SUPPORTING THE FORMULATION OF STABLE, SUSTAINABLE MICROBIAL PRODUCTS FOR SOIL AND PLANT HEALTH

Plant-based products from Roquette answer directly to requirements for high performance formulation ingredients with a low environmental footprint.

The population of microbes in just 5 ml of soil exceeds the number of people on earth,[1] and there is well-established recognition of their beneficial effects on plant health and crop yields. Indeed, the commercial use of microbial inoculants, also known as biorational solutions, biofertilizers, or simply biologicals, dates back to 1896 and the introduction of Nitragin,[2] a nitrogen-fixing product.

In the intervening years, our understanding of the role of microbes, and our ability to develop technologies that manipulate them, have advanced considerably.

Today, increasingly sophisticated products, often containing multiple strains, have a **crucial role to play in optimizing plant microbiomes** thereby helping to increase land productivity while simultaneously reducing reliance on agrochemicals.

Statistics for soybeans, the **heaviest inoculant-consuming crop worldwide**, illustrate the potential. In Brazil, yield increases of around 16% have been observed following co-inoculation with *A. brasilense* and *Bradyrhizobium spp*, and close to 80% of the country's soybean cropping area is now treated annually;[3] state-of-the-art products simultaneously eliminate any requirement for synthetic nitrogen fertilizers.

Such success helps to rationalize predictions of substantial growth in the agricultural inoculant market with estimates suggesting a compound annual growth rate (CAGR) of 10.6% for the period 2020 – 2027.[3]

Identifying microbial strains that can deliver valuable gains is an essential first step towards a successful product. However, **optimal formulation is equally important**, though the topic has historically received far less attention.

The need is for formulations that are stable, cost-effective, high quality and easy-to-apply, and that deliver consistent and marked improvement in the field.

Given the centrality of microbial products in efforts towards greater sustainability, and their role in organic cultivation, the provenance and sustainability of formulation ingredients is a critical concern.

Roquette, a global leader in plant-based ingredients, has developed a range of **plant-derived products that can be used to formulate and manufacture stable biological formulations**.

Collaboration with external partners and experts supports the ongoing refinement of these products to meet **specific applications and the requirements of defined processing routes**. They allow customers to substitute mineral and synthetic chemical ingredients with **natural alternatives** to deliver effective bioformulated inoculants.

In this article, we consider **requirements for formulation** and present experimental data illustrating the **potential of Roquette ingredients, using formulation of a product made by fluidized bed drying as an example**.

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Offering the best of nature™

DEVELOPING EFFECTIVE MICROBIAL PRODUCTS

Microbes play a diverse and complex role in plant cultivation with two clear areas of application. Products that fall under the general classification of **biofertilizers/biostimulants** directly improve plant growth and vigor.

Rhizospheric microbes enhance **nutrient uptake** via well-understood routes such as nitrogen-fixing and phosphorus solubilization while endophytic microorganisms can promote plant metabolism boosting growth and/or improving resistance to stress.

Other microbe-based products can be loosely grouped under the "**biocontrol"** heading and include biopesticides, biofungicides, bioherbicides and bioinsecticides.

Both types of microbial product have potential to support efforts to **improve or maintain crop yields while reducing reliance on synthetic chemicals**, and interest in their use is intensifying as growers target greater sustainability.

Via the application of techniques such as next generation sequencing and metagenomics, our understanding of the plant microbiome is increasing rapidly, providing a foundation for the more effective use of this powerful technology.

Developing a successful commercial biocontrol product from a promising microbial strain is a substantial challenge, and success rates are currently relatively low.

Transfer into the field is complicated by the fact that commercial products are used under very different conditions from those applied during laboratory studies. They are added to a **unique microbiome**, in an environment that varies considerably with respect to important parameters such as **salinity, temperature and humidity**.

Appropriate formulation is vital to success and should ensure safety, ease-of-application, consistent product quality and sustained impact in the soil. Key goals[4] are to:

- **Protect the microbial strain**, delivering the microenvironment needed to support cell viability over a prolonged period such that the product offers a commercially acceptable shelf life and produces a viable microbe population at the point of use.
- **Promote proliferation of the microbial strain** to ensure high soil–plant colonization and the establishment of a persistent population in the prescence of competing native microorganisms
- **Reduce losses at the point of use** as a result of depredation by microfauna.

Microbes may be delivered in solid or liquid form, but solid formulation, often for dissolution at the point of use, has some important advantages.

Solid products weigh less, reducing the cost of shipping, and are also less prone to contamination. **Solid formulation strategies are therefore an important focus** that calls for appropriate ingredients to optimize processes such as freeze drying, fluidized bed drying, spray-drying and granulation.

USING PLANT-BASED INGREDIENTS TO SYNTHESIZE STABLE MICROBIAL FORMULATIONS.

Roquette offers a portfolio of plant-based ingredients for the formulation of stable microbial products that are made by processing crops such as corn, peas, potatoes, and wheat (see figure 1) via the following steps:

- Solubles extraction, via steeping (extended contact with water)
- Fermentation
- Enzymatic hydrolysis
- Separation, including filtration and centrifugation
- Concentration, notably low temperature drying.

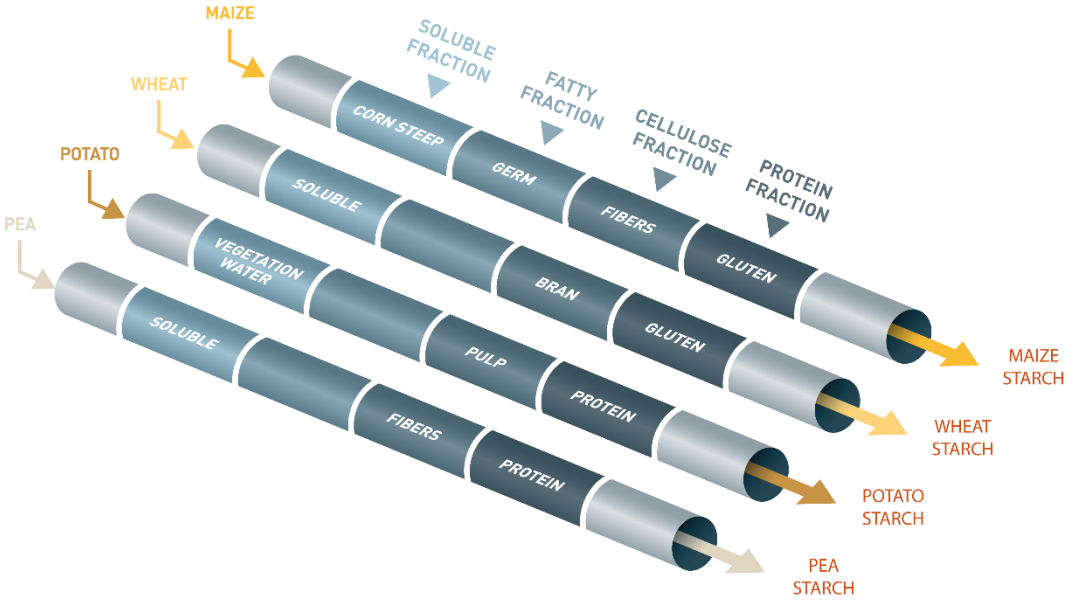

Figure 1: Roquette produces highly consistent products for microbial stabilization via the progressive refining of plant feedstocks.

This portfolio includes maltodextrins (GLUCIDEX®), microcrystalline cellulose (MICROCEL®), dextrose and a range of polyols notably sorbitol (NEOSORB®) and mannitol (PEARLITOL®).

All are derived from **renewable plant-based feedstocks of known provenance**, making them well-aligned with the efforts towards greater sustainability that microbial products support. Furthermore, there are OMRI (Organic Materials Review Institute) compliant options for those that need them.

It is crucial to note that these products are manufactured using closely controlled, often patented, processes that ensure a highly consistent product, even in the face of feedstock variability. Operating solvent-free and under relatively benign conditions, these processes have a rigorously minimized environmental impact.

In the **development and manufacture of stabilized microbial formulations**, Roquette products can act as:

- High performance, biodegradable, readily soluble drying supports
- Water absorbent fillers that promote granule disintegration
- Binders, flow and extrusion aids, and compression agents to improve the efficiency of wet and dry granulation processes
- Stable fillers/bulk excipients with excellent biocompatibility
- Cryoprotectants for use in freeze drying applications
- Functional ingredients for the reduction of water activity

Many products offer **multifunctionality with respect to microbial stabilization** and at the same time have been securely associated with other beneficial outcomes.

For example, polyols, even in the absence of microorganisms, have been linked with **increased resistance to abiotic stress**. Each microbial stabilization challenge is unique, but with this portfolio, formulators have an excellent core toolkit for the formulation of inoculants that combine high performance with a low carbon footprint. The following study illustrates what is achievable.

CASE STUDY

FORMULATING MICROBIAL ISOLATES (BACTERIA) ON AN INERT, BIO-BASED CARRIER MATERIAL BY FLUIDIZED-BED DRYING

A study was carried out to identify ingredients to make a **biocontrol formulation for spray application**. Key aims were to achieve a shelf life of at least 12 months with a final microbial cell count of 10^9 CFU/g (Colony Forming Units/g); the minimum acceptable level was 10^8 CFU/g. In addition, the formulation was required to be water soluble or able to pass through sprayer nozzles.

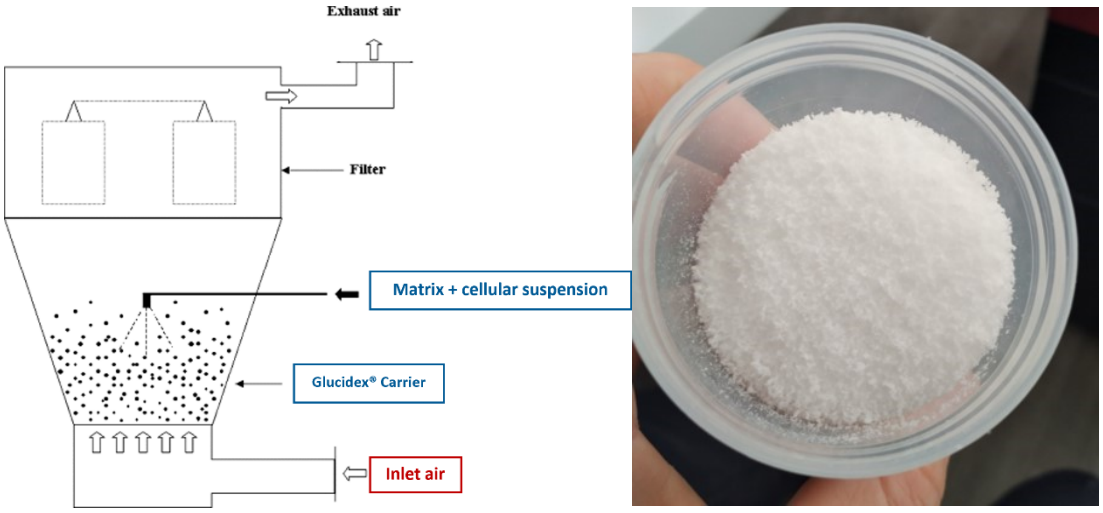

Figure 2: Schematic of a fluidized bed dryer set-up for microbial stabilization studies (left); an example product from the study (right).

Cellular suspensions of the bacteria were made up from samples produced via a process of cell culturing, inoculation, and centrifugation. These were mixed with different matrices at concentrations of 10, 20 or 30%, to produce formulations which were subsequently applied to an inert carrier, GLUCIDEX® IT 19 maltodextrin, using a table-top fluidized bed dryer (see figure 2). Figure 3 shows data from stability/viability studies of the resulting products, involving measurements of cell count over a period of 30 days.

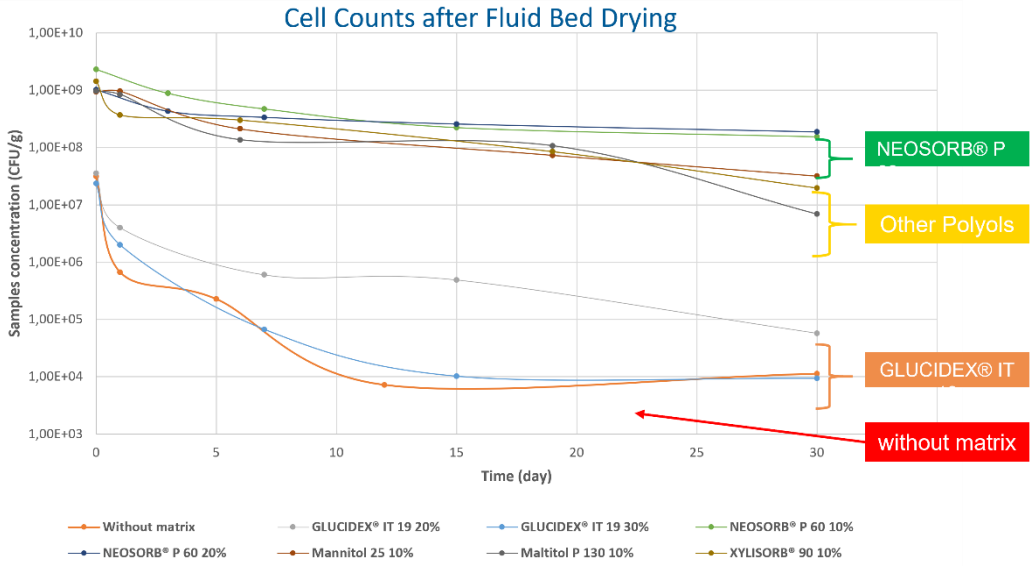

Figure 3: NEOSORB® P 60 sorbitol-based formulations exhibit optimal stabilization for this bacterial strain but other polyols also perform well and almost all matrices improve performance relative to the baseline "no matrix" case.

These results show that for this particular species and fluidized bed process, NEOSORB® P 60 sorbitol offers optimal performance though the other polyols tested – MANNITOL 25, MALTITOL P130 and XYLISORB® 90 xylitol – also perform well.

All these matrices protect the bacteria during the drying process, as evident from higher Day 0 cell counts, and at the same time enhance stability.

Cell counts with NEOSORB® P 60 sorbitol are in excess of the 10^8 minimum at 30 days and relatively stable suggesting good shelf life, especially when compared with the formulation containing no matrix. No significant concentration effect is observed.

In summary, in this example, a formulation containing 20% NEOSORB® P 60 sorbitol, in combination with a GLUCIDEX® IT 19 maltodextrin carrier produced a stable microbial product that maintained the required cell count

throughout the 30-day test period. With a >93% dry matter content and good water solubility, **this product meets all the requirements of the initial brief.**

FORMULATING WITH ROQUETTE PRODUCTS

PUTTING THE SPOTLIGHT ON GLUCIDEX®

Stabilizing microbes remains an empirical, trial and error process with every species presenting a new challenge. Having a good range of grades to work with, for any individual ingredient, can therefore be extremely valuable. Roquette's GLUCIDEX® offers the widest range of maltodextrins available from any commercial supplier; table 1 shows the main grades. A popular carrier choice for microbial formulations maltodextrins are routinely characterized in terms of Dextrose Equivalent (DE) content, a parameter which quantifies the amount of reducing sugars present.

Table 1 : The GLUCIDEX® range offers formulators an unrivaled choice of maltodextrin grade.

MAIN GRADES													
TYPE	**1** Potato based	**2** Waxy Maize based	**6** Waxy Maize based	**9** Potato based	**12**	**17**	**19**	**21**	**29**	**33**	**38**	**39**	**40**	**47**
Dextrose Equivalent (DE)	5 max.	5 max.	5 to 8	8 to 10	11 to 14	15 to 18	18 to 20	20 to 23	28 to 31	31 to 34	36 to 40	38 to 41	38 to 42	43 to 47
Loss on drying (minimum %)	6	6	6	5	5	6	5	5	5	5	5	5	5	5
Carbohydrate composition														
Glucose (%)	0.2	0.2	0.2	0.2	1	1	2	1.5	8	11	15.5	2	16	2
Maltose (%)	0.5	0.5	1	1.5	3	4	5	6	8	10	12	32	12	45
Oligo and Polysacch. (%)	99.3	99.3	98.8	98.3	96	95	93	92.5	84	79	72.5	66	72	51
Poured bulk density (kg / liter)														
Standard grades	0.40	0.40	0.45	0.45	0.45	0.50	0.50	0.50	0.55	-	-	0.55	0.55	-
IT grades	-	-	0.35	-	0.40	-	0.40	0.40	0.45	0.45	0.50	-	-	0.50

Using maltodextrins from across this range formulators can easily assess how DE content impacts on stability, to determine how best to protect any given microbial strain. Some do better with high DE content carriers, whereas others prefer a lower DE content, and may therefore be better formulated with, for example, polysaccharides.

Figure 4 shows data from a typical stabilization screen for a microbe strain that aids phosphorus assimilation.

The results show clearly that survivability passes through an optimal value as a function of a DE content and that the "right" maltodextrin can provide significant improvement relative to alternatives. This is an important finding for formulation studies, facilitated by the availability of a wide range of grades with otherwise identical properties, from a single supplier.

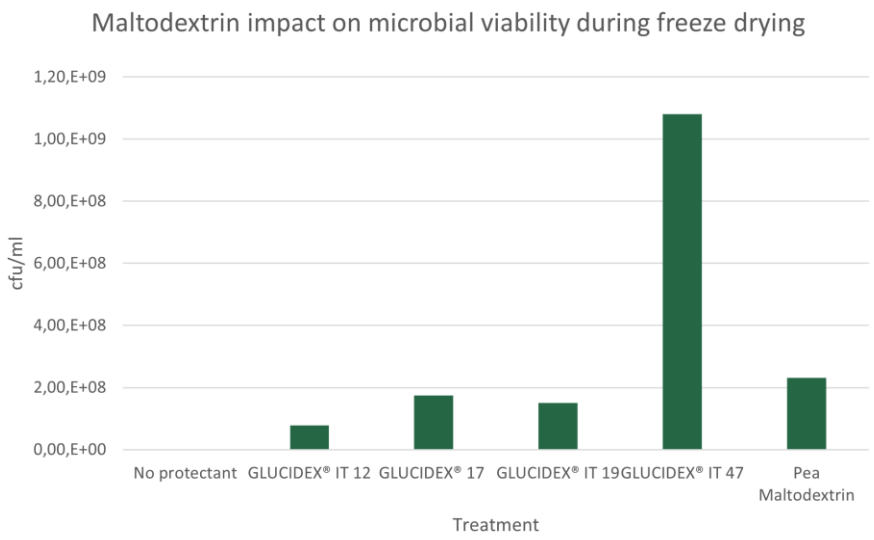

Figure 4: For this microbial strain, a relatively high DE content is optimal for cryogenic protection.

CONCLUSION

Microbial products can help to deliver the land productivity required to feed growing populations while at the same time reducing reliance on synthetic chemicals. Our understanding of the plant microbiome is increasing rapidly underpinning efforts towards a more knowledge-led approach to the identification of useful microbial strains. However, better stabilization and formulation is equally important when it comes to transferring products into the field.

Roquette offers a portfolio of plant-derived products that formulators can use to develop bio-formulated products that are well-aligned with the goals of microbial product use. Experimental data illustrates what can be achieved and how these products can help to deliver effective solutions.

A COMPREHENSIVE PLANT-DERIVED PORTFOLIO FOR MICROBIAL STABILIZATION

Roquette has a comprehensive, established portfolio of plant-derived products for microbial stabilization.

These provide customers with the carriers, filler, binders, flow and extrusion aids, disintegrants, protectants, encapsulation and compression agents they need to effectively deliver bioformulated plant beneficial microorganisms. Specific products for solid- or liquid-based formulation include the following:

Sugars: the **GLUCIDEX® range of maltodextrins** are inert, easily dissolved, highly stable materials with good flowability characteristics. The granulated IT grades are particularly valuable for microbial stabilization offering easier dispersion and dissolution. Primarily used as delivery agents in spray drying, freeze drying and granulation applications, these products also deliver nutritional benefit.

A wide range of dextrose products is available in crystalline powder – anhydrous and monohydrate – or liquid form. These can be used as fillers and also provide a good energy source for microbe proliferation. Powder products of varying particle size distribution are available with flowability characteristics tuned to customer requirements.

Polyols: Our extensive polyols range includes **NEOSORB® sorbitol**, **PEARLITOL® mannitol**, **SweetPearl® maltitol**, and **XYLISORB® xylitol**. Naturally stable/unreactive and highly compatible with other bioactive agents, these compounds are available in liquid form and as powders, in a range of particle sizes. They are especially useful as cryoprotectants and preservatives of microbial functionality. Roquette is a global leader in polyol synthesis and chemistry, delivering products of unsurpassed quality and consistency backed by extensive formulation expertise.

Cellulose: **MICROCEL® microcrystalline cellulose** products are pure, partially depolymerized celluloses with a highly reproducible particle size distribution ideally suited to tablet, granule and pellet manufacture. They can act as binders, compression agents, flow and extrusion aids and can also deliver highly effective water absorption thereby enhancing disintegration characteristics.

Alongside this portfolio, Roquette provides quality ingredients for the production and cultivation of microbial strains including carbon sources and nitrogen/growth factors, all derived from plant proteins.
To find out more about these products and how they can help you formulate an optimized plant care solution for your needs: Check our website and contact us.

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