the emergence of resistance development—particularly in the context of Bt toxins expressed in transgenic crops—and the inherently narrow spectra of activity of traditional strains have highlighted the urgent need for a more diversified and knowledge-based approach [4].

Importantly, recent findings indicate that while the 1.0 model provided the "what" (the successful strains), it often overlooked hidden layers of activity. Closely related taxa, previously dismissed or under-explored by traditional screening, are now revealing unexplored insecticidal and nematicidal potential. For instance, strains of *Bacillus toyonensis* biovar Thuringiensis have been identified as novel entomopathogenic and nematicidal agents, demonstrating activity against both lepidopteran and coleopteran pests, as well as phytonematodes [5–7].

3. From Empirical Screening to Mechanistic Design

Advances in genomics have catalyzed a transition from descriptive microbiology to mechanistic and predictive frameworks [8]. Bioinputs 2.0 represent this next-generation paradigm, moving beyond traditional empirical selection toward knowledge-driven design. By utilizing the successful microorganisms of the 1.0 model as a structural pillar, this framework leverages Whole-Genome Sequencing (WGS) and functional annotation to systematically open the "black box" of microbial activity. This shift allows researchers to transition from merely observing a microorganism's output to understanding the precise molecular pathways and genes that dictate its performance within a biological system.

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The core of the Bioinputs 2.0 model lies in its ability to uncover latent biotechnological potential that remains invisible to standard phenotypic screening. Through the integration of advanced bioinformatics tools—such as antiSMASH, Bakta, and specialized pipelines—it is now possible to identify Biosynthetic Gene Clusters (BGCs) and secondary metabolites that may remain "silent" or unexpressed under laboratory conditions [9,10]. This genomic mining approach reveals that a single strain, traditionally valued for a singular function, may actually harbor a multifactorial arsenal for plant growth promotion, nutrient mobilization, and broad-spectrum biocontrol, ensuring the development of more robust, predictable, and reproducible agricultural products.

4. Expanding Functional Traits Beyond Classical Categories

Within this emerging paradigm, microbial functions extend far beyond traditional categories. In bioinsecticides, *Bacillus thuringiensis* produces not only Cry and Cyt toxins but also vegetative insecticidal proteins (Vip), which exhibit distinct modes of action [4].

The identification of additional toxin families—such as Mpp, Xpp, and Gpp—further highlights the complexity of microbial systems [11], challenging the traditional focus on single mechanisms and supporting a shift toward multi-factorial approaches that leverage a strain's entire pesticidal repertoire.

Beyond pest control, the Bioinputs 2.0 framework integrates a broader spectrum of traits that are increasingly recognized as critical to agronomic success. Traits such as plant immune modulation (Induced Systemic Resistance), abiotic stress tolerance, and microbiome assembly are no longer viewed as secondary effects but as integrated, context-dependent components of the plant–microbe interactome. By considering these functions as a unified system, Bioinputs 2.0 provides a more holistic and effective approach to crop resilience and productivity.

5. From Single Strains to Functional Consortia: Toward Microbial Systems Design

A major limitation of conventional microbial products is their reliance on single strains. In contrast, Bioinputs 2.0 promotes a higher level of complexity, where synthetic microbial consortia are designed to integrate complementary functions and better reflect the dynamics of natural microbial communities [3]. In this context, advances in genomics have fundamentally improved our understanding of microbial signaling, functional complementarity, and the strategic advantages of combining strains with distinct but synergistic traits.

AZOTOBAC, a biofertilizer developed by INTA, effectively exemplifies this transition [12]. This formulation combines *Priestia megaterium* and *Azotobacter chroococcum*, integrating a multi-factorial suite of plant growth-promoting mechanisms such as phosphate solubilization, phytohormone production, and biological nitrogen fixation. This synergistic design specifically addresses key agronomic constraints—particularly during crop establishment—by enhancing early root architecture and improving plant performance during the most vulnerable growth stages.

Beyond its direct functional traits, AZOTOBAC highlights the role of formulation as an integral component of biological performance. Its matrix incorporates microorganisms with intrinsic tolerance to environmental stress, thereby ensuring high viability and enabling anticipatory inoculation strategies. Furthermore, its compatibility with broader co-inoculation schemes reflects a shift toward more modular and combinatorial biological systems, where different functional units can be layered to meet site-specific agricultural needs.

6. Beyond Living Cells: Expanding Functional Units and Ecological Niches

A defining feature of the Bioinputs 2.0 framework is the expansion of what constitutes a functional biological input. Traditionally, microbial products have been conceived as formulations based on viable cells, with efficacy linked to microbial survival, colonization, and activity in the field. However, growing evidence suggests that biological function can be partially decoupled from

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cellular viability and instead mediated by diffusible compounds, secreted metabolites, or structurally stable biomolecules.

In this context, metabolite-driven bioactivity emerges as a critical and still underexplored dimension of microbial-based technologies [10]. Secreted enzymes, toxins, lipopeptides, and siderophores can exert biological effects independently of the producing organism, enabling alternative modes of application and formulation. This perspective opens the possibility of developing hybrid or postbiotic-like products, where the active principle is a biologically derived compound rather than a living microorganism.

At the same time, the search for novel microbial functions is increasingly extending beyond conventional environments such as the rhizosphere and rhizosheath. Non-traditional ecological niches—particularly those shaped by multi-organism interactions—represent promising reservoirs of bioactive microorganisms, as they impose strong selective pressures that drive the evolution of specialized metabolic capabilities.

A notable example is *Serratia odorifera* strain INTA L401-1, isolated from the hemolymph of *Tenebrio molitor* larvae infected with the entomopathogenic nematode *Heterorhabditis bacteriophora* [10]. This ecological context highlights the potential of complex host–microbe–parasite systems as sources of novel functional traits. The strain exhibited strong nematicidal activity against the nematode model *Panagrellus redivivus*, largely mediated by secreted metabolites, as demonstrated by the persistence of activity in cell-free supernatants.

Importantly, the retention of bioactivity in the absence of viable cells challenges the conventional paradigm of microbial inoculants and supports the inclusion of metabolite-based or hybrid formulations within the Bioinputs 2.0 framework. WGS further revealed genes encoding nematicidal factors such as serralysins and chitinases, as well as bioactive compounds including serrawettins and siderophores, linking genomic potential with observed functional outputs.

In addition to its nematicidal properties, greenhouse assays showed improvements in plant growth parameters, suggesting a dual functional role as both a biocontrol agent and a plant growth-promoting bacterium. However, the limited translation of nematode suppression under greenhouse conditions underscores a persistent challenge: bridging the gap between controlled experimental systems and complex field environments. Taken together, these findings illustrate a broader conceptual shift: Bioinputs 2.0 is not restricted to living microorganisms but encompasses a wider spectrum of biological functions, delivery formats, and ecological origins.

7. Formulation as a Determinant of Functionality

Formulation is increasingly recognized as a central determinant of microbial product performance, as environmental stresses such as UV radiation, temperature fluctuations, and desiccation significantly affect microbial viability and activity.

Advanced strategies—including encapsulation, protective carriers, and controlled-release systems—are essential to ensure consistency between laboratory and field conditions [13]. While these approaches have traditionally focused on preserving cell viability, emerging knowledge on microbial metabolites and functional traits is shifting attention toward function-preserving formulations, where the stability and delivery of bioactive compounds become equally relevant.

Within the Bioinputs 2.0 framework, formulation is thus not only integrated into the design process but also informed by mechanistic and functional insights. This enables the development of bioinputs that maintain or deliver specific biological functions regardless of cellular viability, ensuring that the active principle reaches its target in a stable and effective state.

8. Challenges and Future Directions

Despite significant advances, important challenges remain. Regulatory frameworks are often not yet adapted to complex microbial products, such as syntetic consortia or genomically characterized strains. Furthermore, transitioning to industrial-scale production requires maintaining strict genetic

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stability and functional consistency to ensure that the 2.0 design translates accurately from the bioreactor to the field.

At the same time, genome sequencing not only enables precise identification but also provides critical insights into biosafety, supporting its role as a baseline requirement for the development of microbial bioinputs. In specific cases, this genomic knowledge may guide the use of beneficial metabolites under controlled conditions, potentially offering effective solutions without the need for the large-scale environmental release of the producing microorganism.

Field validation remains a major challenge due to inherent environmental variability. Addressing these hurdles will require interdisciplinary approaches integrating microbiology, genomics, agronomy, and regulatory science. Looking forward, the consolidation of the Bioinputs 2.0 framework will depend on the ability to integrate functional, genomic, and ecological knowledge into scalable and context-specific solutions, effectively bridging the gap between laboratory innovation and consistent field performance.

9. Concluding Remarks

Bioinputs 2.0 represents a profound conceptual shift from empirical discovery to knowledge-driven design, moving beyond classical, phenotype-driven approaches toward mechanistic, genomics-informed strategies. As summarized in Table 1, this evolution redefines the core pillars of the industry—methodology, design focus, technical depth, reliability, and the role of formulation. This framework emphasizes that Bioinputs 2.0 is not merely about identifying new microorganisms, but about decoding and engineering their functions within complex biological systems.

While this transition is most advanced in biocontrol agents, where modes of action and target specificities are more readily defined, it offers an equally transformative basis for PGPR and microbial inoculants. By providing a mechanistic framework to interpret field responses, the 2.0 model allows for the incorporation of additional layers of functional complexity, stability, and performance. Innovation in this space now lies at the intersection of improved formulation, multi-mechanism combinations, and systems-based approaches.

As global agriculture faces increasing demands for sustainability and climate resilience, Bioinputs 2.0 challenges the scientific and industrial communities to rethink microbial technologies. They are no longer static, "one-size-fits-all" products, but dynamic, context-dependent biological systems capable of meeting the precision requirements of modern agriculture.

Table 1. Comparative overview of Bioinputs 1.0 and the derived Bioinputs 2.0 paradigm.

Feature	Bioinputs 1.0	Bioinputs 2.0
Methodology	Isolation and phenotypic screening	Genomics, bioinformatics, and data mining (known and latent functions)
Design focus	Simple functions / Viable cell count	Multi-functions / Genes, metabolites, and synthetic consortia
Technical depth	“Black Box” (observable effect)	Mechanistic (gene/pathway identification and functional design)
Reliability	Proven agronomic fitness	Predictable and stable performance with integrated formulation strategies
Formulation	Secondary step (viability preservation)	Integral to design (function-preservation and modularity)

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