MicroFunction Workshop ROSTOCK

AGROSAVIA
Corporación colombiana de investigación agropecuaria

Hydrogel Capsules as Carriers for Growth-Promoting Bacteria Consortia in Bovine Compost Enrichment for enhancing Avena (*Avena sativa*) yields

Martha Chaparro[1], Jonathan Mendoza[2] , Daniel Torres[2], German Estrada[2], Martha Gómez[1], Sergio Pardo-Díaz[2], Daniel F. Rojas-Tapias[2], **Mauricio Cruz[1*]**

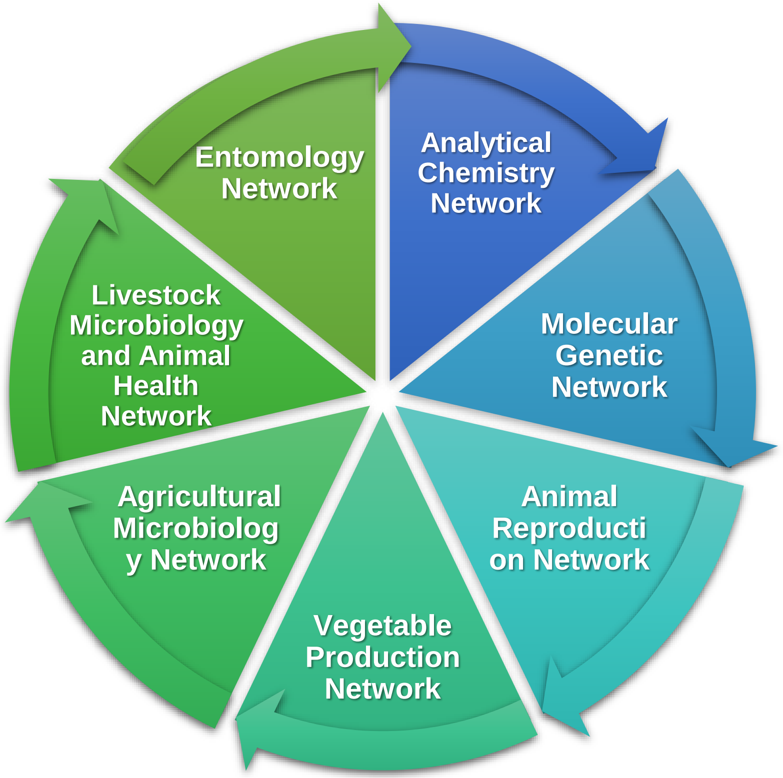
1. Bioproducts Department, Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Km 14 vía Bogotá a Mosquera, Mosquera, Colombia

2. Agricultural Microbiology Laboratory, Tibaitatá Research Center, Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Km 14 vía Bogotá a Mosquera, Mosquera, Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

KoLFACI

Research and Services: Laboratory network



Cutting-edge laboratories

Bioproducts Department

Biofertilizers Pilot Plant

Biopesticides Pilot Plant

Animal Health

Viral propagation

Fermentation facilities
Harvesting
Drying

Definition and Bio-inputs approach

Product that is used for **integrated pest management** purposes or to **improve the productivity of crops and soil**, based on **free-living microorganisms**, viruses, naturally-occurring products or **biochemical products**.

Antibiotics, toxins, genetically modified organisms (GMOs)

BIOPESTICIDES

BIOFERTILIZERS

ANIMAL HEALTH ADDITIVES

Bioinsecticides and biofungicides

- Bacteria
- Fungi
- Viruses
- Yeast

Biofertilizers/Biostimulants

- N-fixing (bacteria)
- P-solubilizing , K-solubilizing
- Plant Growth Promoting MOs

- Bio-remediators (Cd, As y Pb)

Eubiotics

- Prebiotics
- Probiotics
- Plant Extracts
- Oils

Definition and Bio-inputs approach

>100 scientists NETWORKING

Scientific capacity- Research groups

intellectual property
Market Analysts
Technology transfer

National and international research centers, Companies and Universities

Bioproducts and Agricultural Bioprocesses
19 scientists

Biological control of agricultural pests
32 scientists

Innovation in animal health and welfare
23 scientists

Bioprospection of Biomolecules and Microorganisms with Agricultural Applications
25 scientists

Microbiology and Animal Nutrition of the Tropics
38 scientists

Sustainable Agricultural Systems
28 scientists

Definition and Bio-inputs approach

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

Marketing/ distribution
ALLIES

Registration
AGROSAVIA

Research and technological development
AGROSAVIA

Scaling-up
Early-adopters
ALLIES

Protection of intellectual property
AGROSAVIA

Participation in public policy

Encourage the bio-inputs at the national level.

Definition and Bio-inputs approach

AGROSAVIA´S BIOPRODUCT DEPARTMENT

TRL: Technology readiness level

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

Colombian Germplasm Bank for Food and Agriculture

Farmers needs
Industry

2) Proof of Concept

Validation

4) Scaling-up

Product ready to industrialize

4-6 years multidisciplinary team

1) Idea

Research Groups/Networks

3) Developing

Prototype

5) Registration and launch

Product ready to market

(Philip Kotler & Gary Armstrong, 2012)

Bio-input developing: Idea

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

Innovation networks

- Livestock and minor species
- Permanents
- Roots and tubers
- Agro-industrial
- Vegetables and aromatics
- Cacao
- Fruit trees

Meet the demands of the territories and **generate a relevant technological offer (TO)** that responds to the demands of the producer.

Research centers or industry

cenipalma

Casa Luker

ceniflores
Centro de Innovación de la Floricultura Colombiana

Outcome

Idea that can lead to a bioproduct

Bio-input developing: Proof of Concept

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

Active Ingridient

Germplasm banks

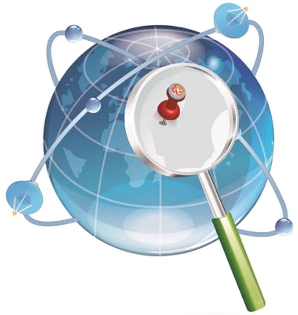
high diversity microorganisms

Access permits to genetic resources

Isolation

Mechanisms, toxicology…

Identification and characterization (Biological act.)



Market relevance

- ✓ **Preliminary production method**
  Laboratory scale
- ✓ **Frequencies/doses under controlled conditions.**

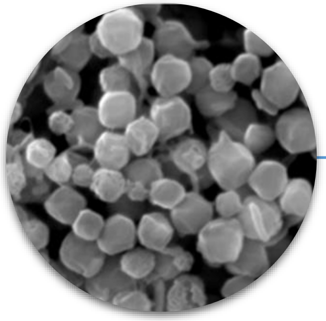
Outcome: Bioproduct profile and value proposition

Bio-input developing: Development

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

Technological Processes

Characterization AI



Production

- Culture media design
- Macro-Micro Nutrients

Yields and viability

Harvesting

- Liquid extraction-dry harvesting
- **Yields and viability**

Delivery system

- Pre-formula
- Formulation

Protection-abiotic stresses

Efficacy

Shelf-life

- ✓ Solid: WP, WG
- ✓ Liquids: CS, CEmulsion

Bio-input developing: Development

AGROSAVIA
Corporación colombiana de investigación agropecuaria

Biological processes




Modes of action

Frequencies

Eficacy controlled conditions

Agrochemical compatibility

Effect on beneficial organisms

Marketing processes

- ✓ Value elements
- ✓ Target segments
- ✓ Market potential
- ✓ Production cost and application

Outcome: Product prototype and value equation


Bio-input developing: Scaling-up

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

Product ready for industrial scaling

- Process optimization
- Scale-up
- Efficacy (Application Recommendations)
- Validations in productive systems

Validation of the value equation

- Financial models
- business strategies
- Value demonstration
- Production and application costs

Outcome:
Approach to the industry

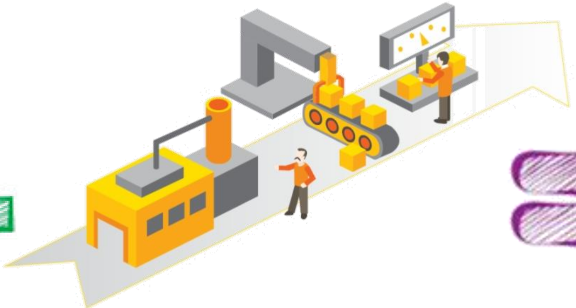
Bio-input developing: Registration-Transfer

AGROSAVIA 30 años
Corporación colombiana de investigación agropecuaria

Efficacy tests in productive regions

ica

Registration
Shelf-life, toxicology, trademark, labeling

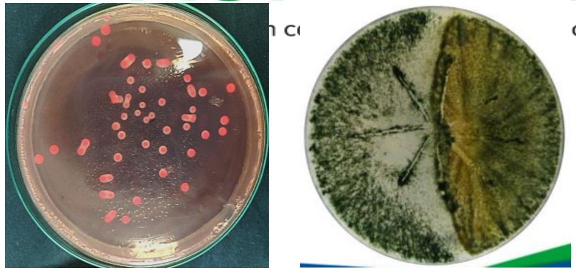


Commercial production

Outcome: Product on the market

Early-adopters
Technical assistant

• Bio-inputs Innovation



01 **Active Ingridient**

Consortia (Bacteria, Mycorrhizae)

02 **Production**

Co-cultivation, Semi-SSF and Submerged fermentation

03 **Delivery System**

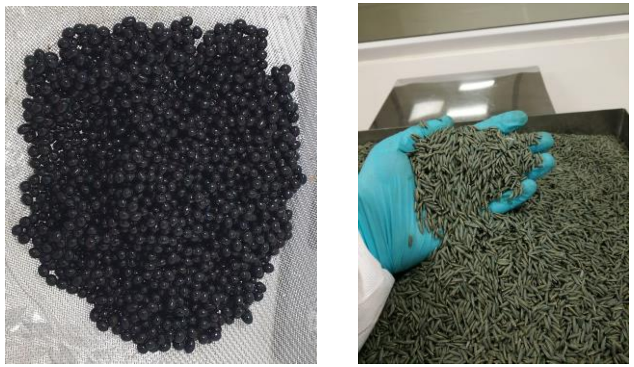
Nano film coating, Capsules

04 **Applications**

Small Farmers

05 **Customer**

Technical assistant



< 1% of research articles on (PGPBs) have been related to formulation

Herrmann and Lesueur (2013)

Corporación colombiana de investigación agropecuaria

Hydrogel Capsules as Carriers for Growth-Promoting Bacteria

Mauricio Cruz Barrera PhD

fcruz@agrosavia.co
https://orcid.org/0000-0002-9208-7902

1. Bioproducts Department, Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Km 14 vía Bogotá a Mosquera, Mosquera, Colombia

Universität Rostock

Traditio et Innovatio

AGROSAVIA30
Corporación colombiana de investigación agropecuaria

- Colombian agricultural sector (**3,8 % PIB**), livestock (**80 %**), **agriculture (20%)**.
- **31 million hectares** dedicated to pastures and forage.
- Colombia **importing 75% of agrochemicals**.
- **Price >200 %**

An alternative is **composting bovine manure** enriched with plant growth-promoting bacteria (PGPB).

AGROSAVIA and KolFaci (ID: 1001556)

KoLFACI

(MADR, 2018; UPRA, 20,19; Wu et al., 2020; Bonilla et al, 2013)
https://www.tierragro.com/collections/nutrimon
https://www.larepublica.co/especiales/crisis-en-ucrania/de-los-fertilizantes-usados-en-el-agro-colombiano-42-provienen-de-rusia-y-ucrania-3310266

Organo-Mineral Fertilizer

Improve Soil structure, Organic matter

Reduce use of imported inputs

Nutrient ciclying

Organic fertilizers and low solubility rocks

+

Biofertilizers (consortia)

Adapt to climate change

Soil Health

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

Sustainable Agricultural Systems Research Team

Alliances: FENAVI Federación Nacional de Avicultores de Colombia; EMPRESA DE FOSFATOS DE BOYACA

Pilot plant for the development of a"smart fertilizer" C.I. Tibaitatá

Application C.I. Tibaitatá

Selected Plant Growth-Promoting Bacteria (PGPBs)

Herbaspirillum sp. AP21

- ✓ Endophytic
- ✓ Increases leaf biomass by 44.8% in Red clover
- ✓ Alleviates water deficit (co-inoculation) in Ryegrass

Azospirillum brasilense D7

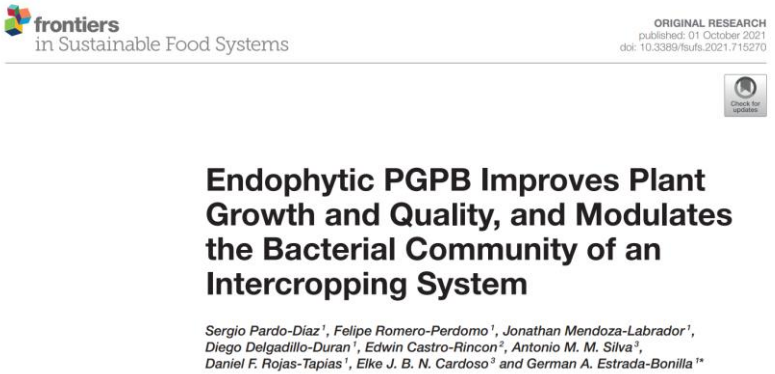
- ✓ Endophytic
- ✓ Alleviates water deficit (12%)
- ✓ Increases leaf biomass (30%) (co-inoculation) in Ryegrass

Rhizobium leguminosarium T88

- ✓ Rhizobacteria
- ✓ Promotion of plant growth in Red Clover (30.46%)
- ✓ Ryegrass (20.31%), P-solubilizing

Gram – Negative
No spore-forming

(Cortes, 2020; Santos, 2020).
https://agroactivocol.com/wp-content/uploads/2020/08/trebol-rojo_8844524b_1280x720.jpg


frontiers in Sustainable Food Systems

ORIGINAL RESEARCH
published: 01 October 2021
doi: 10.3389/fsufs.2021.715270

Endophytic PGPB Improves Plant Growth and Quality, and Modulates the Bacterial Community of an Intercropping System

Sergio Pardo-Díaz[1], Felipe Romero-Perdomo[1], Jonathan Mendoza-Labrador[1], Diego Delgadillo-Duran[1], Edwin Castro-Rincon[2], Antonio M. M. Silva[3], Daniel F. Rojas-Tapias[1], Elke J. B. N. Cardoso[3] and German A. Estrada-Bonilla[1]*

- **Ryegrass—red clover** intercropping system
- *Herbaspirillum* sp. AP21 and *Azospirillum brasilense* D7
- N fertilizer rates (50, 75, 100%)
- 16S rRNA metataxonomics.
- PERMANOVA

	Pr(>F)
Inoculation	0.0461
N fertilizer rate	0.2519

- PGPB alters the bacterial diversity regardless N
- **Actinomycetales** (28.8%), **Rhizobiales** (13.5%), **Burkholderiales** (12.7%)
- **AP21, D7, and AP21 + D7 fertilized with 50% and 75% N cluster with the positive control treatment fertilized with 100% N**. Positively associated with crude protein, shoot N content, and shoot dry weight increments

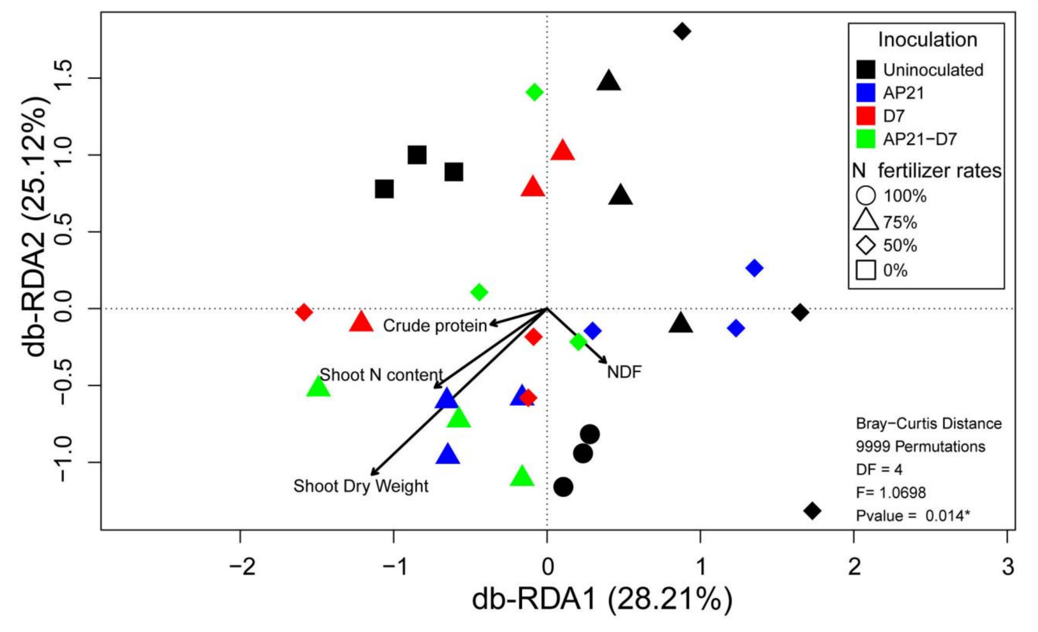
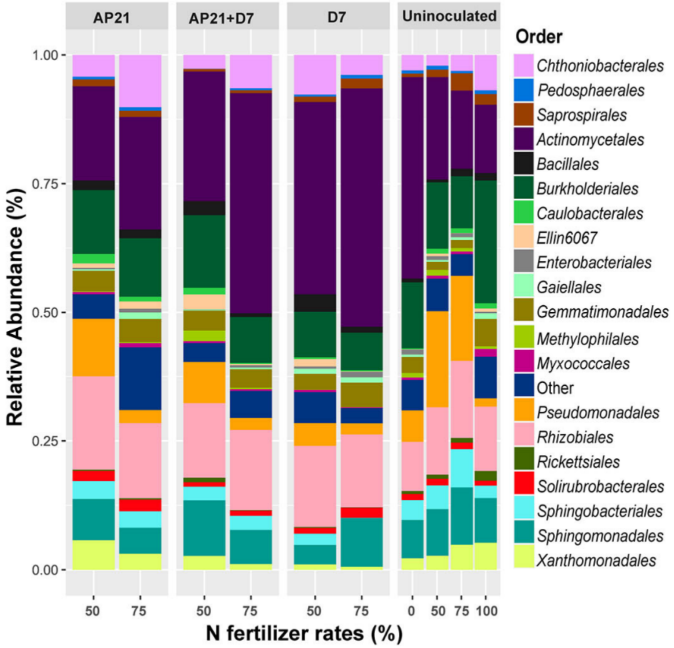
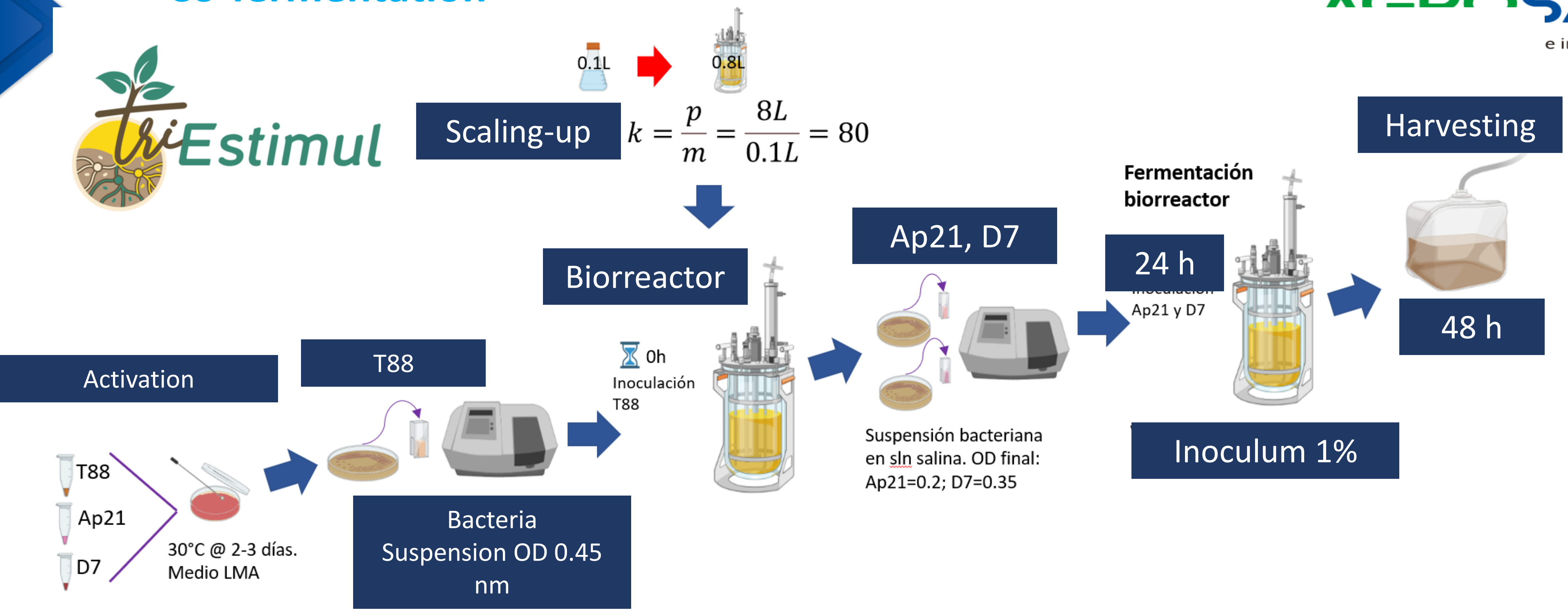


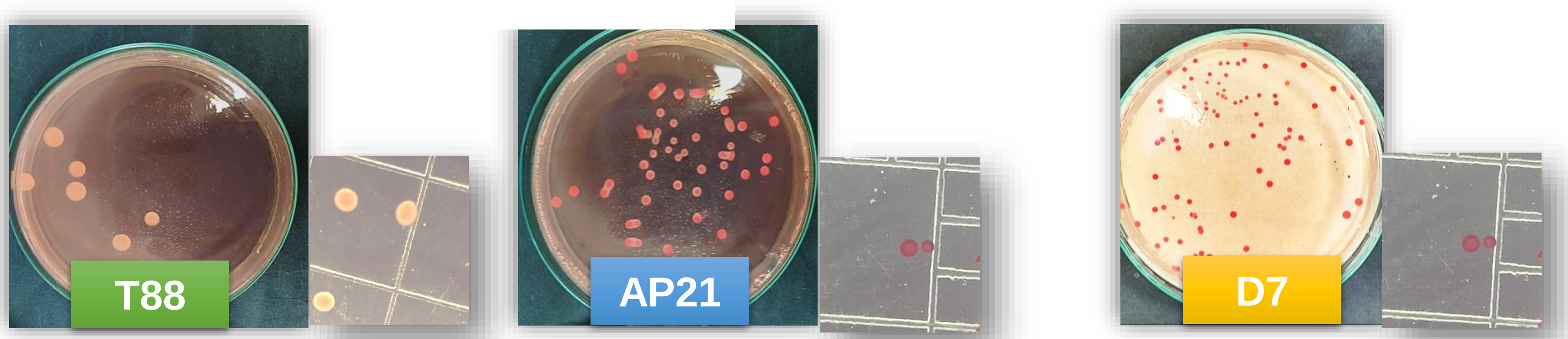
FIGURE 4 | Distance-based redundancy analysis (db-RDA) plot illustrating the bacterial community structure of the rhizosphere in relation to plant parameters. The db-RDA was significant ($p < 0.05$).



Relative abundance of bacterial orders the rhizosphere for inoculation with PGPB (Ap21, D7) under different rates of nitrogen fertilization

Co-fermentation





METHODS

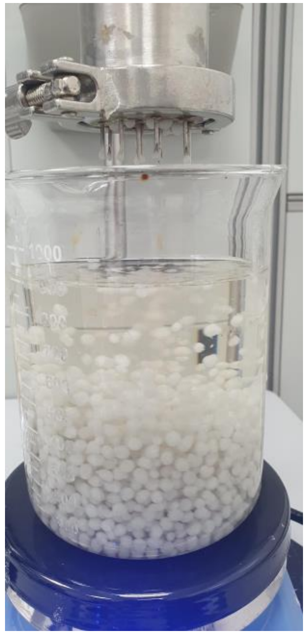
AGROSAVIA 30 años
Corporación colombiana de investigación agropecuaria

Applied Microbiology and Biotechnology (2023) 107:6671–6682
https://doi.org/10.1007/s00253-023-12699-7

ENVIRONMENTAL BIOTECHNOLOGY

Hydrogel capsules as new approach for increasing drying survival of plant biostimulant gram-negative consortium

Martha Chaparro-Rodríguez[1,2] · German Estrada-Bonilla[3] · Jaiver Rosas-Pérez[2] · Martha Gómez-Álvarez[1] · Mauricio Cruz-Barrera[1]



Sensitive to drying processes

Dry formulations promote transportation, product shelf life, and prevent contamination

ENCAPSULATION

BIOPOLYMER MATERIALS (HYDROGEL CAPSULES)

I. Increasing tolerance (biotic and abiotic factors)
Protection against the drying process
II. Improving shelf life in storage

IONIC GELATION

UNIVERSIDAD NACIONAL DE COLOMBIA

(Cruz Barrera et al., 2020; Meng et al., 2008; Song et al., 2014 ; Vemmer & Patel, 2013)

METHODS

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

Preparation of formulation prototypes

P1 = Ps01
P2 = Ps10
P3 = Gr01

Formulation excipients	***Prototypes (%p/p)***		
	P1	***P2***	***P3***
Ps13	*4*	*4*	*4*
Sp30	*5*	*5*	*5*
Ps17	*5*	*5*	*5*
Ps16	*0,1*	*0,1*	*0,1*
Ps01	*1*	*-*	*-*
Ps10	*-*	*1*	*-*
Gr01	*-*	*-*	*1*

Microbiological characterization

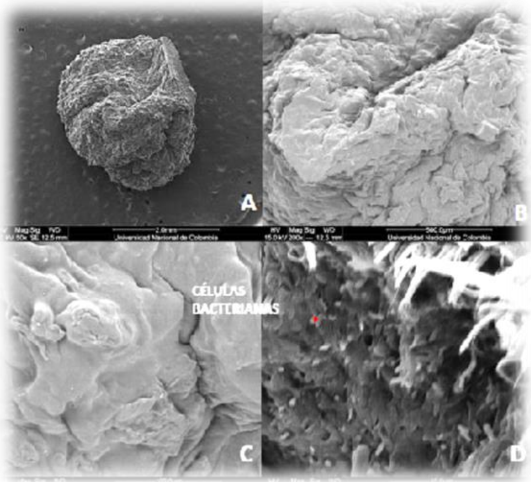
- Determination of the concentration of the active ingredient of each strain D7, AP21, and T88 (CFU/g)
- Determination of contaminant content (CFU/g)

- Preliminary accelerated stability of the active ingredient in capsule prototypes (CFU/capsule)

Time: 0, 15, 30, 60, 90 days.
Temperature: 6,18 y 28±2ºC

Physicochemical characterization

I. Particle size (mm)
II. Sphericity factor
III. Moisture content (%)
IV. a_w
V. SEM



Completely randomized design with a factorial arrangement adjusted for repeated measures over time (SAS Enterprise Guide 8.3)

METHODS

Biological features
Greenhouse

- Avena assay, completely randomized experimental design
- 3 repetitions per treatment
- Experimental unit: 2 kg pot with one Altoandina Avena sativa plant Fertilized with Hoagland nutrient solution

TREATMENT	*DESCRIPTION*
T1	*Control 1 (Hoagland completo 100%)*
T2	*Control 2 (compost + Hoagland 100%)*
T3	***Control 3 (compost +Hoagland 50%)***
T4	***Cell-Broth (compost +Hoagland 50%)***
T5	*P1 (Ps01) + PGPB (compost +Hoagland 50%)*
T6	*P2 (Ps10) + PGPB (compost +Hoagland 50%)*
T7	*P3 (Gr01) + PGPB(compost +Hoagland 50%)*
T8	*Carrier P1 (Ps01) (compost +Hoagland 50%)*
T9	*Carrier P2 (Ps10) (compost +Hoagland 50%)*
T10	*Carrier P3 (Gr01) (compost +Hoagland 50%)*

(Galvani & Gaertnert, 2006)

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

45 days after planting (tillering stage)

Total dry biomass and **nutritional parameters** using NIRS (Near-Infrared Spectroscopy)

Nutritional parameter yield

Crude protein (Kg/ha)
Phosphorus (Kg/ha)
Potassium (Kg/ha)
Nitrogen (Kg/ha)

En SAS Enterprise Guide 8.3: "Sequential analysis, Dunnett test p <0.05".
En Statgraphics Centurion versión XVI: "Análisis secuencial, prueba de Duncan p <0,05".

RESULTS PHYSICOCHEMICAL

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

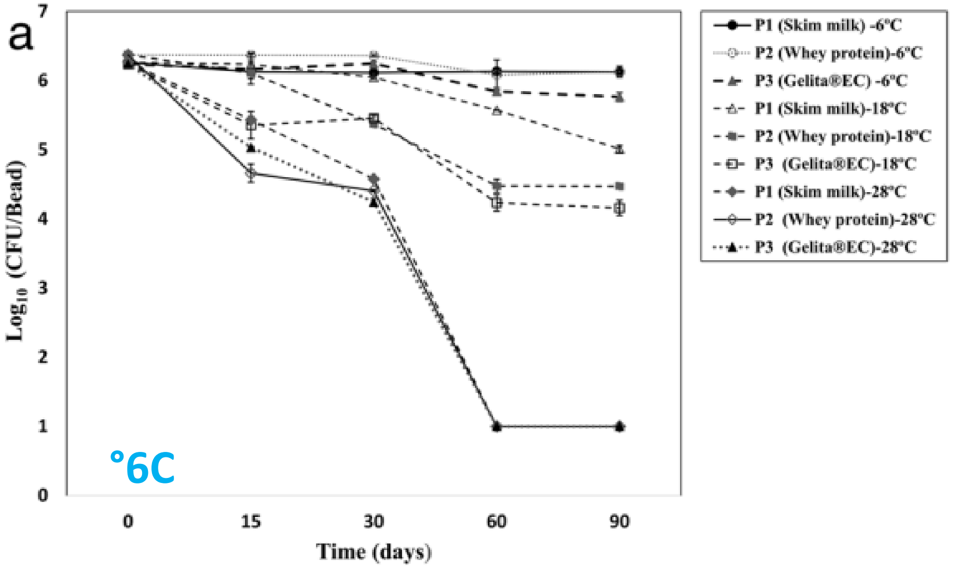
Microbiological characterization of the three formulation prototypes.

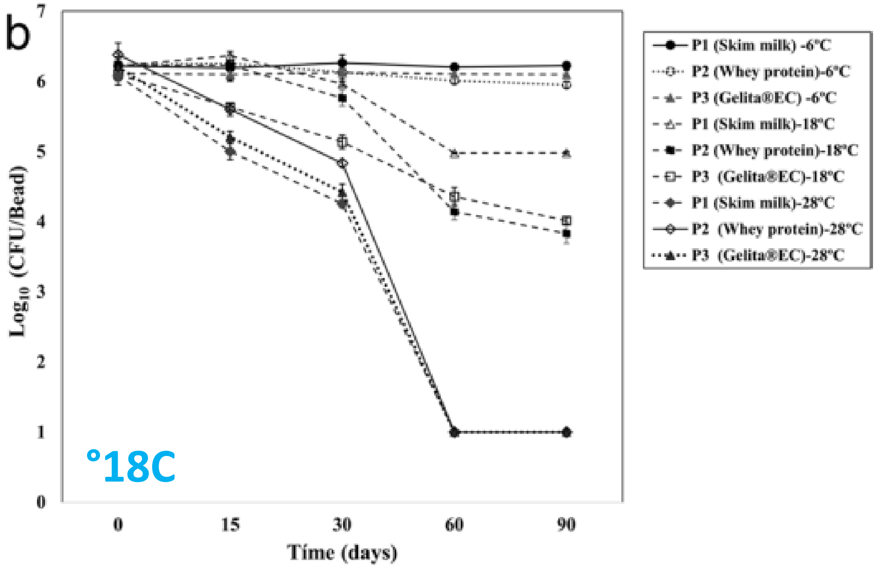
Strain /Prototype (CFU/g)	P1 (Ps01)	P2 (Ps10)	P3(Gr01)
Strain D7	3.74×10^8	4.74×10^8	3.48×10^8
Strain T88	3.46×10^8	3.66×10^8	2.54×10^8
Strain AP21	2.07×10^7	2.20×10^7	2.14×10^7
Total (CFU/g)	**7.41×10^8**	**8.62×10^8**	**6.24×10^8**
Contaminats (CFU/ g)	1.73×10^5	1.33×10^5	1.60×10^5

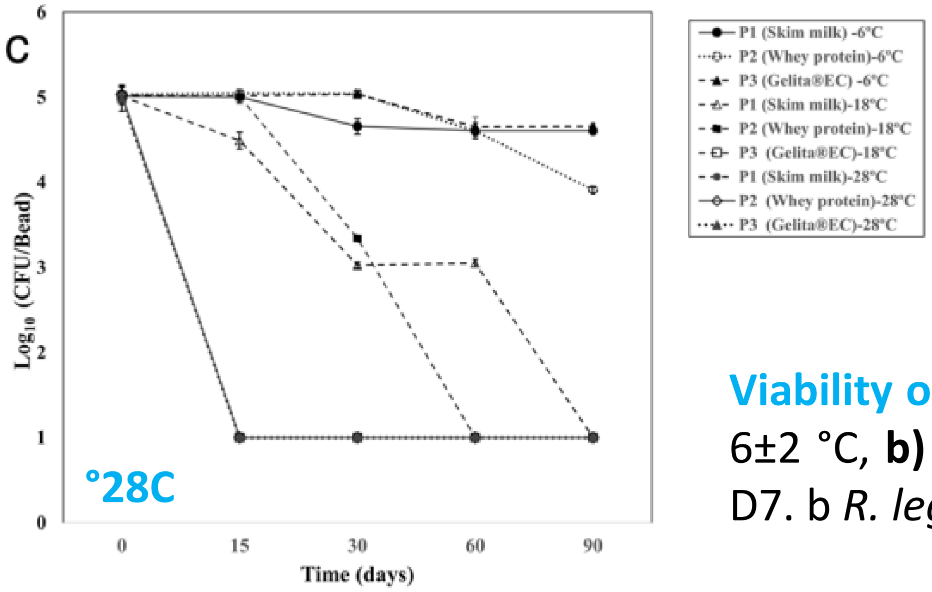
Physicochemical characterization of the three formulation prototypes.

Prototipo /Parámetro	P1 (Ps01)	P2 (Ps10)	P3(Gr01)
Moist (%)	3,11±0,09	3,23±0,15	2,26±0,20
Water activity (a_w)	0,62±0,01	0,61±0,005	0,62±0,01
Particle size (mm)	2,55±0,28	2,30± 0,26	2,43±0,24
Spherecity	0,010±0,28	0,002±0,26	0,005±0,24

(Icontec, 2008; ICA, 2021; Benintende, 2010; Reetha et al., 2014; Kurtmann et al., 2009; Cruz, 2019; Cruz Barrera et al., 2020; Mendoza-Labrador et al., 2021; Lais et al., 2017; Amorij et al., 2008; Chan et al., 2009; Wu et al., 2011, 2012)



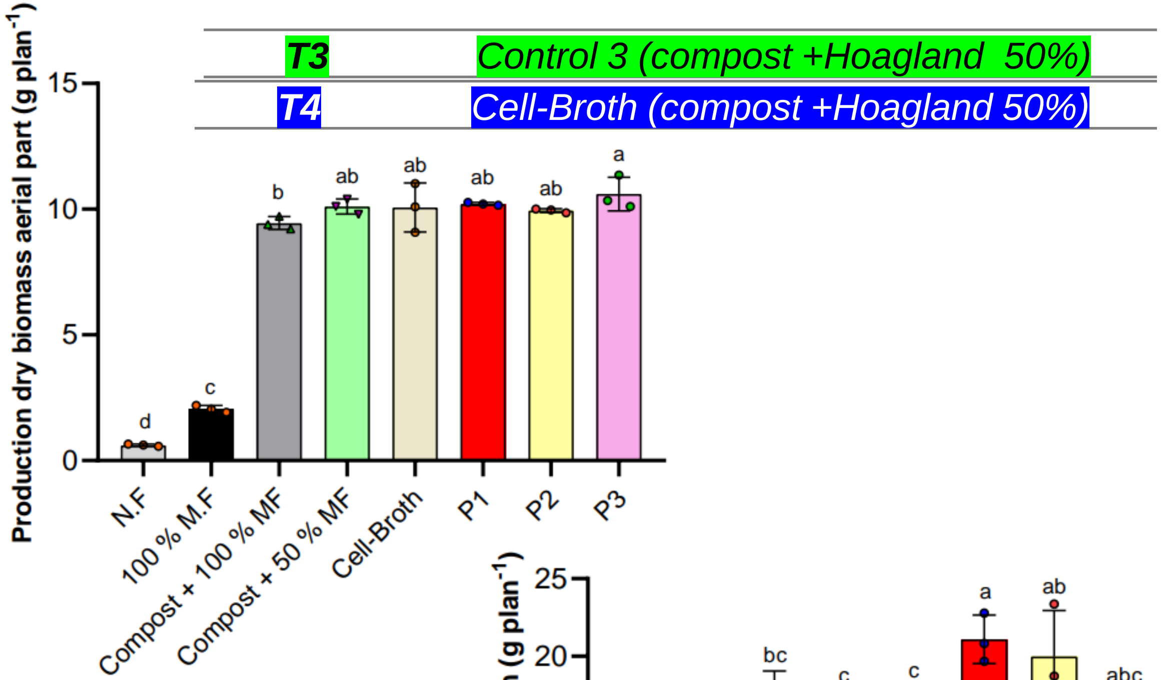




Viability of bacteria (log CFU/bead) within prototypes stored at **a)** 6±2 °C, **b)** 18±2 °C, and **c)** 28±2 °C during 90 days. a *A. brasilense* D7. b *R. leguminosarum* T88. c *H. frisingense* AP21

31,36% (P1)
20,55% (P2)
34,94% (P3)

20,56% (P1)
8,08% (P2)
24,70% (P3)



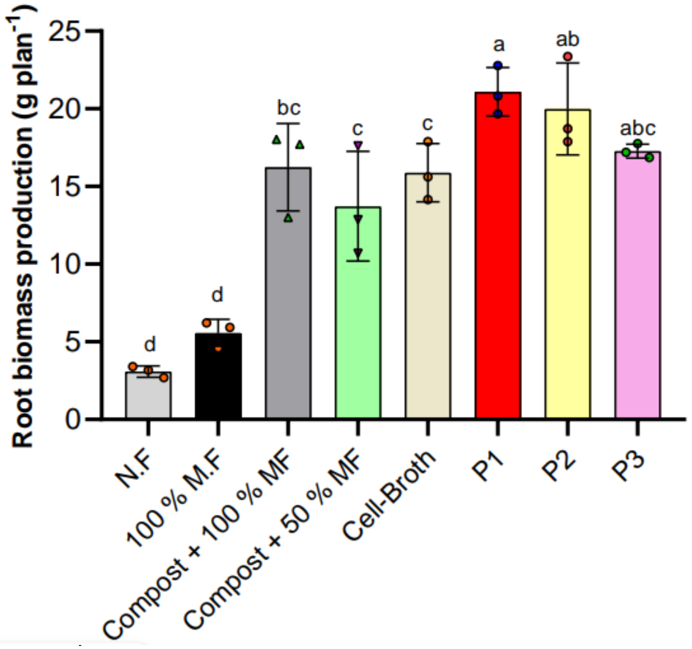


Fig. 1. Root biomass of Altoandina Avena sativa A. Control (T1) B. Control (T3). C. Bacterial suspension (T4) D. P1 (T5) E. P2 (T6) F. P3 (T7)

Fig. 2. Aerial biomass of Altoandina Avena sativa A. Control (T1) B. Control (T3). C. Bacterial suspension (T4) D. P1 (T5) E. P2 (T6) F. P3 (T7)

Altoandina Avena sativa

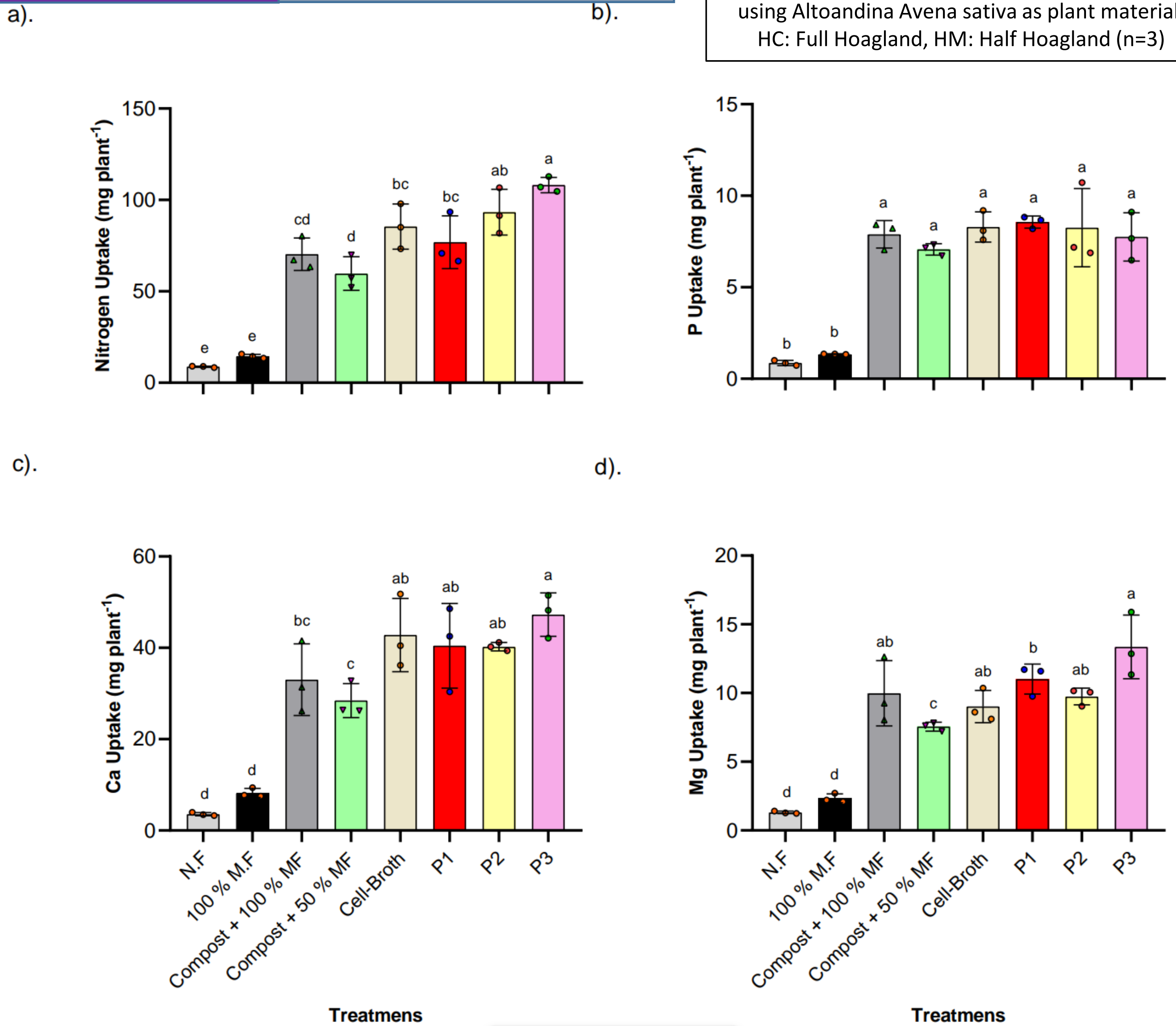
(Mendoza-Pedroza et al., 2018; Aguilar-Benítez et al., 2020; Liu et al., 2016; Ra

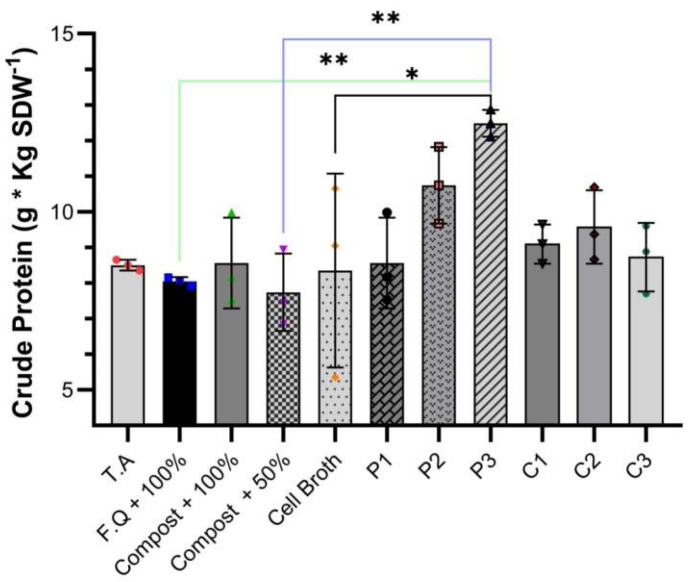
Pérez-Montaño et al., 2014; El-Komy, 2001; Amalraj et al., 2015)

RESULTS

PLANT NUTRIENTS

NIRS analysis with the three formulation prototypes P1 (Ps01), P2 (Ps10), and P3 (Gr01) using Altoandina Avena sativa as plant material. HC: Full Hoagland, HM: Half Hoagland (n=3)





CONTROL T3 | **S. BACTERIA**

CRUDE PROTEIN

CONTROL T3	S. BACTERIA
35,98% (P1)	23,85% (P1)
44,77% (P2)	34,29% (P2)

NITROGEN

CONTROL T3	S. BACTERIA
36,02% (P1)	23,81% (P1)
44,82% (P2)	34,29% (P2)

POTASSIUM

CONTROL T3	S. BACTERIA
15,14% (P1)	18,04% (P1)
22,18% (P2)	24,84% (P2)

PHOSPHORUS

CONTROL T3	S. BACTERIA
16,12% (P3)	6,45% (P3)

CONCLUSIONS

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

- Three prototypes **enrich the compost**, **increasing the dry biomass and nutritional parameters** in the plants by 31.36% with P1, 20.55% with P2, and 34.94%

- The **mixture of bovine compost and PGPB encapsulates** could help **reduce chemical fertilization** by up to 50%.

- The nutrient content showcased significant increments, with upticks of **32.44% in Ca, 4.34%** in **P, and 36.48%** in **Mg.**

UNIVERSIDAD NACIONAL DE COLOMBIA

OUTLOOK

- Explore Ps01-like substances to **increase AP21 resistance to drying** and stability.
- Conduct stability studies with **commercial packaging** and scaled batches.
- Biological activity of capsule formulations without the presence of compost (forages and other crops).
- Determine the capacity/kinetics of **PGPB release from hydrogel capsules in soil systems**.
- **Field studies, chemical fertilizers (Salamanca, Spain)**

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

UNIVERSIDAD NACIONAL DE COLOMBIA

VNiVERSiDAD
Ð SALAMANCA

MiRAT

PROYECTO TRICOECHO

Co-aplicación de formulados de *Trichoderma* con Fertilizantes NPK en cultivo de Trigo

Figura 1. Representación esquemática del proceso de formulación de cepas de Trichoderma mediante la técnica de plato granulador

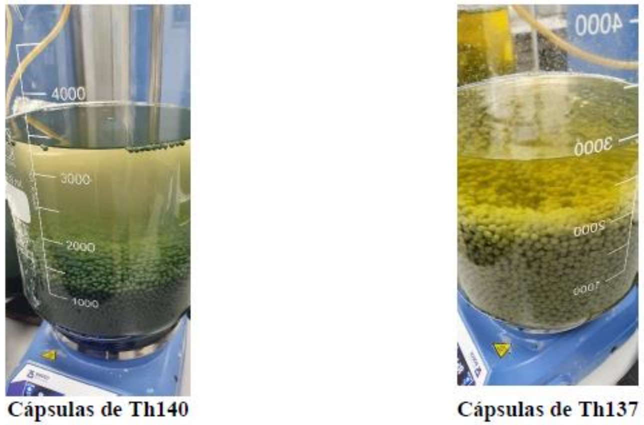
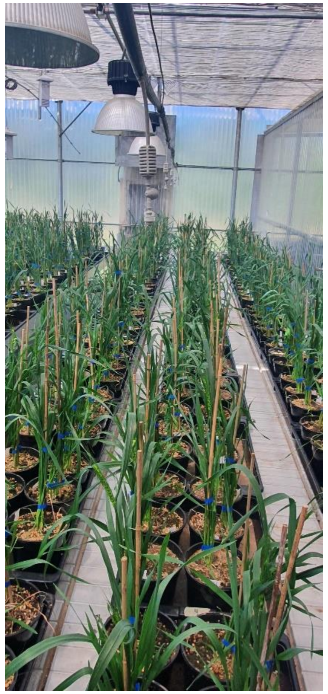


Figura 2. Formación de cápsulas de las de cepas de *Trichoderma* USAL Th140 y Th137 mediante la técnica de gelificación iónica



Outlook, The road Ahead

AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

- ✓ Novel **cost-effective** high-efficiency bioproducts **will encourage** their adoption and widespread use.
- ✓ There are still great **limitations** in the industrial production of **high-tech** bioproducts at the national level.
- ✓ Strategic **alliances with leading actors** must therefore continue to address the current and **upcoming challenges** in agriculture and climate change
- ✓ Multi-microorganism **consortia** vs single-strain
- ✓ **Bio-inputs** remain underutilized for a sustain agriculture
- ✓ Eeasily **available** for **small farmers-Training**
- ✓ Bio-inputs could **recover soil microbiome-soil regeneration**

https://www.agrosavia.co/noticias/agrosavia-lanza-oficialmente-proyecto-de-investigacion-de-quinua-en-cauca-el-mayor-productor-en-colombia

"AGROSAVIA is growing with the farmers and to the farmers"

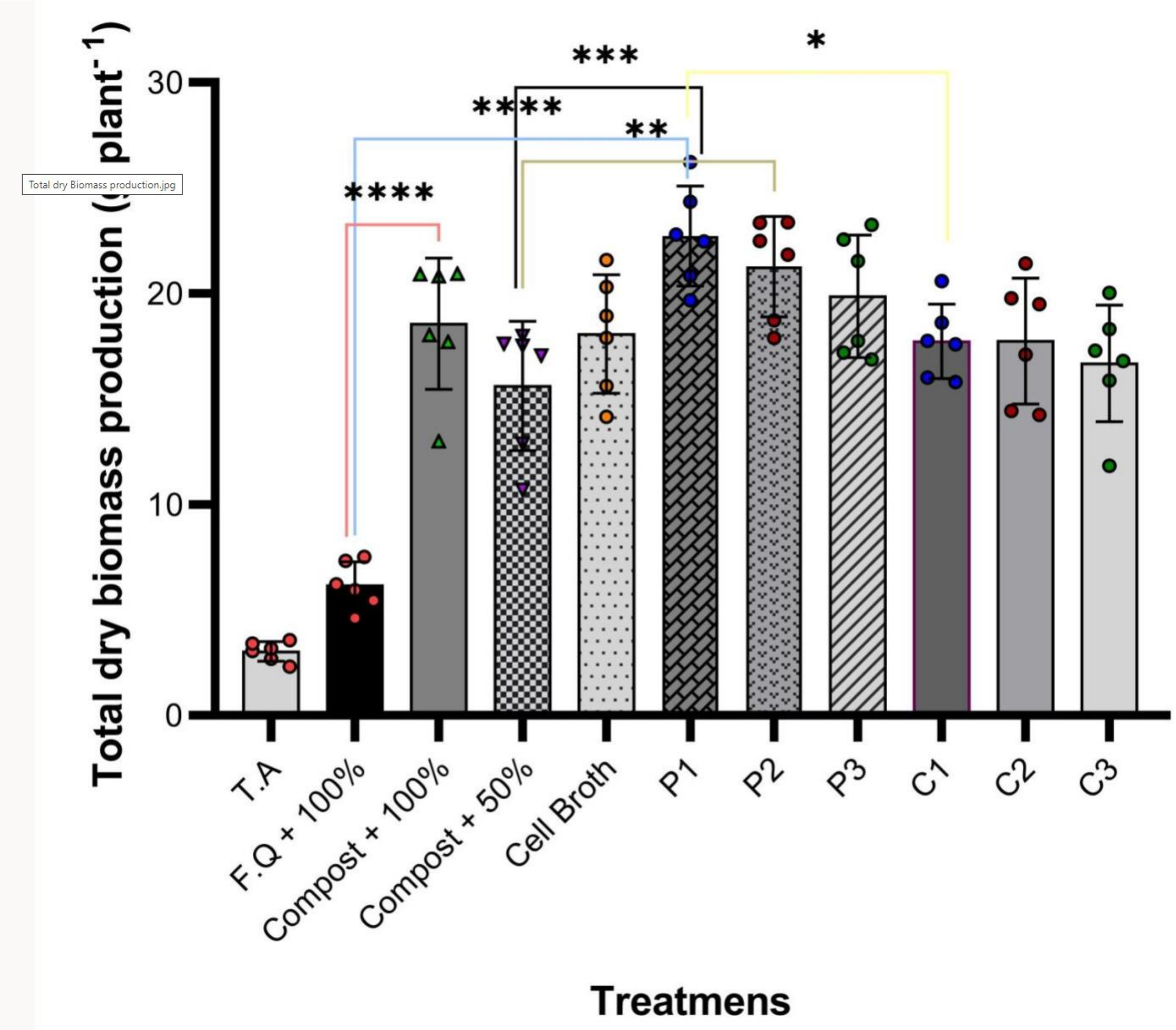
AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

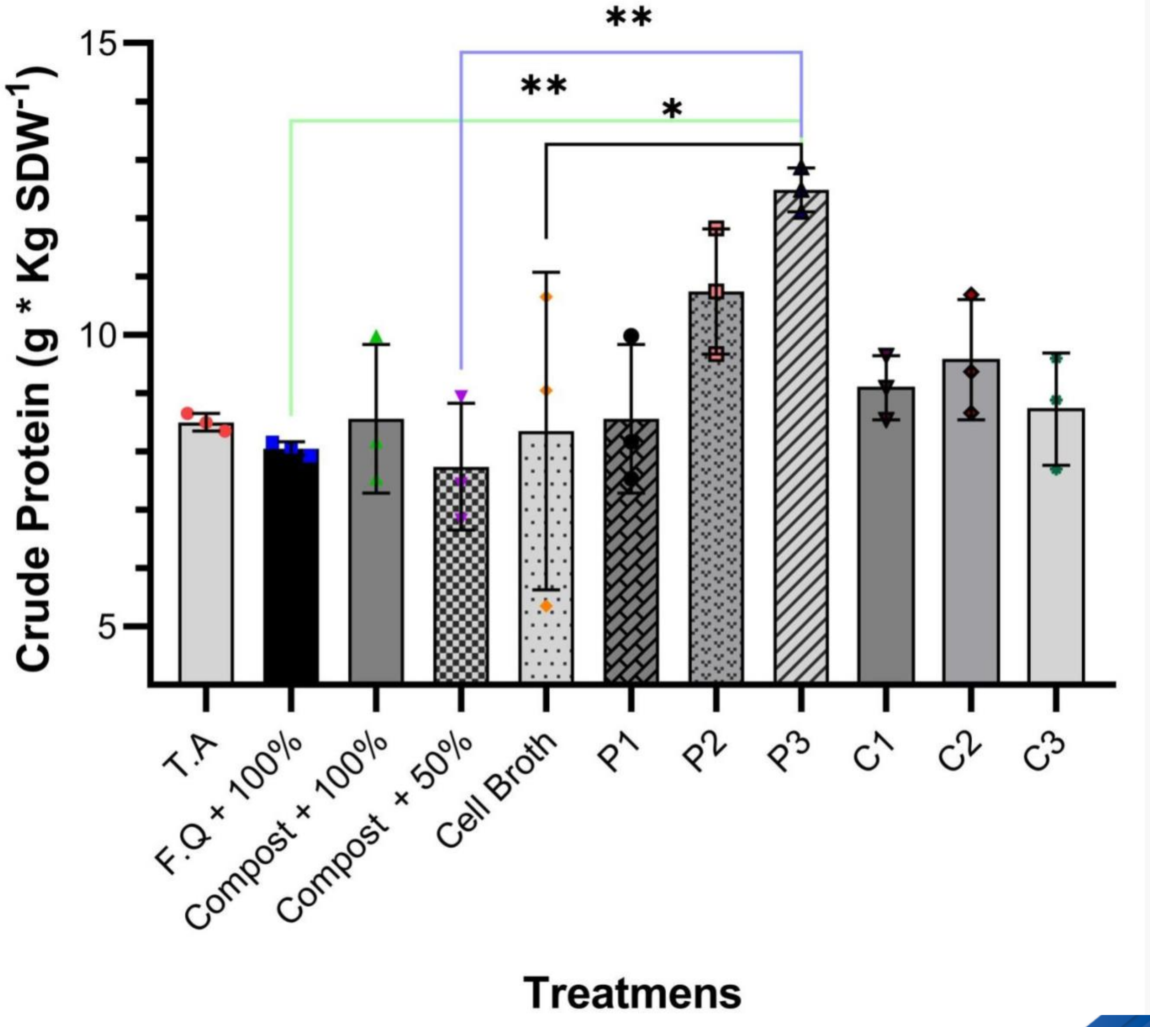
THANK YOU ¡¡¡

BIOPRODUCTS DEPARTMENT AT AGROSAVIA, COLOMBIA

Carriers

Corporación colombiana de investigación agropecuaria

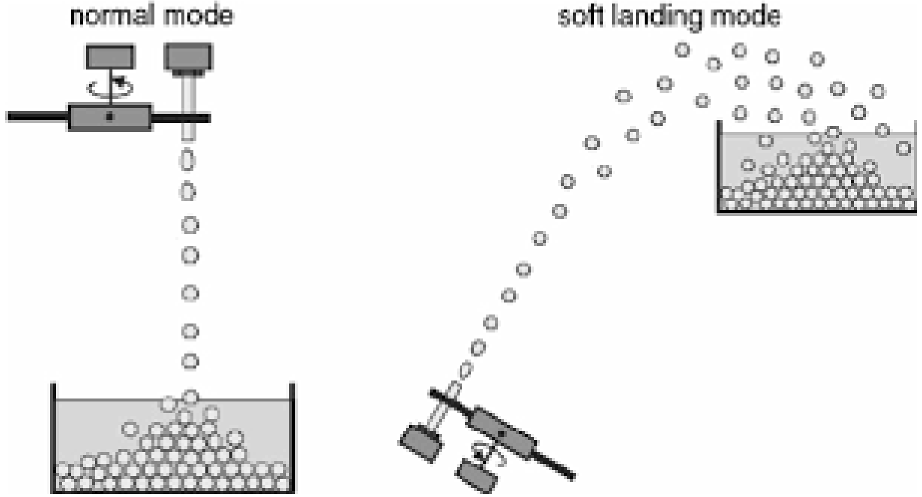




AGROSAVIA 30 AÑOS
Corporación colombiana de investigación agropecuaria

JetCutter (200 kg/hour)

Efficient and transferable for scaling up



Upcoming Bio-inputs

Biopesticide based on *Rhodotorula glutinis* for the control of *Penicillium expansum*, *Botrytis cinerea* and *Colletotrichum gloesporoides*, in post-harvest stages (flowers and fruits)

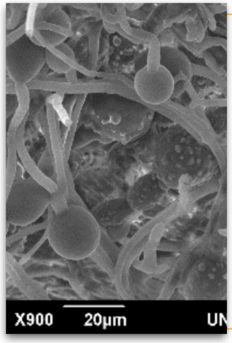
Growth promoter based on the bacteria *Bacillus velezensis* for nursery or seedbed stages, for **vegetables and fruit trees**

Natibac

Biofertilizer for phosphate fertilization in rotational crops based on the bacterium ***Rhizobium sp. B02***

Fosfotal

Biofertilizer for Cd immobilization and K solubilization for **cocoa crops** based on two bacteria: Pseudomonas sp. and *Bacillus* sp.



Biocontrol of **ruminant gastrointestinal nematodes** based on the **nematophagous fungus *Duddingtonia flagrans***: Targeted indirect control

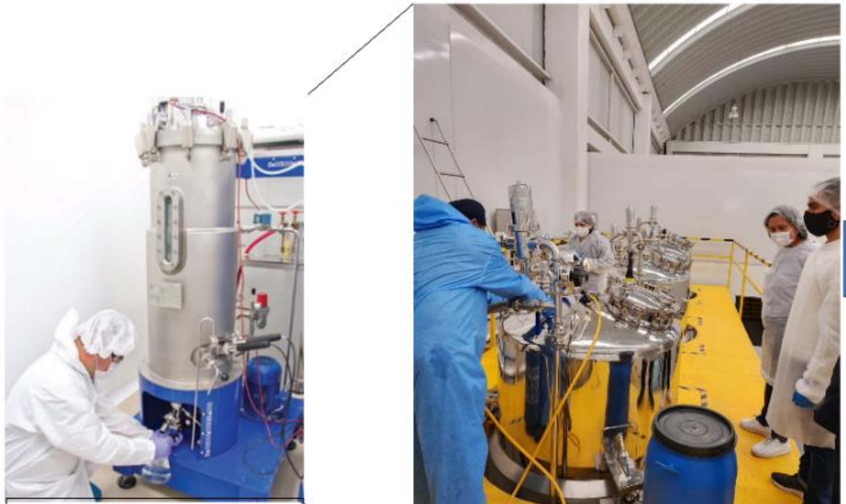
BIOCLAM

Bio-inputs portfolio AGROSAVIA

Accelerating bioproduct scaling-up and registration

AGROSAVIA
Corporación colombiana de investigación agropecuaria

FORBIO



Culture media a formulation design

Scaling-up

Fosfotal

Scaling-up of biofertilizers at commercial level

AGROSAVIA 30 AÑOS COLOMBIA

Global Green Growth Institute

UK PACT

CONVOCATORIA
Mecanismo de Aceleración de Proyectos de Bioeconomía #MAPBIO

Financial aid: Inter-governmental organizations

scaling-up and for efficacy tests

Production initially agreed	Hectares to impact 2022	Maximum capacity that the ally could generate (FORBIO)	Hectares to impact with the maximum capacity
4.000 L/año	2.666 ha/año	**144.000 L/año**	**96.000 ha/año**