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Development of an Indigenous *Azotobacter*, *Rhizobium* and Phosphate Solubilizing Bacterial Consortium for Enhanced Soil Fertility and Sustainable Crop Production

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Abstract

The management of nitrogen (N) and phosphorus (P) through biological means is crucial for sustaining crop production in future years and safeguarding environmental health. The indigenous strains of *Azotobacter*, crop-compatible rhizobia and phosphate-solubilising bacteria (PSB) are interesting inoculants because they address different but inter-dependent constraints non-symbiotic N fixation and rhizosphere stimulation, symbiotic N fixation in legumes and mobilization of sparingly available soil P. However, one cannot assume that their combination outperforms single strain inoculation. The novel consortium performance develops as an emergent property and is determined by host specificity, functional complementarity, competitive fitness, metabolite exchange, formulation stability, resistance to resident microbiomes, soil chemical properties and particular management context. This paper integrates mechanistic and agronomic evidence into a stage-gated framework for developing an indigenous three-guild bacterial consortium. The framework starts with crop- and site-defined sampling, followed by culture-dependent recovery, molecular identification, functional phenotyping, biosafety screening, pairwise and whole-consortium compatibility testing, formulation optimisation, and tiered validation from sterile systems to multi-location field trials. From the formation of halos, it does not follow that P delivery to plant is proven. From acetylene reduction, it does not follow that N transfer to crop is proven. From 16S rRNA identity, it does not follow that safety of strain or symbiotic effectiveness are proven. From greater biomass in pots, it does not follow that durability of performance in field is proven. Research on chickpea, soybean, common bean, wheat, potato and other crops has shown that co-inoculation can improve nodulation, nutrient uptake, biomass, yield, and post-harvest soil fertility; effects sizes, however, are heterogeneous and often decline under field conditions. A defensible consortium must therefore outperform its best component under realistic nutrient regimes, retain each member above a predefined viable threshold, its N and P delivery being crop-relevant and not being pathogenic, antagonistic and ecologically disruptive. The procedure suggests that microbial inoculation is part of integrated nutrient management and not a replacement for mineral fertilizer. Further, it identifies the experiments needed to prove efficacy, reproducibility, safety, shelf life, and agronomic value.

Keywords: *Azotobacter; Rhizobium; biological nitrogen fixation; phosphate-solubilizing bacteria; microbial consortium; indigenous inoculant; biofertilizer; soil fertility; nutrient-use efficiency; sustainable crop production*

1. Introduction

Today's crop production systems are heavily reliant on external nitrogen and phosphorus inputs. Fertilizer applications are invariably inefficient with regard to plant acquisition. Nitrogen is lost easily by leaching, volatilisation, denitrification and immobilisation, phosphorus which is poorly mobile, gets rapidly sorbed or precipitated with calcium, iron and aluminium compounds as per soil pH and mineralogy. As a result, the simple occurrence of a nutrient in soil or application as a fertilizer may not ensure its prompt supply to root.

As such, the agronomic problem is not merely a question of quantity, but the synchronisation of transformation, demand, activity and dynamics.

Microbial inoculants provide a biological means to improve this synchronization. Under appropriate conditions of carbon, oxygen and micronutrients, free living diazotrophs (like Azotobacter) can fix atmospheric N, and may also produce phytohormones, siderophores, exopolysaccharides, and stress modulating metabolites. Rhizobia engage in legume-specific symbiosis, forming nodules and reducing N2 through nitrogenase in a plant-regulated microaerobic environment. PSB enhance soluble orthophosphate via proton release, organic-acid production, ligand exchange, chelation, and enzymatic mineralisation of organic P. These functions are mechanistically complementary: P supports roots, ATP, nodules and energy demanding N2 fixation, while better N nutrition supplies the photosynthetic C to roots and symbionts. Field-grown chickpea positively responded to joint N-fixing and P-solubilizing inoculation; further, the soybean co-inoculation studies show that the simultaneous enhancement of N and P acquisition can exceed certain single-inoculant responses (Afzal et al., 2010; Wani et al., 2007).

Consequently, the conceptual appeal of a three-member consortium is strong, but a catalogue of beneficial traits is not a consortium. When different strains are mixed together, they compete for the carbon, oxygen, iron, attachment sites and space to reach the roots. A strain that grows very well in monocultured fields may be inhibited by the production of bacteriocins, acidification, siderophore-mediated iron deprivation, rapid capture of resources, or unfavourable interactions due to quorum sensing. In the opposite scenario, a small number of the strain may be essential, because it supplies the limiting metabolite, detoxifies the inhibiting compound, stabilizes the biofilm, or modifies rhizosphere such that another member will work. Synthetic-community tests have revealed that plant phenotypes are dependent on taxonomic richness but on strain identity and on higher-order interactions (Herrera Paredes et al., 2018; Niu et al., 2017). The design must thus be hypothesis-driven, quantitatively tested, and crop-specific.

Precision is also required in the term indigenous. It must denote strains isolated from the intended crop, soil, or agroecological zone and selected under conditions simulating the environment of intended use. Indigenous origin can enhance ecological fit. However, it does not provide evidence for agronomic effectiveness, genetic stability, biosafety, or competitive nodule occupancy. While native rhizobia may be highly competitive in nodulating legumes, they may also be inefficient at N fixation. Included, elite strains may fix N efficiently in sterile tests, but fail to occupy nodules in resident communities. As per study findings (Irisarri et al., 2019), field selection of native-naturalized rhizobia observed that competitive nodule occupancy is as important as symbiotic efficiency. Therefore, local origin is a starting criterion, not a performance endpoint.

This article outlines an evidence-based blueprint for a native consortium of (i) a free-living Azotobacter strain, (ii) a host-compatible Rhizobium or an allied rhizobial strain and (iii) a PSB strain. Since there is no original experimental dataset in the present synthesis, the term "development" is employed to refer to a scientifically specified development pathway rather than to imply that a new commercial consortium is already established. The purpose of this document is to present the rationale and mechanics behind striving for three functional guilds: (1) a critical analysis of the evidence for single and combined inoculation (2) definitions of stage-specific selection and rejection criteria (3) a proposed pot and field-validation strategy that is rigorous in approach (4) proffering formulation, quality, biosafety and regulatory requirements (5) identification of the conditions under which association is likely to succeed or fail.

2. Review Approach and Evidentiary Boundaries

This paper employs a systematic critical-review approach. We searched the literature using the terms Azotobacter and rhizobia, Rhizobium, biological nitrogen fixation and phosphate-solubilizing bacteria, microbial consortium, co-inoculation and indigenous inoculant, formulation, shelf life, nodule occupancy,

soil fertility and crop yield through PubMed, publisher databases and Crossref linked records along with backward citation tracing. All the research published from the Year 2010 to August 2026 was given emphasis. Earlier research was kept if it set up commonly used assay or offered direct field evidence. The evidence was assigned a valuation by the extent to which it was relevant to the claim: multi-year field trials and quantitative syntheses were assigned the most agronomic weight, followed by greenhouse trials, plate assays, with genomic or biochemical potential not being considered equivalent to nutrient transfer or yield benefits.

The review is analytical rather than exhaustive, neither does it constitute a systematic review as per PRISMA. The primary evidentiary difference is between capacity, activity, delivery and outcome. The term capacity is used to identify the genes or phenotypes that secure the viability of a function. Activity is defined as the expression or transformation of biochemical in defined conditions. Delivery refers to the biological uptake of fixed nitrogen or mobile phosphorus by plant roots. Outcomes mean improved growth, yield, nutrient-use efficiency or soil function. Weak studies often refer to outcome will follow directly from capacity within evidence categories.

The term Rhizobium is utilized in the title and general discussions as an agronomic technical term for compatible root-nodule bacteria. The strain selected in practice should apply the right current taxonomic scheme, as well appropriate symbiont to the chosen legume,such as: Mesorhizobium ciceri for chickpea, effective Bradyrhizobium strains for soybean. It does not have a defensible universal claim to non-leguminous crops, as the main benefit of the consortium's rhizobial member is node-specific, hence, species-specific. For non-legumes, the formulation would need a different justification, or a crop-rotation strategy in which the rhizobial component benefits the legume phase..

3. Why Combine the Three Functional Guilds?

3.1 Nutrient coupling rather than additive trait stacking

The envisaged consortium must be viewed as a coupled nutrient-delivery system, not three independently functioning biofertilizers in one package. Ample ATP and reductant are needed for nitrogen fixation. A phosphorus deficiency can limit the initiation of nodules, nodule growth, allocation of photosynthates, and nitrogenase activity. Therefore, increasing the availability of P can enhance the efficiency of rhizobial symbiosis, rather than simply supplying an additional nutrient to the plant. Research on wheat found that PSB stimulation of rhizosphere and endosphere diazotrophic activity was correlated with enhanced P status, suggesting that N and P processes can be biologically linked outside of classical legume nodules (Li et al. 2020). Chickpea showed enhanced nodulation, nutrient uptake and yield with triple inoculation of Mesorhizobium, Azotobacter and a phosphate-solubilizing rhizobacterium compared to less complete treatments (Wani et al., 2007).

However complementarity must be established at the level of limiting functions. If the chosen Azotobacter already solubilizes phosphate very well, or if the rhizobial strain produces enough organic acids and phosphatases, then the addition of a third PSB member may provide little benefit and may even reduce stability. On the contrary, an effective PSB that acidifies the microenvironment too much may affect the survival or nodulation of rhizobia. Rational design aims to find the smallest community that delivers reliable, non-redundant functions. Redundancy is useful when environmental variation is buffering and not competition.

3.2 Allocation of Units in Space-Time

Members are not likely to contribute equally at every crop stage. In seed germination, there is usually a predominance of rapid colonisation and auxin production. Through the process of nodule initiation, rhizobial recognition, and development of the infection thread adequate P supply becomes critical. When crops are growing or filling grain, the extent of continued N fixation with root turnover organic-P mineralisation and continued colonisation. Consortium ratios should therefore be considered as dynamic ecological variables rather than ones fixed by equal optical density at mixing.

The timing of events also influences interpretation. An early response by seedlings can be related to hormone-induced root proliferation without measurable nutrient transfer which could be negated later on if the inoculant population collapses. Conversely, it might occur that early competition yields to spatial partitioning as roots expand. Measuring strain abundance, nodule occupancy, soluble phosphorus, plant nitrogen and phosphorus, and functional-gene expression in a time series should therefore be more informative than endpoint colony counts.

3.3 Ecological insurance measures and their shortcomings

Changes in moisture, temperature, pH, carbon supply, and nutrient form may be compensated by functional diversity. Experiements examining soil biodiveristy (SDI) indicate that community composition efficient in multiple ecosystem processes function. Furthermore defined synthetic communities demonstrating that chosen combination of strains results in reproducible phentotypes in plants (Herrera Paredes et al. 2018; Wagg et al. 2014). However, diversity is not necessarily a good thing. With the increase in the number of added members, there are more possibilities for interactions that are antagonistic and higher-order. Moreover, complications in the manufacturing process are quite likely. The decline rates will be unequal during storage. The goal of design is complexity that is just enough: sufficient complementary capacity to survive functional, but few enough members to allow identification, enumeration, quality control causality.

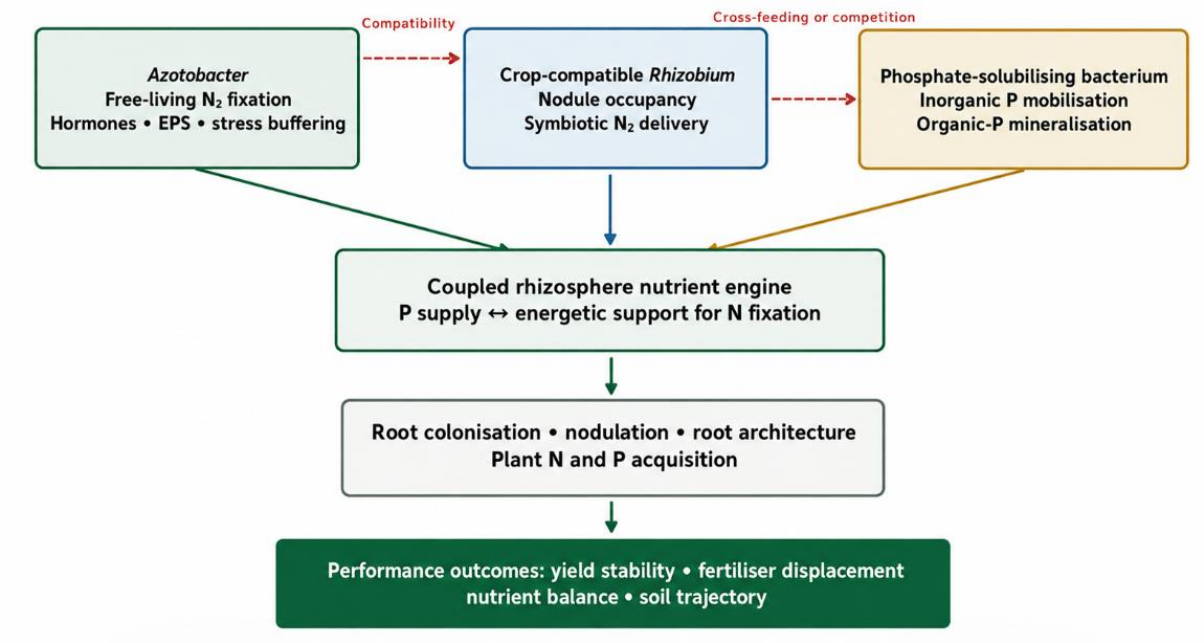

Figure 1. Functional architecture of an indigenous three-guild consortium. Solid arrows represent intended nutrient or plant-growth effects; dashed pathways represent context-dependent interactions that must be experimentally verified.

4. Functional Biology of the Consortium Members

4.1 Azotobacter: aerobic diazotrophy, rhizosphere stimulation, and context dependence

The species of Azotobacter are atypical among the free-living diazotrophs, as they do not lose their oxygen sensitive nitrogenase, in spite of being aerobic because of their very high respiratory rates, their extracellular polysaccharides and regulatory control of the N fixation. At the oxic root-soil interfaces, this physiological capacity supports survival but the carbon cost is high. oxygen-exposure experimentsA. control. The nitrogenase activity of vinelandii shows strong limitations due to oxygen regime even with this protection (Natzke et al., 2018). In soils which are poor in carbon, at strongly acidic pH, low in molybdenum or iron, or in the presence of easily available mineral N, nitrogen fixation can therefore be much lower than plate-based screening suggests. When selecting, focus on rhizosphere carbon use, oxygen tolerance, micronutrient requirements, persistence, and not just growth on N-free medium.

The agronomic impact of Azotobacter is not confined to the fixed nitrogen. Some strains have the ability to produce substances such as indole-3-acetic acid, gibberellin-like compounds, cytokinins, vitamins and vary their excretion of exopolysaccharides and other compounds. They may also solubilize phosphate or other minerals or alter root architecture or stress responses. The increase of biomass grain yield and plant nitrogen in wheat by salinity tolerant strains under controlled condition (Chaudhary et al, 2013) Inoculating Roots with A. The inoculant chroococcum 76A improved tomato performance under salinity and reduced N supply, although the response was weaker with optimal nutrition. This shows that inoculant efficacy depends on the resource environment (Van

Oosten et al., 2018). We should not assume that all growth promotion is due to N fixation.

A designed structure. Bageshwar and colleagues (2017) show that a chroococcum strain with altered nitrogen regulation leads to higher wheat yield and lower urea use in the field. The evidence of biological potential cannot be directly generalised to local wild type isolates. The construction of deregulation alters excretion of N, fitness, containment, and regulatory considerations.

In the case of a normal indigenous consortium, the development question concerns the ability of a naturally occurring strain to support measurable N2 fixation and plant benefit while also coexisting with rhizobia and PSB under target soil conditions.

4.2 Rhizobium Symbiotic efficiency, host specificity, and nodule competition

The contribution of rhizobia differs fundamentally from free-living diazotrophy. Rhizobia that are compatible with the host detect the flavonoids produced by it, produce Nod factors, begin curling of the root hair on infection and then differentiate in the nodules with the help of carbon supplied in plants which is used to support nitrogenase. The host regulates nodule structure and leghemoglobin for oxygen while the bacteria supply reduced N. This closeness allows for a high N delivery potential but comes with strict dependency on the host genotype. A strain's efficacy on one legume species or even one cultivar cannot assume efficacy on another.

In the selection of traits must be considered infectivity, effectiveness and competitiveness of the trait. The ability to form nodules is called infectivity, the quantity of N fixed and delivered is termed effectiveness, and the ability to occupy nodules in the presence of resident strains is competitiveness. Ineffective indigenous rhizobia may build up in nodules and exclude an elite. On the other hand, field soil might negatively affect the performance of a strain that is highly effective in a N-free sterile substrate. Such a strain may fail in field soil with the capacity to perform in N-free sterile substrate as it cannot survive seed desiccation, acidic conditions, heat, pesticides or competition. The selection for locally adapted native-naturalized strains of Rhizobium leguminosarum for white clover, which has been shown to be effective in other legumes, revealed that some of these strains could equal or outperform a commercial strain in terms of nodule occupancy and field performance. This illustrates the practical value of carrying out selection for effectiveness and competitiveness (Irisarri et al., 2019).

The supply of phosphorus is especially beneficial to rhizobial symbiosis because nodule formation and nitrogen fixation require considerable energy. According to Benjelloun et al. (2021), co-inoculation of Mesorhizobium ciceri with phosphorous solubilizing bacteria (PSB) significantly enhanced chickpea nodulation, shoot biomass, grain yield and grain nutrient status in phosphorus-deficient soil although the extent varied with bacterial combination. The key to this a strain-dependent treatment: “rhizobia plus PSB” is not reproducible unless you specify the strains, ratios, formulation, crop genotype, soil, and nutrient regime.

4.3 Phosphate solubilizing bacteria Traps and measuring systems

Soil P exists as a variety of inorganic precipitates, in mineral-sorbed pools, and as part of a variety of organic compounds. PSB can aid in the process of solubility of P through various pathway. The release of phosphates is directly associated with gluconic and 2-ketogluconic acid from chromatography (Hwangbo et al., 2003); glucose can be oxidized through a pyrroloquinoline quinone-dependent glucose dehydrogenase. Other organic acids can give protons and ligands that dissolve calcium phosphates or complex metal cations. However, their efficiency depends on pH, P saturation and mineral surface chemistry (Oburger et al., 2011). Another processes might be proton extrusion, ammonium assimilation, and siderophore production and chelation. Mineralisation of organic P takes place due to acid and alkaline phosphatases, phytases and C-P lyases; combined organic-anion production and phytase activity can enhance plant acquisition from calcium phytate (Giles et al., 2014). The relevant pathway associated with any P limitation is decided upon whether P limitation is dominated by Ca-bound P, Fe/Al-associated P, organic P, diffusion, or poor root exploration.

The use of standard agar halo is useful to get an early screen, but poor predictor of field value. The size of the halo is determined by the diffusion, blending of colonies, buffering and source of P. Certain strains discharge significant amounts of soluble P in the broth, creating no halo whereas there are some which acidify the agar but are unable to colonize the root or compete in soil. Tricalcium phosphate is not a universal selection substrate and can favour strains whose

laboratory phenotype is little related to the target soil (Bashan et al., 2013).

As such, quantitative screening should be conducted using buffered liquid media with multiple P sources, measure pH and biomass, determine soluble orthophosphate, profile organic acids if possible, and determine whether P release is sustained in both non-sterile target soil. Screening on NBRIP medium gives a more reproducible quantitative screen than Pikovskaya agar (Nautiyal, 1999).

Effects of plants may also occur without bulk-soil P solubilisation. PSB has the ability to modify the root architecture, hormone equilibrium, microbial community structure or microscale P fluxes at the vicinity of root surfaces. The effects of PSB on wheat's root traits and plant physiology are stronger than those changes inferred using bulk rhizosphere P alone (Elhaissoufi et al., 2020). It should not promote a single mechanism narrative. It should test if improved P uptake is attributable to either chemical mobilisation, root-system modification, increased microbial turnover, or combinations of these.

4.4 Interaction hypotheses

The three-guild consortium has at least five testable interaction hypotheses:

PSB-mediated P release increases rhizobial nodulation and N fixation under P limitation.

Azotobacter-derived phytohormones or exopolysaccharides increase root surface area and rhizosphere retention, indirectly improving rhizobial infection and PSB access to root exudates.

Rhizosphere carbon released by a better-nourished plant supports free-living diazotrophy and P mobilisation, creating a positive plant-mediated feedback.

Cross-feeding of vitamins, organic acids, or siderophore-bound iron improves consortium persistence.

Competition for carbon, oxygen, iron, or root attachment suppresses one or more members and nullifies the intended complementarity.

These hypotheses are mutually compatible; positive and negative interactions can occur simultaneously and vary over time. A valid study must therefore quantify net consortium performance relative to every constituent and relevant pairwise combination.

Table 1. Functional roles, evidence requirements, and principal failure modes

Guild	Intended contribution	Minimum proof	Common false inference	Principal failure mode
Azotobacter	Free-living N fixation; phytohormones; stress tolerance; rhizosphere conditioning	Strain persistence plus N-fixation activity and plant N gain under target conditions	Growth on N-free agar proves agronomic N delivery	Carbon limitation, mineral-N repression, low persistence, or competition
Crop-compatible rhizobium	Nodulation and symbiotic N delivery	Nodule occupancy, effective nodules, 15N or validated N-balance evidence, and crop response	More nodules necessarily mean more N fixation	Host mismatch or occupation by competitive ineffective residents
PSB	Mobilisation/mineralisation of soil P; root stimulation	Soluble P from relevant substrates, root colonisation, plant P uptake, and P balance	Halo diameter predicts field performance	Buffering, sorption, carbon limitation, or poor survival in soil
Whole consortium	Coupled N-P acquisition and functional resilience	Performance exceeding the best member under realistic soil and nutrient regimes	More strains automatically produce synergy	Antagonism, ratio drift, storage instability, or ecological exclusion

5. Indigenous Strain Discovery: From Sampling to Candidate Selection

5.1 Define the target product before sampling

The process of sampling should be initiated after the crop and cultivar, soil constraints, climatic envelope, application method and fertilizer-displacing claim have been defined. An inoculant containing rhizobia and plant growth-promoting bacteria (PGPB) for chickpea grown in alkaline, P-deficient dryland soil need to be different from those present in a soybean inoculant for acidic, high-rainfall soil. If a non-leguminous target crop is to be obtained, a rhizobial strain should not be included on the grounds of nodulation and it must be reconsidered the title or crop strategy. One might defend the proposition: "a seed

applied consortium for chickpea grown in alkaline alluvial soil that maintains yield at 75% of the recommended P dose without reducing N fixation." The first statement can be tested; the second one "improves sustainable agriculture" cannot.

The samples should be representative of the ecological niches that the formulation must colonize: healthy nodules for rhizobia; rhizosphere and rhizoplane soil for PSB and Azotobacter; and, where relevant, root endosphere material.

Source: Reference Guide for Practical Applications of Inoculants Samples should be taken from a wider scope of the target area, not just a single performing plant. Information about coordinates, crop history, cultivar, previous inoculant used, fertilizer regime, pesticide exposure, pH, electrical conductivity, texture, organic carbon, available N and P, moisture and sampling date should be included in metadata. The term indigenous has little ecological meaning without this data.

5.2 Separation and Identity verification

Enrichment of Azotobacter on N-free media establishes presence, but not necessarily diazotrophy. It is essential to repeat the purification of colonies and test these colonies morphologically, biochemically and for reproducible growth. Isolation of rhizobia from yeast extract mannitol medium must be verified by the reinoculation of the homologous host under axenic N-free conditions from surface-sterilised, internal pink nodules. Nodule formation fulfills Koch-like symbiotic authentication; colony morphology on selective medium does not (Somasegaran & Hoben, 1994). PSB can be recovered on Pikovskaya or NBRIP media containing defined insoluble P sources and quantitative confirmation in broth.

It is essential to go beyond just a short 16S rRNA sequence for molecular identification. The divergence between rhizobial taxonomy and its symbiotic function is attributable to the nodulation and nitrogen-fixation genes′ location on plasmids or symbiosis islands. An efficient minimum requires high-quality identification from 16S rRNA together with evidence from nodC and nifH for rhizobia and from nifH for Azotobacter. The use of whole-genome sequencing is preferred for elite candidates, as it can resolve strain identity, functional gene complements, plasmids, secretion systems, virulence-associated loci, antimicrobial-resistance determinants and genome stability. For PSB, the genes gcd and the pqq cluster can support a gluconic-acid hypothesis, but the phenotype must still be shown.

5.3 Function screening utilizing pertinent limitations

Initial assessment should be broad enough to capture complimentary function yet stringent enough not to pick laboratory specialists. Candidates of Azotobacter should be compared for their N-fixation activity, N accumulation, exopolysaccharide and biofilm formation, root adherence, carbon-source use, and tolerance to target pH, salinity, temperature and moisture stress.

Although the acetylene-reduction assay gives good relative data on nitrogenase activity, it does not quantify N transferred to the plant. Therefore, selected candidates should go to 15N2 addition, 15N isotope dilution or highly controlled N-balance experiments.

The target crop and cultivar should be screened for days to nodulation, nodule number and dry mass, nodule colour, shoot N, plant biomass under N-free conditions, and competitive occupancy against representative resident strains. The nodule effectiveness and occupancy of the strain are actually more important than the maximum production of nodules. Competent values of PSB in the presence of tricalcium phosphate, rock phosphate, and Fe- or Al-phosphate, as applicable to the soil, should be compared. In addition, soluble P should be standardized to biomass, while pH should also be measured. When organic P is critical, phosphatase and phytase assays are necessary.

Some secondary plant traits such as producing IAA, ACC deaminase, producing siderophores, potassium or zinc mobilisation, pathogen suppression can strengthen the candidate and not weaken it. It is essential to use quantitative assays as well as physiologically appropriate substrate concentrations in trait screens. A colour change occurring in an assay's performance is a claim generator, not a product claim.

5.4 Biosafety as an early selection gate

Biosafety assessment should precede costly agronomic trials. Taxonomic or genomic evidence demonstrating clinically significant pathogenicity, strong haemolytic activity, presence of transferable antimicrobial-resistance determinants, toxigenic potential or unacceptable plant pathogenicity should lead to exclusion of candidate strains. It is especially crucial for PSB since species that incorporate effective solubilizers such as Pseudomonas, Burkholderia, Enterobacter, and Serratia are also opportunistic pathogens. Names at the species level cannot convey safety, as risk is strain-specific and not possessing an

identified virulence gene is not a guarantee of safety. Whole-genome analyses should complement phenotypic tests and an assessment of the planned exposure route.

Being native doesn't necessarily mean it is safe. Local strains might have mobile resistance genes or persist outside the intended niche. Development must document antibiotic susceptibility for quality-control purposes without deliberately selecting resistance marker for field-tracking. Studies of persistence and occupancy would benefit from molecular barcodes, strain-specific qPCR, naturally discriminating genomic loci, or non-antibiotic reporter systems.

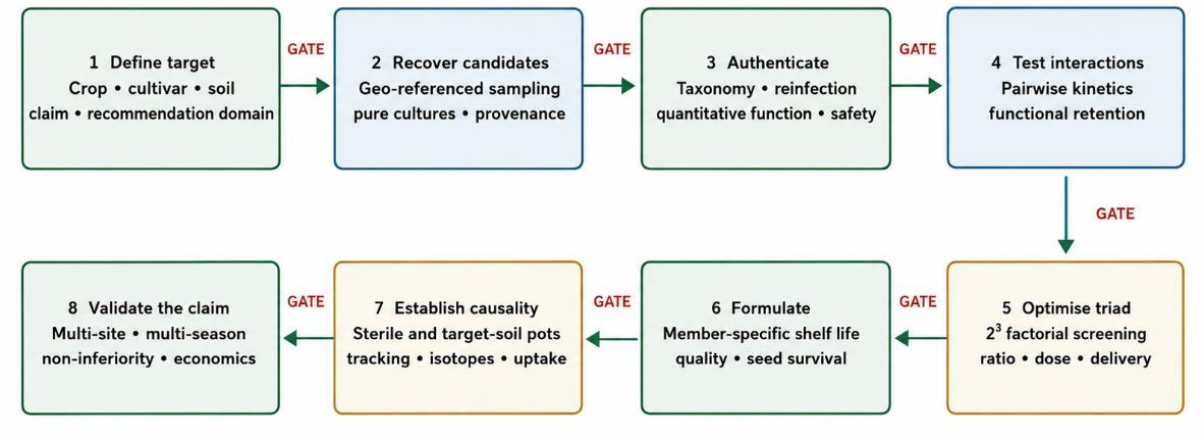

Figure 2. Stage-gated development pipeline. A candidate advances only when it meets predefined functional, compatibility, safety, stability, and agronomic criteria; failure at any gate triggers reformulation or rejection.

6. Consortium Assembly and Compatibility Testing

6.1 Why plate compatibility is necessary but insufficient

Strong antibiosis can be identified by cross-streaking, dual-culture inhibition and agar-well diffusion and overlay assays which are good first exclusion tests. Because of the significant differences in diffusion, concentration of nutrients, oxygen, pH buffering, spatial structure, and others from soil, they cannot establish compatibility in rhizosphere. Absence of any visible inhibition zone just means that no diffusible antagonist has been detected under that assay condition. Candidates who pass plate tests should be examined in co-culture of liquid carrier formulations sterile non-sterile soil and plant-associated microcosms.

Before testing, compatibility must be evaluated pairwise. This allows attributing failure to the interactions, Azotobacter - rhizobium, Azotobacter - PSB and rhizobium - PSB. Over time, viable counts or strain-specific molecular quantification of each pair and the entire consortium compared to monocultures. Outputs of interest include lag time, peak population, decline rate, pH, dissolved oxygen, biofilm mass, metabolite profiles, functional activity and strain ratio maintenance A consortium producing the desired aggregate function in the absence of one and whose composition is transient is not necessarily stable; rather, its effective composition differs from its label.

6.2 Complementarity has to exceed the best component

Synergy ought to be measured quantitatively The consortium is compared, only with an uninoculated control. A more robust definition indicates that the consortium should outperform the best single strain with a particular interaction contrast pre-specified prior to analysis. A factorial pot or field experiment should utilize a statistical model that estimates main effects and interaction terms, as opposed to merely treatment-wise multiple comparisons. If the sum of the triangle is the strongest single stress, then the extra complexity must be justified by the better stability, higher environment tolerance, lower fertilizer requirement, or some other predeclared advantage.

This standard, several co-inoculation studies show, is needed. In soybean, the yield and nutrient status was enhanced by N-fixing and P-solubilizing bacteria however the responses were pot and field condition dependent (Afzal et al., 2010). In chickpea, specific Mesorhizobium-PSB combinations produced big improvements in P-deficient soil whereas alternative strain combinations were less effective (Benjelloun et al., 2021). In pea, C. pinetorum strengthened nodulation in pots but did not increase seed yield in two years of microplots containing abundant resident

rhizobium (Martyniuk et al., 2015). Zeffa et al. (2020) found that soybean co-inoculation improved nodulation and biomass across the evidence base. However, co-inoculation did not have a consistent positive effect on grain yield, suggesting that biological processes evident early in maturation do not always lead to increased harvestable yield.

6.3 Optimizing Ratio and Ecological Modeling

Mixing equal volumes is useful for laboratory procedures and has no biological justification. Using a mixture experiment or response-surface experiment, the initial ratios should be optimised that constrains the total inoculum while varying the proportion of each strain. Outcomes must include the response of the plant and the abundance of each member. A high-performing ratio that collapses during storage or immediately after soil application is not commercially relevant.

Choosing ratios can be informed by metabolic profiling. The overlap in carbon use can anticipate competition while the complementary use of organic acid can also support cross-feeding of the organisms. Using genome-scale metabolic reconstructions to produce interaction hypotheses does not substitute for empirical co-culture since gene expression and supply of rhizosphere resources are context-dependent. The correct workflow is experimentation rather than the revision of models.

6.4 Spatial arrangement and application pattern

Simultaneous delivery is not the best option. Rhizobia depend on swiftly reaching emerging root hairs; PSB is likely to perform better when applied to soil alone or as granules close to un-soluble P; and placement with organic C or protective polymers would benefit Azotobacter. The development programme should evaluate co-formulation, simultaneous separate application, and sequential application. A two-component package at sowing may protect biological complementarity without forcing storage compatibility if a mixed bottle reduces viability or causes antagonism.

Seed coating imposes more restrictions. Desiccation, osmotic stress, seed coat antimicrobials, pesticide dressings and local high salt concentrations decrease cell viability before germination. Testing at label rates and realistic contact times for compatibility with commonly used fungicides and insecticides must occur. When chemical seed treatments are incompatible, in-furrow application or polymer separation or delayed inoculation should be the first consideration, not assuming a field failure indicates poor strain biology.

Table 2. Stage-gated selection criteria for consortium development

Stage	Core measurements	Advancement criterion	Rejection trigger
Ecological definition	Crop, cultivar, soil constraints, climate, management, intended claim	A specific target-use profile and testable fertilizer-reduction claim	Undefined crop or universal claims across incompatible hosts
Isolation and identity	Pure culture, molecular identity, provenance metadata	Authenticated strain with traceable accession and preserved stock	Mixed culture, uncertain identity, or untraceable origin
Primary function	N fixation, symbiotic effectiveness, P mobilisation	Reproducible quantitative activity under target constraints	Only qualitative plate response or activity lost under relevant pH/salinity
Safety	Genome and phenotype-based hazard screen	No unacceptable pathogenicity, virulence, or transferable resistance signal	Clinically significant risk or unresolved identity
Pairwise compatibility	Inhibition, co-culture kinetics, functional retention	No meaningful loss of either member or target function	Antagonism, exclusion, or major functional suppression
Triad optimisation	Mixture design, time-series abundance, plant assay	Triad exceeds best component or provides predeclared stability advantage	No added value, ratio collapse, or non-reproducible response
Formulation	Viability, purity, physicochemical stability, seed survival	Each member remains above product specification through shelf life	Differential decline, contamination, phase instability, or label failure
Pot validation	Full controls, nutrient gradients, target soil, strain tracking	Consistent nutrient delivery and plant benefit in non-sterile soil	Benefit limited to sterile substrate or excessive fertilizer regime
Field validation	Multi-site, multi-season randomised trials and economics	Agronomic benefit with acceptable risk and reproducibility	Site-specific failure without a usable response rule

7. Formulation, Shelf Life, and Product Quality

7.1 Formulation is part of biological design

A formulation comprising living cells must be stable to allow use at the desired ratio, protect cells against temperature and desiccation, allow application and their release in the vicinity of the target niche and must not contain substances which inhibit the crop or other members of the consortium.

A consortium does not work if the strain combination exhibits activity when freshly mixed in the lab, but not after storage. As a result, formulation should occur during strain selection rather than post efficacy testing.

Carrier-based products are derived from peat, lignite, charcoal, composted material, biochar, vermiculite, or other appropriate matrix having the capacity to hold water, pH, sterile or microbial cleanliness, particle size, etc. Liquid formulations may support higher initial counts and easier mechanised application but the cells remain metabolically interactive and may decline at different rates. Use of protective additives can reduce osmotic and desiccation injury. Some protective additives are polyvinylpyrrolidone, carboxymethyl cellulose, gum arabic, alginate, glycerol, trehalose, or other compatible polymers. The effects of such compounds are strain-specific and need testing within the complete formulation.

A liquid PSB formulation based on Bacillus licheniformis and Pseudomonas putida containing two strains benefitted the potato yield and P nutrition across two field seasons and maintained the stability of the formulation reflecting the significance of integration of strain performance, formulation and agronomic testing (Bansal et al., 2025) The outcome is informative, but we cannot apply it directly in the case of three-guild scenarios where spore-forming Bacillus is added together with non-spore-forming rhizobia or Azotobacter because these exhibit different storage kinetics. If they manufacture equal amounts, they can become very unequal before using.

7.2 Quality attributes

Count each strain separately rather than total viable count only in quality control. A product may fulfill a total count specification, but it could lose its rhizobial component and thus its essential function. The use of strain-specific selective plating, qPCR combined with viability discrimination, flow cytometry or validated molecular enumeration. The batch release testing should ideally include identification, viable counts per member, contamination tests, pH, moisture or viscosity, activity, physical stability and absence of unacceptable pathogens.

Shelf-life studies must use several production batches and target packaging. The counts must be determined at manufacture and at regular intervals under recommended storage, elevated temperature plus temperature cycling. Functional assays should accompany counts as viable cells may lose plasmids, symbiotic traits, P-solubilizing performance or stress tolerance. It is vital that applications of seed-applied viability are made at realistic intervals between coating and sowing.

The Bureau of Indian Standards specifications that are important in India include carrier-based and liquid-based rhizobial, Azotobacter and PSB microbial inoculants and IS 17755:2022 for consortia of microbial inoculants. These standards ensure regulatory quality at a minimum but not agronomic efficiency. According to Bureau of Indian Standards in 2023; The development of the product must comply with the current applicable Fertiliser (Control) Order and BIS specifications; and must also verify the current legal text before registration or commercial sale.

7.3 Reproducibility in Manufacturing

All strains should be produced under defined fermentation conditions and combined at a controlled physiological stage. One of the attractive features of co-fermentation is that it may reduce the number of steps needed to make the product but unmonitored co-fermentations can mask competitive exclusion and batch drift. An early stage product is better achieved through a separate fermentation and controlled blending. Master and working seed lots ought to be cryopreserved, authenticated and kept to a low passage number. Parameters critical to fermentation performance include inoculum age and dissolved oxygen, and also pH, carbon source, antifoam, and harvest density and time between harvest and formulation.

Measurement contamination control should match intended use. Farm inoculants are not sterile medicines as uncharacterized contamination can impact their efficacy and safety. There should be consistency in identity, count, and function in the batches. The compatibility of the formulation with packaging materials, transport vibration, storage light and on-farm dilution water quality should also be tested.

8. Experimental Validation Strategy

8.1 Tier 1: mechanistic microcosms

The first tier of validation must show some form of causality under controlled or strong conditions. To compare an uninoculated control, each single strain, all three pairwise combinations, and the triad, use sterile growth substrate or gnotobiotic systems. Choose a mineral-N control, mineral-P control, and complete N-plus-P nutrition options. The design should include P-sufficient and P-limited conditions, otherwise the PSB hypothesis cannot be tested meaningfully. With similar significance, high mineral N can hinder nodulation and mask rhizobial and Azotobacter contributions.

The measurements taken should include germination, root architecture, shoot and root biomass, nodule number and dry mass, nodule occupancy, plant N and P, substrate-available P, pH, and strain abundance over time. The activity of nitrogenase can be screened via the acetylene reduction assay, but actual N contribution should be measured either by 15N2 incorporation, either a 15N isotope dilution, or a validated N-difference method with appropriate controls. In assessing P mobilisation one should look at plant P uptake and P balance and not soluble P.

The sterile systems have no resident-community competition by design, hence the former is a mechanistic test, not an efficacy claim. Any combination that fails at this stage should be rejected in general. Any combination that passes the test must be transferred to non-sterile soil.

8.2 Tier 2: non-sterile target-soil pot trials

Pot trials must use characterized field soil from the intended region and maintain its indigenous community. A structure of factorial is preferable. One factor represents inoculation (control, single strains, pairs and triad) while another represents fertilizer regime, for example 0%, 50%, 75% and 100% of recommended P with deliberately justified N regime. For legumes, uninoculated full-N offers a yield target, but high starter N should not be applied willy-nilly because it can repress nodulation.

It is recommended that four to six biological replicates be employed based on expected variation and power analysis. To regulate the greenhouse gradients, pots should be randomised and shifted at intervals. When feasible, the investigators who will be assessing biomass, nodulation, and chemical endpoints should be blinded to the treatment codes. Data collected at repeated measurements should use mixed effects models rather than conducting separate ANOVAs for each date.

The key criterion for transition must be a notable departure from the uninoculated control. In order to fit the outline of the triad of management practices mentioned in the last chapter, a new inoculant should show one or more pre-declared benefits: high yield or biomass, equal performance at lower fertilizer dose, more stable performance during a stress treatment, greater N or P recovery, greater persistence at no extra ecological cost.

8.3 Tier 3: multi-environment field trials

Field validation should include multiple sites and seasons because soil pH, texture, P buffering, resident rhizobia, rainfall, temperature and management history can reverse inoculant effects. A randomised complete block design may suffice on site that is relatively homogeneous; if many treatments are anticipated or there are strong spatial gradients, alpha-lattice or incomplete-block designs are preferable. It is important to inform the blocking, dimensions of the plot, border rows, sowing density, inoculation method, fertilizer placement, irrigation, and use of pesticides.

The minimum treatment set in the field should consist of an uninoculated control; recommended fertilizer practice; each single strain; best-performing pair; the triad; and the triad with one or more reduced fertilizer regimes. An experiment needs to be designed for non-inferiority or equivalence and not only superiority over an unfertilized control, if the claim is fertilizer displacement. Testing a pre-specified non-inferiority margin for yield, plus economic and nutrient-use outcomes, has greater decision relevance.

The primary endpoints should be few in number and announced ahead of time normally, it is grain or marketable yield, plant N and P uptake, and for a legume, biological N fixation. Secondary endpoints could be emergence, biomass, nodulation, chlorophyll, root traits, soil available N and P, enzyme activity, microbial abundance and product quality. Control of multiple testing's been happening. If we want our inference to apply beyond our sampled sites, we should treat site and year as random effects in a mixed model.

8.4 Track the inoculant, not merely the response

Without evidence that the strains did survive and occupied the relevant niches, the causal link between the intended consortium and the field response cannot be established. To assess rhizobial nodule occupancy strain, one must use specific molecular markers. The

abundance of Azotobacter and PSB should be monitored in the rhizosphere and, where applicable, the rhizoplane or within roots. At emergence, flowering, peak nodulation, and harvest, sampling can change transient exposure to persistent colonisation.

It's better to have absolute abundance than relative amplicon-sequencing abundance. A strain may seem to increase proportionately when the total biomass decreases of microbes. Community profiling should, therefore, be complemented by qPCR, digital PCR, selective counts, or internal-standard metagenomics. A functional-gene abundance cannot be taken as an indication of activity; transcripts, proteins, metabolites, isotope tracing, or process rates would be needed for such a claim.

8.5 Soil fertility must be measured as a trajectory

The absorption of nutrients by crops that experience enhanced growth may deplete available soil nutrients more rapidly. As a result, the soil test after harvest is lower despite enhanced uptake of nutrients. On the other hand, an increase in extractable P may reflect mobilization without plant uptake, thereby increasing loss risk. Consequently, soil-fertility interpretation should integrate initial and post-harvest pools, plant removal, fertilizer input, microbial biomass, and residual nutrient fractions.

When N is involved, measurement of mineral N, total N (where justified), potentially mineralisable N, plant N removal and N derived from atmosphere. When the mechanism is central, measure an agronomically appropriate available-P test plus selected P fractions for P. Soil health may be more than just a cover crop or farming practice, but a series of short trials should limit claims of improving soil health in a lasting way.

Table 3. Recommended experimental treatment structure

Code	Inoculation treatment	Fertilizer context	Purpose
C0	No inoculant	No N or P beyond basal non-test nutrients	Biological baseline
CF	No inoculant	Recommended fertilizer practice	Conventional agronomic benchmark
AZ	*Azotobacter* alone	Defined reduced-N/P regime	Isolate free-living diazotroph effect
RH	Compatible rhizobium alone	Defined low starter N and P regime	Isolate symbiotic effect
PS	PSB alone	Defined P regime	Isolate P-mobilisation effect
AZ+RH	Pairwise consortium	Same nutrient regime	Test interaction between N-fixing guilds
AZ+PS	Pairwise consortium	Same nutrient regime	Test free-living N-P complementarity
RH+PS	Pairwise consortium	Same nutrient regime	Test P support of nodulation and BNF
TRI	Three-member consortium	Same nutrient regime	Test net consortium effect
TRI-R	Three-member consortium	Reduced fertilizer, e.g., 50-75% of target nutrient	Test fertilizer-displacement claim
TRI-F	Three-member consortium	Recommended fertilizer practice	Test additivity and possible fertilizer inhibition

9. Evidence from Crop Studies: What Can and Cannot Be Concluded

9.1 Legume systems provide the clearest biological rationale

The most compelling justification for the suggested triad happens to be a legume cultivated under

N and P constraints taken together. Chickpea is the most-promising of the first-validation cropping options with evidence from the field supporting the same three functional guilds. According to a study conducted in India by Wani et al (2007), field-grown chickpea improved on inoculating with M. ciceri-A. chroococcum-PSB as evidenced with improved nodulation, nutrient uptake and yield. Later work on chickpea showed that M. According to Benjelloun and colleagues (2021), ciceri alongside carefully chosen PSB could yield significantly greater responses than one-off inoculation. Bacillus co-inoculation improved yield and nodulation of chickpea, but its response varied with strain and environment Elkoca et al. 2008

Counter-evidence gives us equal instructions. In an indigenous chickpea program, the Mesorhizobium-Pseudomonas pair yielded better than the Mesorhizobium-Azotobacter-Bacillus triad (Verma et al., 2013). Chickpea is thus the most defensible provisional focal crop, but the exact triad must still win

out on its complexity against the best pair. If chickpea adoption is happening, the rhizobial component should normally be identified and selected as a compatible Mesorhizobium ciceri strain than treated as a generic Rhizobium.

Soybean research also backs N-P co-management. In their pot-and-field research, Afzal et al. (2010) found out that co-inoculation with N-fixers and P-solubilizers improved yield and seed nutrient status. However the field response was smaller than the pot response. It's normal these are weaker as there is competition, spatial heterogeneity, weather, nutrient buffering from field soils. A meta-analysis has confirmed that rhizobial inoculation can improve the traits of soybean. However, actual improvement phenotype will depend on the identity of the inoculant, the edaphic conditions and the experimental framework (Thilakarathna & Raizada, 2017).

The common bean and other promiscuously nodulating legumes face another challenge in that resident rhizobial populations may occupy their nodules and not fix N efficiently. In these systems, a consortium can improve both P availability and root growth but fails to enhance N delivery if the inoculant rhizobium loses the occupancy contest. The count of nodules may increase without any change to the grains N or yield. It is essential to have competitive occupancy as an endpoint.

9.2 Evidence supporting some components, not the title, from non-legumes

Azotobacter and phosphate-solubilizing bacteria (PSB) have been found to promote the growth of cereals as well as horticultural crops. Azotobacter strains that are tolerant to salinity stimulated the growth of wheat (Chaudhary et al. 2013), and PSB influenced the characteristics of wheat roots, P nutrition, and associated diazotrophic activity (Elhaissoufi et al. 2020; Li et al. 2020). A formulated combination of PSB increased yield and P availability in potato in multi-season field testing (Bansal et al., 2025). The relevant functional guilds are supported by these studies, but the involvement of a rhizobial component for a non-legume is backed only if the rhizobium has independent validation as a beneficial endophyte or plant-growth-promoting agent, and the claim is qualified as such.

The most scientifically coherent primary target for the precise consortium title is therefore a legume, particularly chickpea, pea, lentil, common bean, mung bean or soybean with the appropriate symbiont. A cereal or vegetable may be chosen as a rotational secondary crop to examine residual soil effects, but it should not be prescribed to infer rhizobial symbiotic N fixation.

9.3 Quantitative syntheses imply heterogeneity

A worldwide meta-analysis indicated that biofertilization could positively enhance crop yield and nutrient-use efficiency, with the response conditioned by climate, soil properties, crop group and inoculant function (Schütz et al., 2018). Averages should inform what to expect, not replace local trials. The responses being distributed have failures and negative affect. Publication bias, effects related to small studies, differences in product quality, and inconsistent reporting inflate beliefs about reliability.

Inoculation benefits of rhizobial meta-analyses are not consistent. Even if average yield gains are positive, the ultimate outcome will depend on factors such as host genotype, resident rhizobial abundance, soil pH and P status, inoculum dose and management. The question of concern is not if microbial inoculants work but rather whether this defined consortium works predictably for the specified crop and recommendation domain.

10. Mechanistic endpoints and analytical methods

10.1 Conversion and sending of nitrogen

Growing on N-free medium is for enrichment and not a quantitative test. Acetylene reduction is a highly sensitive measure of the ethylene produced by nitrogenase, but the conversion of ethylene to fixed N is system dependent and can be affected by gas diffusion and oxygen, incubation time, and hydrogen evolution. It is most suitable for comparative screening under standard conditions. Confirmation methods should either use 15N methods or a N balance which is defensible in whole-system terms.

Rhizobia should take into account not only nodule number, nodule dry mass, leghemoglobin and nitrogenase activity but also shoot N, total N, nodule occupancy. The percentage of N from the atmosphere and amount of total fixed N answer different questions. For instance, a high percentage of N in a small plant could represent less N in total than a moderate percentage in a larger high-biomass plant. Both conclusions are not misleading interpretations.

10.2 Mobilisation And Delivery Of Phosphorus

Quantitative P assays need to include the source of insoluble P, buffering of the medium, incubation time, inoculum density, biomass final pH and certainty of

the method. The molybdate-blue method is extensively used, but matrix chemistry can affect it; calibration and uninoculated controls are necessary (Murphy and Riley 1962). The mechanism can be supported by organic-acid profiling (HPLC or LC-MS), while gene and transcript assays can test gcd, pqq, phosphatase, phytase or C-P lyase hypotheses.

Tests for available-P used in soils and plant trials should be consistent with soil chemistry and regional practice. Olsen P can sometimes be used in neutral to alkaline soils, while Bray or Mehlich extractants may be more appropriate elsewhere. Different extractants yield results that cannot be interchanged. Plant phosphorus (P) uptake calculated from tissue P concentration multiplied by biomass is often more informative than P concentration alone, because the increased growth with nutrient addition dilutes P concentration but increases total uptake.

10.3 Root colonization and community response

The colonization of roots should be assessed in both space and time. Rhizosphere counts do not demonstrate attachment to the rhizoplane or nodule occupancy. Strain-specific qPCR, fluorescence in situ hybridization, confocal microscopy, contained-use tagged strains, and recovery from surface-sterilized tissues distinguish the niches. While amplicon sequencing can reveal major shifts in communities, it often lacks strain resolution and provides measures of relative abundance.

A stated ecological theory should be tested through microbiome analysis. Descriptive complexity results from sequencing every treatment without linking community changes to function. Some useful hypotheses are that the consortium; (i) displaces beneficial native taxa;(ii) enriches P-cycling genes; (iii) alters network stability; and/or (iv) leaves a legacy that is present after harvest. Compositional data should be accompanied by absolute microbial load, functional measurements and soil chemistry.

10.4 Plant Performance And Nutrient Use Efficiency

There should be a progression of agronomic endpoints starting there at emergence and root traits until biomass and phenology. The terminology of nutrient-use efficiency should be defined. Experiments targeting the different processes underlying these measurements will require different controls. A consortium will be able to increase the yield per unit of fertilizer applied without an increase in recovery, if it modifies root architecture or water relations.

A broad economic analysis will include inoculant production, storage and transport, application labour, altered fertilizer cost, value of additional yield, risk of non-response. An increase in biomass that is statistically significant and small, may have no farm level value. Yield equivalence at a lower fertilizer rate can offer economic and environmental benefits, even if it does not outperform full fertilizer.

11. Mathematics, Statistical Logic, Causal Attribution and Decision Thresholds

The complete biological design is a 2^3 factorial experiment, which crosses the presence or absence of Azotobacter (A), rhizobium (R), and PSB (P) under the same environment and nutrient conditions. This design provides an estimate of the average contribution of each organism, as well as the degree to which its effect depends on the others. It is not enough for the labels to be about treatment; the design must preserve factor structure. A linear model for the response consists of main effects for A R and P; pairwise interactions A R A P and R P; and three-way interaction A R P. In case inference is intended to be drawn out to non-sampled environments then block site season and their justified interactions enter as random effects for field experiments.

The three-way contrast is the difference between the observed triad response and the response that would be expected given all lower-order components. If the same response is generated by the top single strain or a combination of two strains, then a positive triad average cannot be considered evidence of synergy. The term “synergy” should be limited to a positive interaction contrast, building against a zero overlap uncertainty interval, which is large enough to be agronomically relevant. A competitive interaction may occur alongside a net benefit; for example, the triad may surpass an uninoculated control while underperforming an effective rhizobium-PSB pair. A two-member product is supported by this kind of result, not a three-member product.

Mixture ratio tests are different from presence-absence tests. The factorial design establishes which members are necessary; a constrained mixture design then establishes their proportions at a fixed and total inoculum. Optimization needs a desirability function that relates yield or nutrient uptake to individual survival and functional retention. Merely enhancing plant biomass has the potential to choose an unstable ratio, with one organism dominating.

There is mutual relationship between microbial counts, soluble P, root colonisation, and plant growth. Observations collected repeatedly from the same flask, pot, plot or site are correlated and should be analysed using mixed-effects or generalized mixed-effects models. Data on colony-forming units (CFUs) are typically subjected to log transformation or a specific counting model. Meanwhile, populations of nodules can be overdispersed and would fit a negative-binomial model. In this case, the nodule occupancy is binary. Lastly, the endpoint relating to time-to-population-loss can be regarded as a survival one. Residual diagnostics should dictate the final model and not an automatic transformation convention.

The experimental unit is the entity randomly selected independently. When multiple plants are planted in one pot, or one plant produces multiple nodules, or one technical PCR well is split into several, or one extract generates multiple chemical readings, these are subsamples, not independent replicates. It results in inflated estimates of precision and pseudoreplication. Averaged or included at appropriate nested level analytical repeats. It is necessary to model the greenhouse position, field block, batch and assay plate when they produce systematic variation.

The number of samples is based on one main endpoint, a biologically meaningful effect, expected variance, desired power and planned model. A factorial design which has many treatments isn't automatically well powered for interaction effects as interactions require generally more information than main effects. It is important to interpret pilot variance estimates with caution because small pilots underestimate uncertainty. Power analysis with simulation is appropriate for multi-site mixed models in non-inferiority designs.

Confirmatory inference should be anchored by a primary agronomic endpoint. Only a few key secondary endpoints can be employed to test the causal pathway. These are nodule occupancy, N derived from the atmosphere (NDA), and total plant P (TPP). Big panels of enzymes, metabolites, genes, and community taxa are exploratory and require false-discovery control or explicit hierarchical interpretation. Results should report estimated effects, 95% confidence intervals, and raw distributions instead of reporting just letter-group displays and p values.

If the claim is that the consortium will allow a lower N or P dose, the decision question will be whether reduced fertilizer plus inoculant retains an acceptable proportion of performance achieved by recommended fertilizer. A non-significant difference does not imply equivalence. Developers need to specify a non-inferiority margin that reflects agronomic risk before trialling the trait. This margin may be expressed as the upper limit of yield loss, which farmers are willing to accept in return for lower input cost or reduced environmental burden. The yield difference's confidence interval must then be higher than that negative margin.

If it is accomplished by mining soil nutrient reserves, a non-inferior yield is inadequate. A fertilizer-displacement claim should be backed by evidence of plant nutrient removal, crop post-harvest soil pools, partial N and P balances, and residual effects, and ideally, any benefits to the following crop. For any legume, the credibility of reduced mineral N only arises when biological N fixation and plant N acquisition have been demonstrated. For P, a short-term increase in extractable soil P shall not be viewed as improved P-use efficiency unless there is evidence of plant uptake and an acceptable residual balance.

The following is a defensible causal chain: inoculum delivered -> each strain survives -> the strains colonize their target niches -> target functions are expressed -> N and P become available at the root -> the plant acquires them -> growth/yield change. Insufficient efforts are made to measure intermediate steps. More biomass might arise from auxin-mediated root growth rather than fixed N; higher amounts of soluble P in a flask might not actually reach roots; more nodules might be formed from resident rather than inoculant rhizobia; and a changed microbiome might be a consequence rather than a cause of vigorous growth.

To strengthen attribution in mechanistic experiments, isotope tracing, strain-specific occupancy assays, targeted metabolites and temporal sampling can be used. Although still a useful way of ranking nitrogenase activity, the acetylene reduction reaction should not be converted to N fixed using a universal factor. Hardy et al. (1968) Where N contribution is a major claim, ^{15}N methods provide stronger whole-plant evidence (Unkovich et al., 2008). It is possible to measure nodule occupancy by validated strain-specific molecular assays (Osei et al., 2017). There is no need for these measurements to appear in every commercial trial, but the development programme must show the claimed mechanism operates in representative soils.

12. Failure Modes, Biosafety, and Product Stewardship

12.1 Predictable routes to failure

The most informative negative result is one that identifies the failed link in the causal chain. Consortium development should therefore use diagnostic rejection rules rather than repeatedly testing poorly characterised mixtures. Table 4 connects common field outcomes to competing explanations and corrective actions.

Table 4. Diagnostic interpretation of major consortium failure modes

Observed outcome	Most plausible explanations	Diagnostic evidence	Corrective decision
High counts in package, low counts on seed	Desiccation, pesticide toxicity, osmotic injury, long coating-to-sowing interval	Member-specific CFU immediately and 2-24 h after coating; pesticide-contact assay	Change polymer or application route; separate chemical and biological treatments
Stable total CFU, loss of one member	Differential decline masked by aggregate enumeration	Strain-specific culture or viability-qPCR during shelf life	Reformulate, alter starting ratio, shorten expiry, or use separate sachets
Good plate compatibility, poor co-culture	Resource competition, pH drift, siderophore-mediated exclusion	Time-series abundance, pH, oxygen, metabolites, spent-medium tests	Adjust medium/ratio or replace antagonistic strain
Strong P halo, no plant P gain	Unrealistic P substrate, soil buffering or readsorption, poor colonisation	Soil-specific P fractions, organic acids, root colonisation, total P uptake	Re-screen with target soil and P forms; reject plate-only specialist
Many nodules, little plant N	Ineffective nodules or occupation by resident strains	Nodule occupancy, shoot N, %N derived from atmosphere, relative effectiveness	Replace rhizobium or improve competitive delivery
Pot benefit, no field yield response	Resident microbiome resistance, weather, nutrient sufficiency, low persistence	Baseline rhizobial population, inoculant tracking, site covariates, absolute yield	Restrict recommendation domain or reject broad claim
Yield benefit only at zero fertilizer	Inoculant cannot function under farmer nutrient practice; mineral N represses fixation	Nutrient-gradient interaction, nodulation and N-fixation measures	Reframe for low-input systems or redesign nutrient strategy
Early vigour, no harvest benefit	Hormonal effect without sustained nutrient delivery; later resource limitation	Time-series biomass, N/P uptake, population persistence, yield components	Do not claim yield enhancement; identify limiting later-stage process
Yield maintained but post-harvest nutrients fall	Increased nutrient mining rather than improved fertility	Full nutrient balance, residual soil pools, following-crop response	Withdraw soil-fertility claim; adjust fertilizer replacement rate

The mismatch between hosts and competition among residents merits particular focus. The competitiveness and fixation efficiency of rhizobia are independent traits (Bourion et al., 2018), whereas small indigenous populations can smother the reaction of an introduced strain (Thies et al., 1991). Pairwise competition can favour a poorly beneficial and fast-colonising rhizobium; this can reduce benefits to the host. On the other hand, structured diversity of beneficial strains can enhance host growth for specific host-genotype combinations; thus, the very value of diversity is itself conditional (Mendoza-Suárez et al., 2024). Therefore, using a high inoculum dose or a more diverse mixture does not substitute the need to select competitive strains compatible with the crop and check their occupancy.

12.2 Biosafety should be specific to strains

Agronomic traits must be combined with acceptable risk. To be elite, candidates should display an unambiguous taxonomic identity, a high-quality whole-genome sequence, and the absence of virulence, toxin and acquired antimicrobial-resistance determinants of concern. Members of closely related taxa may differ sharply in risk; several more genera that are often reported as PSB include opportunistic human, animal or plant pathogens. The Bacillus cereus group, Pseudomonas aeruginosa, Burkholderia cepacia complex, Klebsiella, Enterobacter and Serratia's isolates should receive special attention. A “plant-growth-promoting” status at the genus-level is not a safety assessment.”

Whole-genome screening must encompass assembly quality, contamination, average nucleotide identity, mobile elements, plasmids, secretion systems, known toxins, virulence factors and antimicrobial-resistance genes. Tools like AMRFinderPlus check known resistance determinants; however, a negative database result does not prove absolute safety

(Feldgarden et al., 2019). Genomic evidence must include relevant phenotypic tests as per BS-EN standards, e.g. susceptibility, haemolysis, cytotoxicity, phytotoxicity and environmental-persistence tests. The general WGS logic followed by the EFSA for intentionally applied microorganisms‘ identity plus systematic evaluation of traits of concern provides an interesting assessment model for risk assessment, even when the product falls under a different legal regime (European Food Safety Authority, 2024).

Field-released antibiotic-resistance markers should not be introduced via strain tracking. Genomic places that are naturally discriminating , strain-precise qPCR or digital PCR , and nonantibiotic tags in contained mechanistic studies are most preferable . Environmental monitoring should verify the persistence of the GM crop beyond the lifecycle of the crop, interactiveness (potential for horizontal-gene transfer if mobile elements are present), an impact on representative non-target functions, and does its application alter the (pathogen) abundance or (antimicrobial-resistance) profile.

12.3 Evidence of efficacy is not a minimum evidence

In India, the development of the product must comply with the Fertiliser (Control) Order as amended and applicable standards. According to Bureau of Indian Standards (2023), standard relevant documents are as follows: IS 8268:2020 on rhizobial inoculants, IS 9138:2020 on Azotobacter (whose liquid counterpart is IS 17134:2020), IS 14807:2021 on phosphate-solubilizing bacterial inoculants (liquid IS 17135:2019), IS 17137:2019 on akal-like bacterial inoculants (liquid equivalent IS 17136:2020, and IS 17755:2022 on consortia of microbial inoculants. At the registration and batch release, check the amendments and the complete standard texts from the Gazette.

Field performance is not determined by any legal minimums, such as viable count, contamination limit, pH, moisture, plate-zone activity, or positive nodulation test. Each individual on a scientific specification must define the strain identity, member-specific viable counts at expiry, retained functional potency, formulation homogeneity, seed survival and performance in the recommended stated domain. The label must mention the compatible crop or crop group for the rhizobial component, application route, dose, storage conditions, expiry, pesticide restrictions, and evidence-based fertilizer regime.

13. Making crops bio-efficient for sustainable production

13.1 Set the scope of recommendations

The reliability of microbial product claims, particularly those made by companies, depends on the context, particularly the use. The recommendation domain should specify compatible legume species and cultivars, soil pH and salinity range, relevant P status and dominant P forms, climate or moisture regime, baseline resident rhizobia where known, fertilizer background, cropping history, seed treatment and application method. A reaction model can consequently differentiate circumstances that hold a high likelihood of gaining from those which the product will not refund its expenditure.

Evidence from local rhizobia illustrates this principle. Native common bean rhizobia have been competitive when true formulation and local adaptation were addressed (Pastor-Bueis et al., 2019); indigenous soybean symbionts have enhanced yield, but strain × cultivar and environment effects gn0020966aed remained significant (Omari et al., 2022); and extensive multi-location faba bean work has demonstrated location × strain × cultivar interaction (Allito et al., 2021). The data supports local selection while the stronger assumption that being native is a guarantee for broad adaptation didn’t hold.

13.2 The suitable objective is integrated nutrient management

An inoculant can enhance fertilizer recovery, allow a precise reduction in one nutrient or stabilize performance under stress without replacing the entire external input. The three guilds themselves also remain resource dependent: Azotobacter requires carbon and micronutrients, rhizobial fixation requires a compatible host and adequate P, and PSB require appropriate carbon and an accessible P substrate. Excessive mineral N has the potential to suppress nodulation and diazotrophy, while serious P deficiency can hinder root growth and the energy supply needed for N fixation. Recent functional and proteomic work in A. The process of diazotrophy and nitrogen limitation in chroococcum are tightly coupled and not independent functions (Biełło et al., 2023).

Recommendations for fertilizer use, therefore, should be developed through response surfaces, not single treatment. In soils moderately deficient, consortium may offer the greatest value. Extremely deficient soils still call for corrective fertilization while nutrient-rich soils may show little response. The

expression of the functions of the inoculant depends on micronutrients, pH correction, organic matter management, water availability and placement.

13.3 Examining sustainability through various assessable outcomes

The term "Sustainable Crop Production" is not defined by yield increase in a single season. The claim should at least include agronomic productivity, nutrient balance, input displacement, economic return and absence of unacceptable environmental or biological effects. Measure nitrate leaching, N2O emissions, energy use, climate impact and greenhouse gas intensity per unit product, wherever feasible. In the early tests, if the direct measurement is impossible, consider these outcomes as untested and not beneficial ones.

The farm evaluation should report yield, product quality, cost of inoculant and application, saving in fertilizers, net return, and probability of non-response. Storage and labour needs to be estimated in a partial budgeting. The risk issue is important. A treatment that has a higher average return but suffers more frequent large losses may be less likely to be adopted than one that offers a smaller but more consistent benefit. Mixed models, as well as stability, statistics can quantify this trade-off in different environments.

Proving long-term soil-fertility claims requires multiple seasons at the least, residuals measurements, and a following crop. A legume-phase consortium may add to a rotation through biologically fixed N, changes in N and C residues, improved P acquisition or root-derived carbon. The right rotational comparison is not just inoculated versus uninoculated legume. It should account for nutrient inputs; harvested outputs; residue management; response of subsequent crop; and change in soil pools. Changes in soil organic carbon typically necessitate extended observation exceeding the duration of one season in a pot or field.

14. Experimentation Priorities and Knowledge Gaps

To begin with reference to constraint,N and P acquisition interaction must be quantified. Many studies measure diazotrophy or P solubilisation in isolation, but the consortium hypothesis depends on their coupling. Using a combination of isotopes, metabolites and time-based expression can allow us to uniquely differentiate whether P either mobilizes to relieve an energetic constraint on N fixation or P simply mobilizes due to enhanced growth.

Moreover, strain identity and function should be examined at the appropriate level. A brief 16S rRNA sequence is not sufficient for disentangling elite-strain identity or taxonomic distinction from horizontally transferred symbiotic genes. Analyses of multiple genomic locations as well as full genomic sequences are to be correlated with the occurrence of reinfection, fixation, and the phenotype of P-delivery (Menna et al., 2009; Tan et al., 2012). To ensure reproducibility, public genome deposition and authenticated culture-collection accessions are necessary.

Third, rhizosphere ecology should shift from being relative-abundance based to absolute dynamics which are strain-resolved. Communalities defined strain identity and higher-order interactions can drive plant phenotypes (Herrera Paredes et al., 2018; Niu et al., 2017). For ag consortia it is not depth that counts alone, but an experimentally testable relationship with dose, establishment, function and outcome.

Combining formulation research with ecology should be installed. Aloo et al. (2022) and Albareda et al. (2008) show that carrier composition, storage temperature, and time produce organism-specific survival trajectories. Future studies could investigate whether delivering separately (microencapsulation, spatially structured granules, or protective polymers) could better maintain complementarity than a homogeneous liquid. Validation of shelf-life models must involve real-time storage and temperature excursions representative of distribution systems.

Simply averaging responses is not enough in multi-environment trials. Fifth, we should learn why responses differ. Moderators to record should include baseline soil chemistry, resident rhizobial abundance, weather, cultivar, fertilizer regime, and inoculant persistence. Wheat, soybean, common bean and cowpea indigenous strain programs show strong promise but also large strain and environment specificity (Kızılkaya, 2008; Pastor-Bueis et al., 2019; Omari et al., 2022; Simbine et al., 2021). There was failure of even a mechanistically agreed-upon PSB to enhance wheat acquisition of ^{33}P-labelled hydroxyapatite in buffered calcareous soil, reporting laboratory to soil discontinuity (Meyer et al., 2019). The predicted probability of response is more useful than a single pooled mean.

Ultimately, the field requires clear negative evidence. Product-development studies should, where feasible, preregister their primary outcomes, publish exact strain identities and full treatment means, report failed formulations and incompatible combinations,

and deposit analysis code. This will minimise selective reporting and make it clear when a concept is biologically weak and not merely effective but compromised by formulation, delivery or environment.

15. Current synthesis' limitations; possible extensions

This is a review and development framework. It is not a report on a completed isolation campaign or crop trial. No strain accessions, crop variety, sampling coordinates, soil database, formulation batches, raw observations or field results were made available. As such, it claims not to identify a particular indigenous consortium, that more than three organisms are compatible and improve yield or soil fertility. Their proof requires the prearranged evidence described above.

Though the title incorporates the user-selected term Rhizobium, current root-nodule symbionts belong to several genera.

The final name should reflect the identity of host compatible strain. Proof can differ between crops, soils, inoculant compositions and experimental scales, with some cited systems containing Bradyrhizobium, Mesorhizobium, Azospirillum or PSB genera that are mechanistically informative, but different to that of the product. What their results do not establish is efficacy of this untested formulation.

16. Conclusion

It is biologically plausible to use an indigenous azotobacter-rhizobium-psb consortium because it can couple free-living and symbiotic N fixation with P mobilisation, root stimulation and stress buffering. However, this complementarity is conditional, not automatic. A seemingly favourable mix can be made neutral or antagonistic because of host specificity, resident nodule competitors, soil P chemistry, carbon and oxygen supply, formulation stability, strain ratio and management.

A trustworthy development program should aim at a legume-soil-management target. In addition, it must authenticate and genome characterise each strain. It should also demonstrate crop-relevant N and P functions. Furthermore, any unsafe or antagonistic candidates must get rejected. Member-specific survival must get quantified. Optimize ratio and delivery and validate the product in factorial, nutrient-gradient, multi-site, and multi-season experiments. The key criterion is not growth on selective media, number of nodules or bigger seedling. The delivery of nutrients and agronomic value over time is comparable to the best regulator and appropriate fertilizer benchmarks with acceptable biosafety, shelf life, economics, and nutrient balance.

Unless that data ever comes to light, we should call the consortium an evidence-based development candidate, not a verified biofertilizer. After the evidence package is completed, the same framework offers a clear path to a crop-specific quality-controlled product claim and a robust assessment of the soil fertility and sustainable crop production contributions.

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