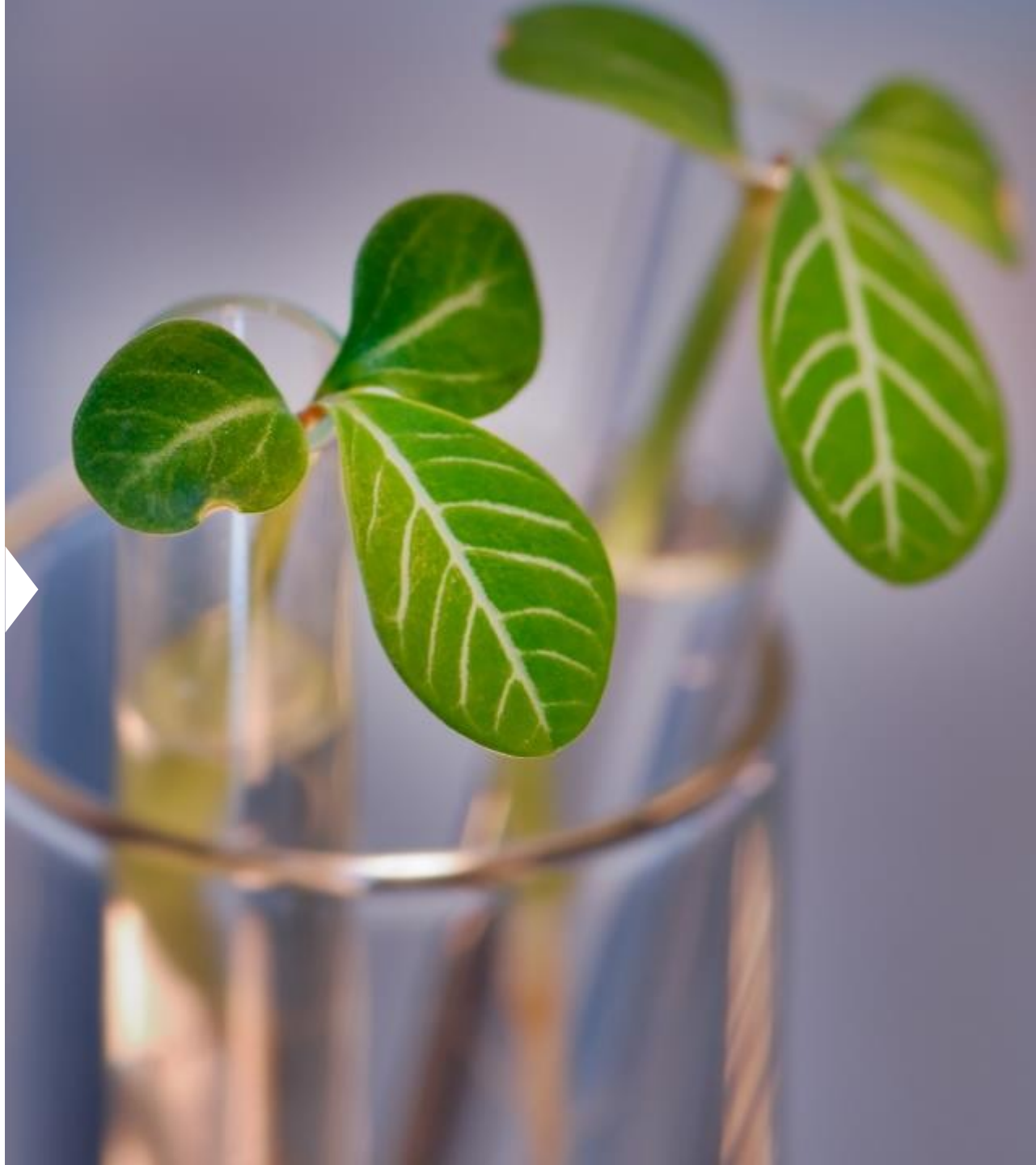
SOLVAY

Progress beyond

Microbial Biopesticides

Adjuvants & Co-formulants toolbox



Agenda

1. **Biocontrol introduction**
   - What is Biocontrol?
   - Market Overview

2. **Biocontrol Formulation Challenges**
   - Overview
   - The case of Bacillus spores
   - The case of conidial species

3. **Solvay Capabilities for Biocontrol Formulation design**
   - Overview
   - R&I global organisation
   - Zoom: Biotechnology and Microbiology facilities in Brazil

4. **Solvay's Approach and Solutions**
   - Approach
   - Solvay's Adjuvant and Co-formulants toolbox
   - Case Studies
     - Guar as retention/ rainfastness additive for biopesticides
     - Resolving microbial dispersion challenges with Solvay solutions
     - Adressing Needs for Seed treatments with biologicals

5. **Q&A**

What is Biocontrol ?

Biocontrol comprises **using living organisms or natural substances** to prevent or reduce damage caused by harmful organisms (animal pests, weeds and pathogens):

4 categories of Biocontrol products / Biopesticides:

- Macro-organisms (eg Coccinellidae, trichogramma)
- **Micro-organisms (eg *Bacillus Thuringensis, Beauveria Bassiana*)**
- Semiochemicals (eg Pheromone)
- Natural substances of mineral, plant or animal origin (eg S, Cu, Plant Extracts)



One of the most promising tools for **sustainable agriculture**, complementing the use and reducing the risk of resistance to synthetic agrochemical actives, thus strengthening **integrated pest management (IPM) adoption**

Biocontrol – Market Overview

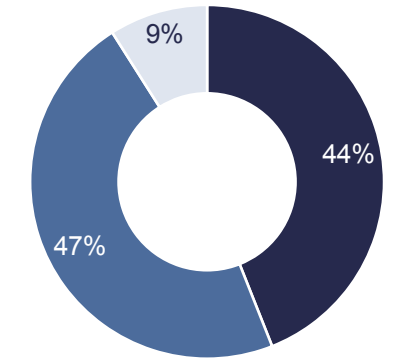
Market Size

4.0
bn$

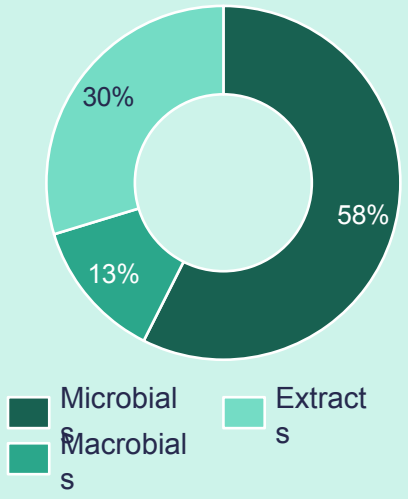
CAGR% 2019-25

~15%

Products



Technologies



Growth Drivers

- **Ag transition** from synthetic pesticide to biologicals with **Integrated Pest Management**
- **Growing demand** from end consumer **for organic & residue-free** food
- Regulatory:
  - **EU Farm to Fork strategy 2030 targets**: *Making 25% of EU agriculture organic*; *Reduce by 50% the use of Pesticides;*
  - ***Brazil new National Bio-input Program***

Formulation is key for biological efficacy of microbial pesticides

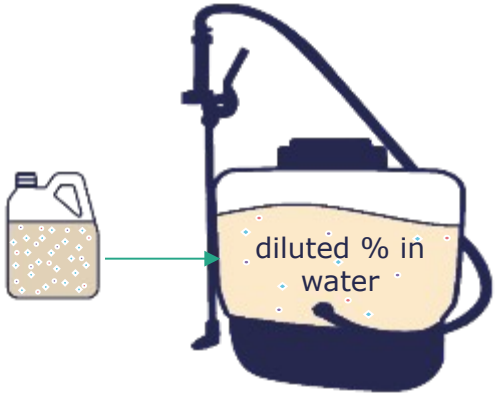
- Microorganism species (bacillus spores vs conidial) have different sensitivity to chemicals, temperature, UV, humidity.
- Microorganism strains and fermentation process also impact performance and formulability

 There is no universal solutions for formulating microorganisms. ***Formulation toolbox needs to be adapted.***

Key formulation success criteria:

- **Optimal Shelf-life**
- **Homogeneous dispersion upon dilution, ease of handling**
- **Retention & protection on target surface**
  - **Rainfastness**
  - Protection against **environmental** factors (UV, temperature/drought resistance)
- Enhanced **microbial bioactivity after application**

Formulation & Application challenges with microorganisms

FORMULATION ***Liquid Concentrate*** *(SC / FS / OD)* ***Solid Concentrate*** *(DF, WP, WDG)*	*DILUTION* *in the Spray Tank*	*APPLICATION* *on target pest / disease*
		
SHELF LIFE • Microorganism viability (compatibility inerts) • Controlled microorganism growth • Homogenous Dispersion (Microorganism particles sizes usually larger than chemical pesticides, with a tendency to aggregate)	HANDLING • Consistent dispersion quality upon dilution • Prevention of agglomeration and nozzles clogging	ON TARGET • Retention (Droplet size management;Good Wetting / spreading; Adhesion) • Protection (Rainfastness, UV protection, Humectancy – depending on microorganism…) • Activation (Boosting of microorganism growth, biofilm formation + production of 2ndary metabolites)

More than 40 years of Agro formulations expertise worldwide

Solvay Capabilities for Biocontrol formulations design

- ✔ SC, SE, OD, EC, SL, EW, WP, WDG agro formulations, process, characterizations
- ✔ Database of more than **2000 agro formulations**
- ✔ **High throughput** formulation capabilities & modelling tools
- ✔ **State of the art capabilities**:
  Droplet size distributions, contact angle, surface tension, microscopy, granulometry, Zeta potential, foliar retention, rainfastness

Solid analytical capabilities Network
(structural, separation, elementary analysis, powder morphology)

Phys-chem expertise
- *Rheology*
- *Colloidal science*
- *Surface modification*

Technology platforms
- *Polymers incl. from natural source*
- *Mild Surfactants*
- *Green Solvents*

Microbiology Advanced labs

France, Brazil

Solvay's approach

FOCUS ON 4 MAIN MICROORGANISMS

*to understand **key formulations & application challenges,** and therefore **the core functionalities required for desired co-formulants:***

- *Bacillus thuringiensis*
- *Beauvaria bassiana*

BIOINSECTICIDES

- *Bacillus subtilis /amyloliquefaciens*
- *Trichoderma harzianum*

BIOFUNGICIDES

CO-FORMULANTS SCREENING based on:

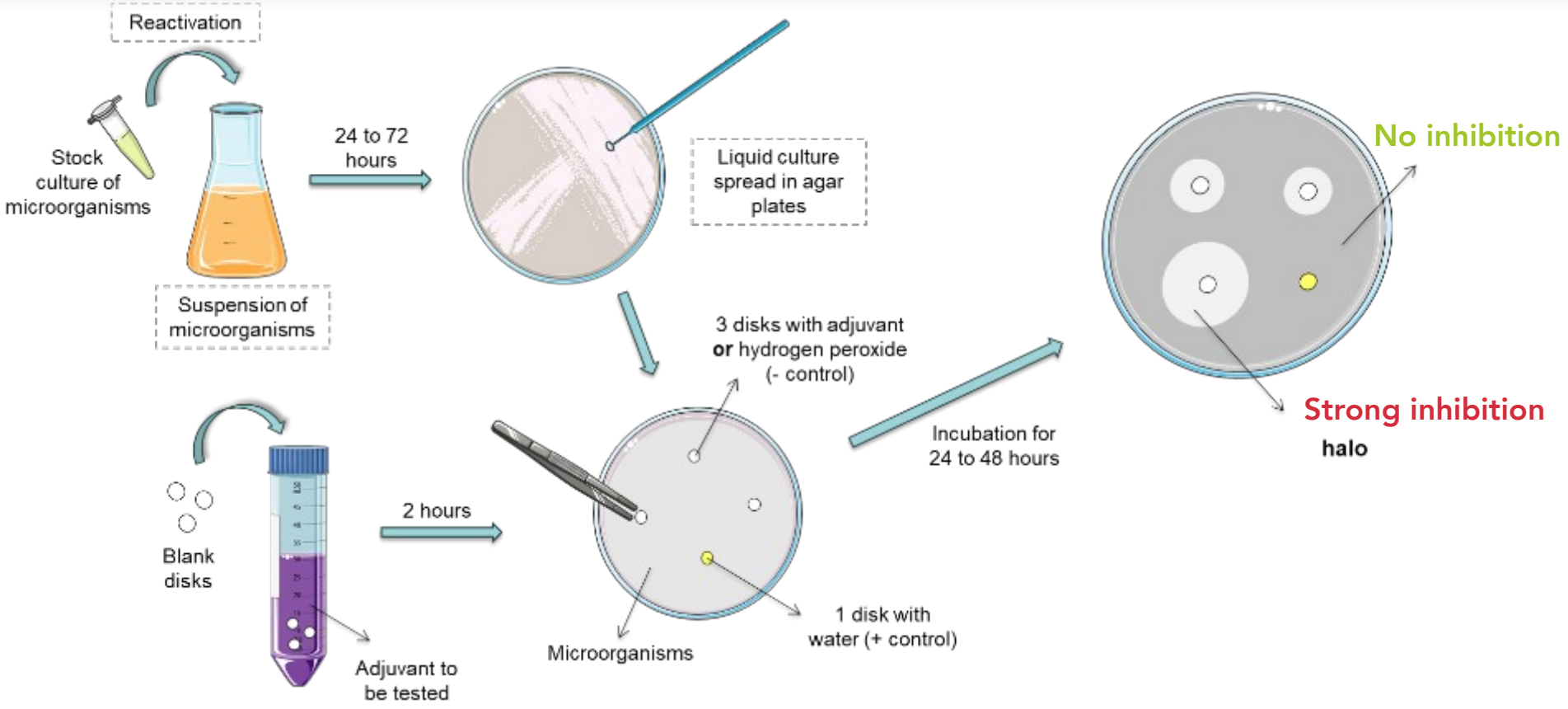
- **Functionality desired and targeted formulation type**
- **Regulatory criteria** (low tox/ecotox profile, REACH & EPA, NOP for organic farming compliance etc.)
- **Biological viability** of selected strains from these 4 main microorganisms **(zone inhibition tests)**

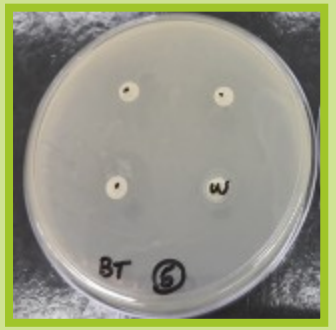
SPECIFIC PRODUCT DEVELOPMENT

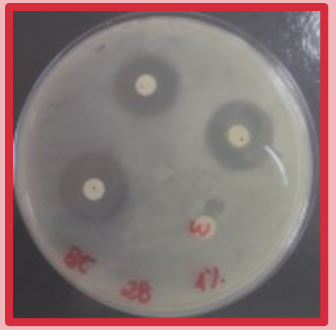
1. Natural adjuvant for biocontrol **retention & rainfastness** leading to **improved biological efficacy**
2. **Dispersibility & desagglomeration aid** designed for dense and hydrophobic microbial systems
3. Solutions for on **seed applications** of biologicals

Biological viability screening

Zone of inhibition (“Halo”) Test







Rapid, qualitative method to assess if a co-formulant has a negative impact on microorganism viability in agar plates

 important test to anticipate potential viability issues, once microorganisms are activated / germinate upon formulation dilution

Solvay's range of inerts for biocontrol formulations

Function (Formulation type)	Product Name	NOP / OMRI Eligibility*	GHS Classification	EPA	Compatibility, Microbial Viability (Zone Inhibition Test)			
					Bacillus Thuringiensis	*Bacillus Subtilis*	*Trichoderma Harzianum*	*Beauveria Bassiana*
Wetting Dispersing Agents (WP/WDG/SC/FS)	**Rhodasurf® 860/P**	No	No if < 1%	180.910 & 180.920	(1%)	(1%)		(1%)
	Rhodasurf® CET/5	Yes	EU : No if < 2.5% UN : No if < 1%	180.910 & 180.920			(1%)	
	Soprophor® BSU	Yes	No if < 25%	180.910 & 180.920				
	Soprophor® 4D/384	Yes	No if < 25%	180.920				
	Soprophor® FL/K	Yes	No classification	180.920				
	Soprophor® TS/29	No	No if < 25%	180.910 & 180.920				
	Soprophor® 796/P	No	No classification	180.960				
	Antarox® 25-R-2	Yes	No classification	180.960				
	Antarox® L-62	Yes	No classification	180.960				
	Antarox® PL 104	Yes	No classification	180.940 & 180.960				
	Antarox® PLG-254	Yes	No classification	180.960				
	Antarox® B 600	No	No if <= 4.2%	180.960				
	Geropon® DA 1349	Yes	No classification	180.960				
	Geropon® T 36	Yes	No if < 18%	180.910 & 180.920				
	Supragil® RM/210-EI	Yes	No classification	180.910 & 180.920				
	Geronol® CF82 CC	Yes	No classification	180.920				

i.e included in EPA NOP compliance List 4A/4B, therefore OMRI eligible

	No inhibition with 1% or 5% adjuvant
(1%)	Some inhibition with 5% adjuvant, no inhibition with 1% adjuvant
	Significant inhibition observed at 1% and 5% (radius inhibition zone [2 – 11 mm])
nt	Not tested

Soprophor® grades (TSP), Antarox® EO/PO, Geropon® DA1349 & T36 are versatile co-formulants with good biological compatibility with tested microorganisms strains. Suitable candidates for WP/WDG/SC/FS design

Microorganisms viability with co-formulants

					Compatibility, Microbial Viability (Zone Inhibition Test)			
Function (Formulation type)	**Product Name**	**NOP / OMRI Eligibility***	**GHS Classification**	**EPA**	***Bacillus Thuringiensis***	***Bacillus Subtilis***	***Trichoderma Harzianum***	***Beauveria Bassiana***
Emulsifiers (OD, EC)	**Alkamuls® T20**	Yes	No classification	180.910 & 180.920				
	Alkamuls® T/85-V	Yes	No if < 25%	180.910 & 180.920	nt	nt	(1%)	
	Alkamuls® OL 40	Yes	No classification	180.910 & 180.920				
	Alkamuls® RC	Yes	No classification	180.960				
	Alkamuls® VO 2003	No	No classification	180.960	nt	nt		(1%)
Thickeners (SC/FS)	**Rhodopol® 23**	Yes	No classification	180.950				

i.e included in EPA NOP compliance List 4A/4B, therefore OMRI eligible

	No inhibition with 1% or 5% adjuvant
(1%)	Some inhibition with 5% adjuvant, no inhibition with 1% adjuvant
	Significant inhibition observed at 1% and 5% (radius inhibition zone [2 – 11 mm])
nt	Not tested

For OD Design, e.g. with *Beauveria Bassiana* conoidal spores, depending on the nature of oil, **Alkamuls® T/85V, OL40, OR-36, VO2003 or T/20** are interesting candidates, with good biological compatibility with tested strains.

Geronol® Odessa

The robust ready-to use solution for Oil Dispersion

GERONOL ODESSA SERIES

A ready-to-use, efficient Oil Dispersion basis, suitable for some microorganisms formulation (eg *Beauveria Bassiana*)

- **Geronol® Odessa 01** for Vegetable Oil based OD
- **Geronol® Odessa 05** for MSO methylated seed oil based OD

All
- Part of the oil
- Emulsifier system

In
- Dispersing agent
- Rheology modifier

One
- Activator

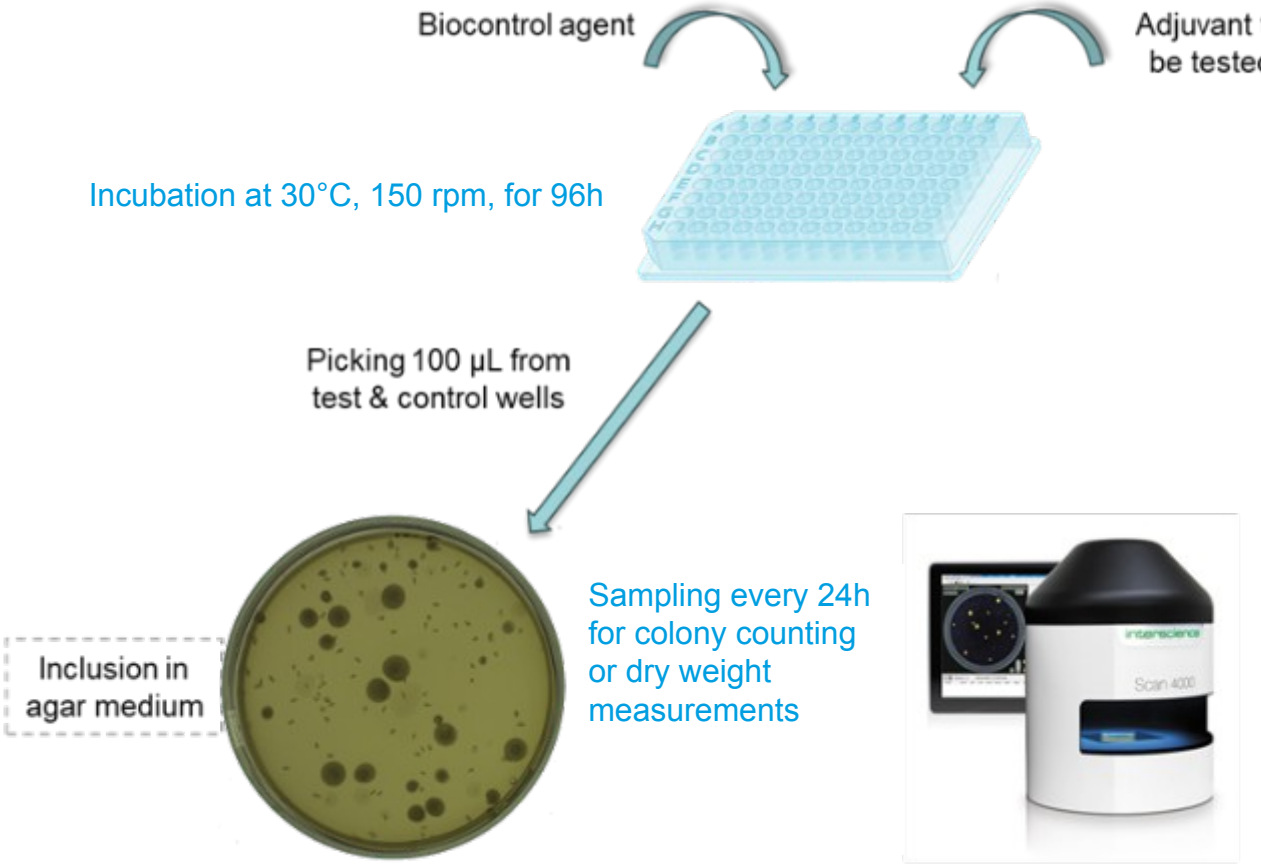
OD MADE EASY

1. Blend Odessa & Oil
2. Add the a.i.
3. Stir, Homogenize & Pack

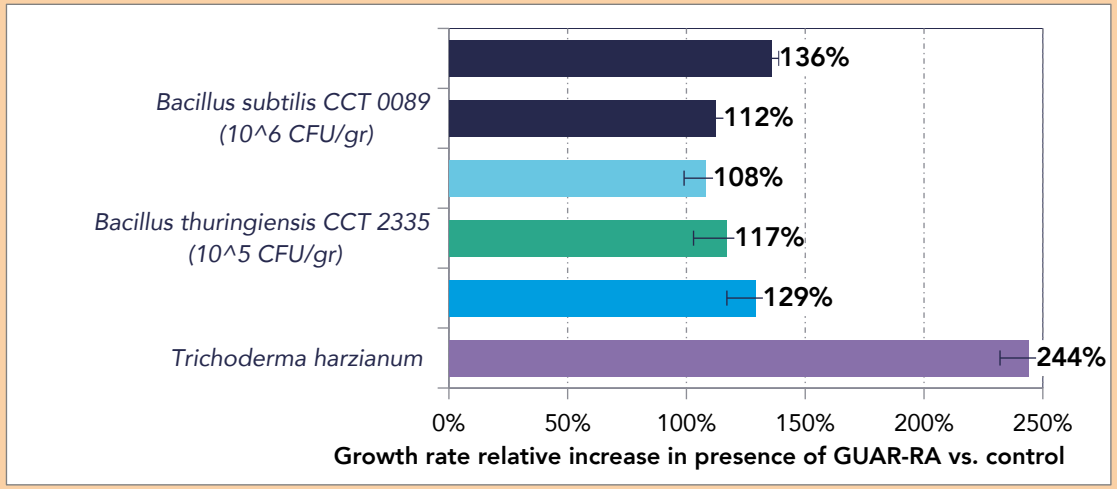
FORMULATION EXAMPLE

Composition	OD#1 (Wt%)
Beauveria Bassiana	20.0
Geronol® Odessa 01	45.0
Soybean Oil	Up to 100
Characteristics	
Appearance	Dark green viscous liquid
Density	0.98
pH (5%)	6.0
Brookfield Viscosity (20°C, 20RPM, mPa.s)	1250
Storage at 0°C for 7 days	stable
Dilution stability (CIPAC D, 30°C, 2h)	OK

Enhancing microbial growth with natural polymer technology



- **Significant increase of microbial growth observed in presence of 1%(w/v) GUAR-RA for all microorganisms tested**

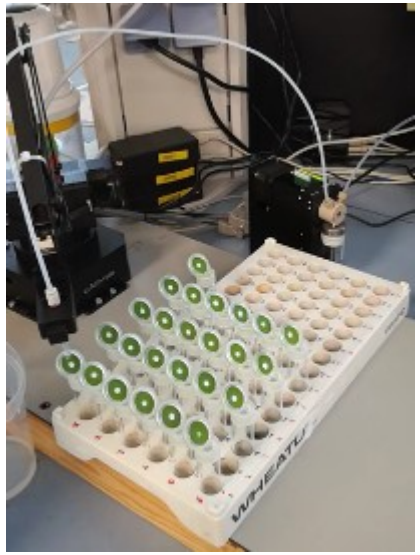


Microorganisms were separately inoculated in the experimental broth containing 1%(w/v) of GUAR-RA and the control media, without it. All flasks were incubated at 30°C, 150 rpm, for 96h, with sampling every 24h for colony counting or dry weight measurements. The growth rate was calculated and compared with control experiments.

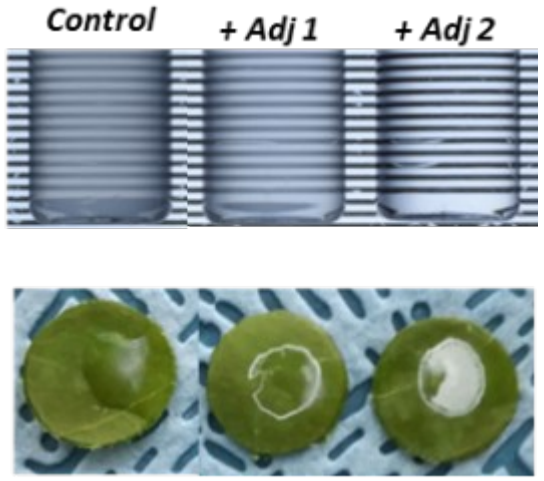
Rainfastness additive for biopesticides

RAINFASTNESS - High throughput screening methodology

Deposit, drying & washing



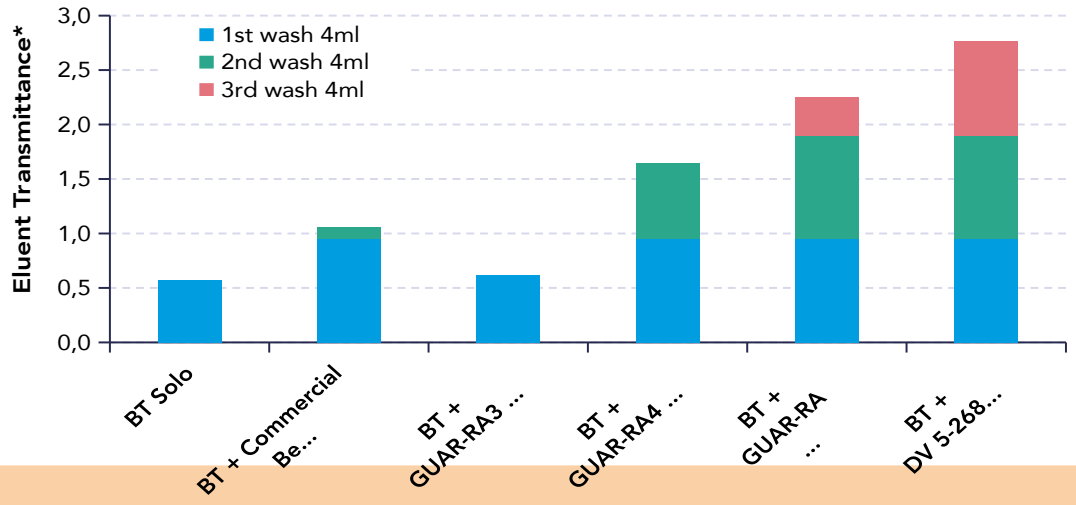
Measurement



- *Calibrated droplets deposit on the surface of the leaves, drying time 1 hour at RT, followed by washing cycles using a specific robotic arm.*
- *Then absorbance measurements of the collected eluents from captured images with a digital camera. A high transmittance value corresponds to a good rainfastness.*

- **Outstanding rainfastness even after 3 rain cycles**
- **Outperfmorms standard synthetic commercial benchmark**

Bacillus thuringiensis var. aizawai WDG (BT) – *2.5 wt% dilution*

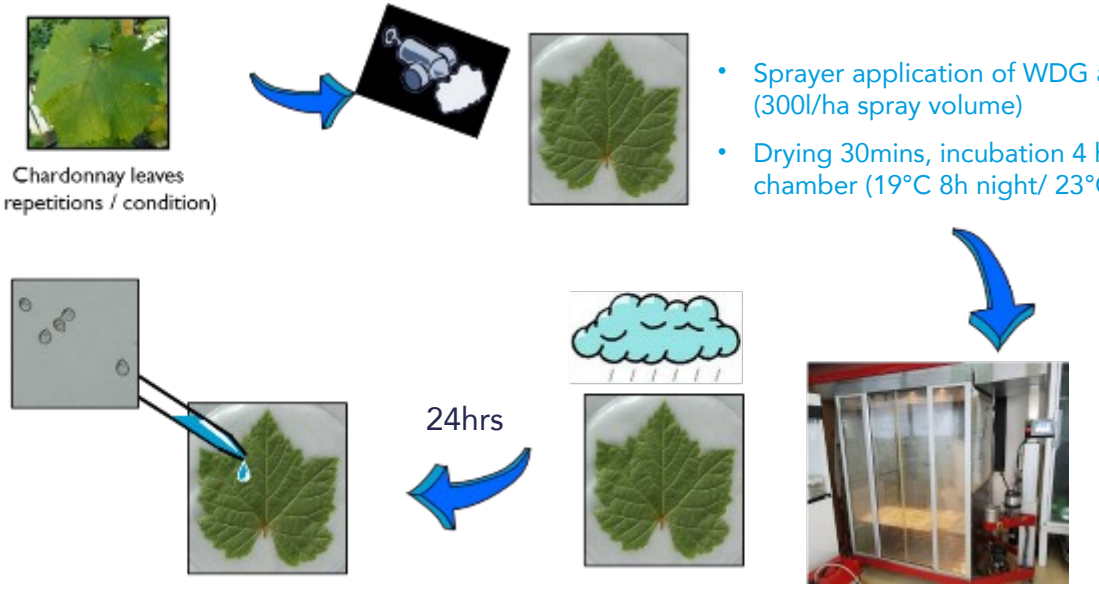


High value of eluent transmittance = good rainfastness effect

** Cumulative eluent transmittance, up to 3 rinsing cycles 4ml water*
[1] For Bt Solo & [Bt + GUAR-RA3], only 1st wash performed, due to limited rainfast after 1 wash
[2] For [Bt + Benchmark] & [Bt + GUAR-RA4], only 1st & 2nd washes performed due to limited rainfast after 2nd wash.

Rainfastness additive for biopesticides

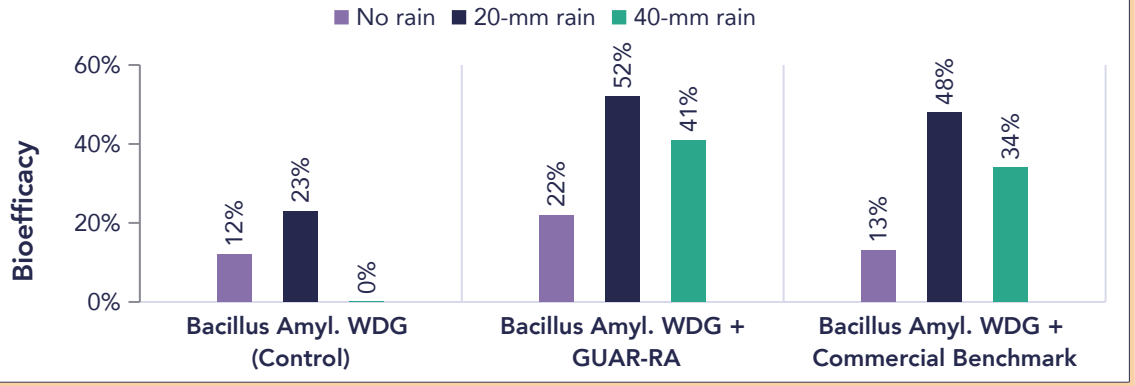
WDG, *Bacillus Amyloliquefaciens strain D747, 5.10¹⁰ CFU/gr*



- Sprayer application of WDG at 2,5kg/ha (300l/ha spray volume)
- Drying 30mins, incubation 4 hours in climatic chamber (19°C 8h night/ 23°C 16h day)
- Inoculation of the lower (abaxial) face of vine leaf with a calibrated suspension of *B. cinerea* strain **(Botrytis)**
- 20 or 40-mm of artificial rain
- Drying 1h
- Incubation 24h in climatic chamber (19°C 8h night/ 23°C 16h day)

BIOtransfer
Growing innovation for your solutions

Bacillus Amyloliquefaciens WDG efficacy against Botrytis on vine



- **Under heavy rain, use of rainfastness technology is critical for performance**
- **Solvay natural product doubles biological efficacy under dry or normal rain conditions**
- **Solvay product consistently outperforms synthetic commercial benchmark**

Resolving dispersion challenges with Solvay solution

DISPERSION CHALLENGES

High tendency to aggregation/agglomeration/sedimentation when diluting most commercial biopesticide products into water (dilution rate range 0.1%~2% m/v)

WHY ?

- *Larger particle sizes distribution of microorganisms / metabolites vs conventional pesticides*
- *Particles density*
- *Hydrophobicity of fermentation broth microorganisms, metabolites etc.*

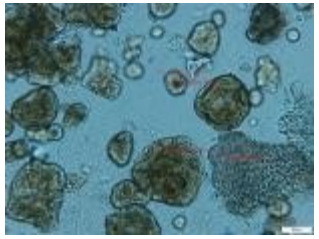
CONSEQUENCES

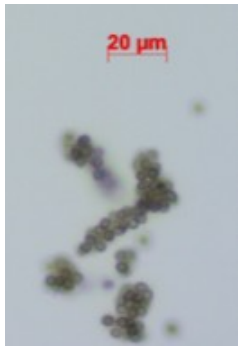
- *Lack of homogeneity of dispersion, nozzles blocking risk*
- *Handling process issues for final user*
- *Potential lack of bioavailability of microorganisms & their metabolites once applied*

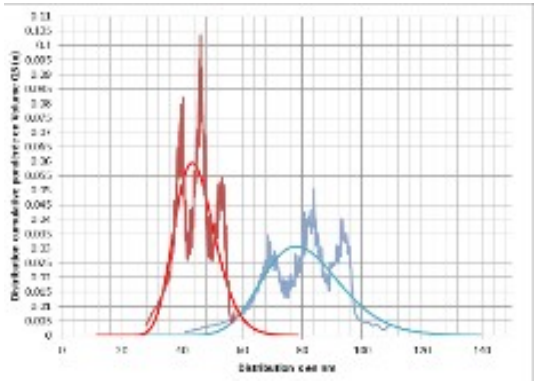
⍰ ***Potential negative impact on final bioefficacy***

0.5% wt.
Bt WDG
tap water
30 min

1% wt.
B. Subtilis SC
tap water
30 min





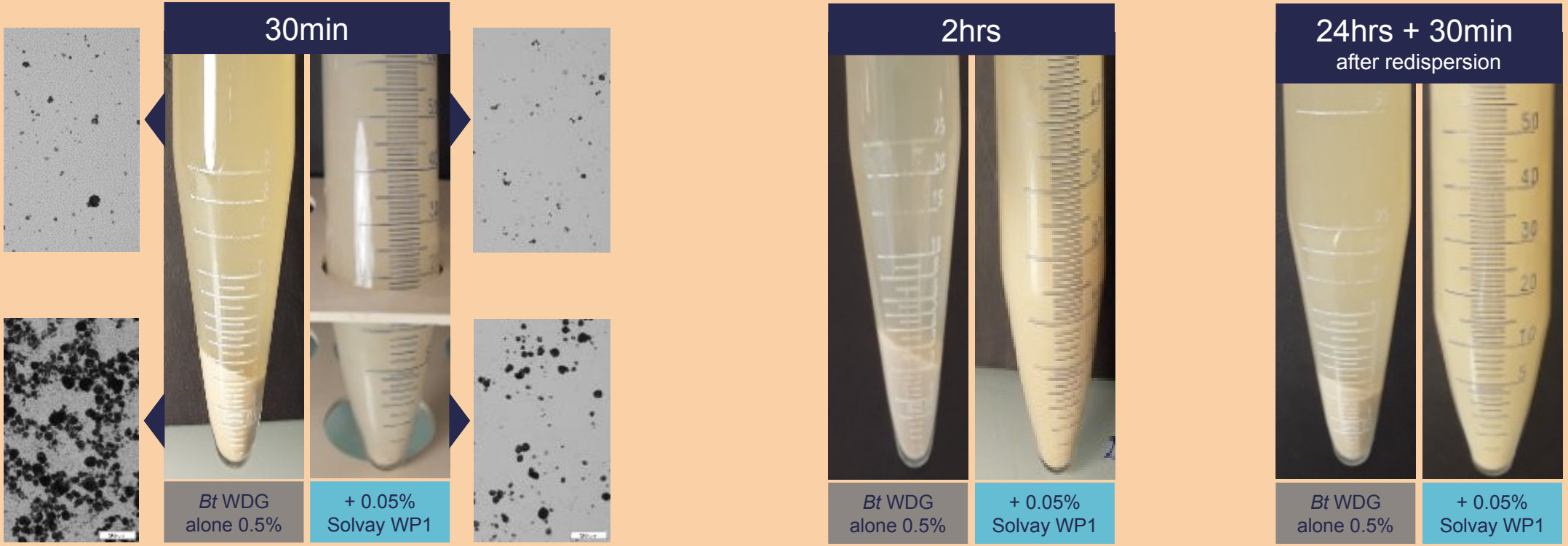


B. Subtilis SC before dilution
(same brand different lots)

Resolving dispersion challenges with Solvay solution

Strong improvement of dispersion quality with Solvay WP1.

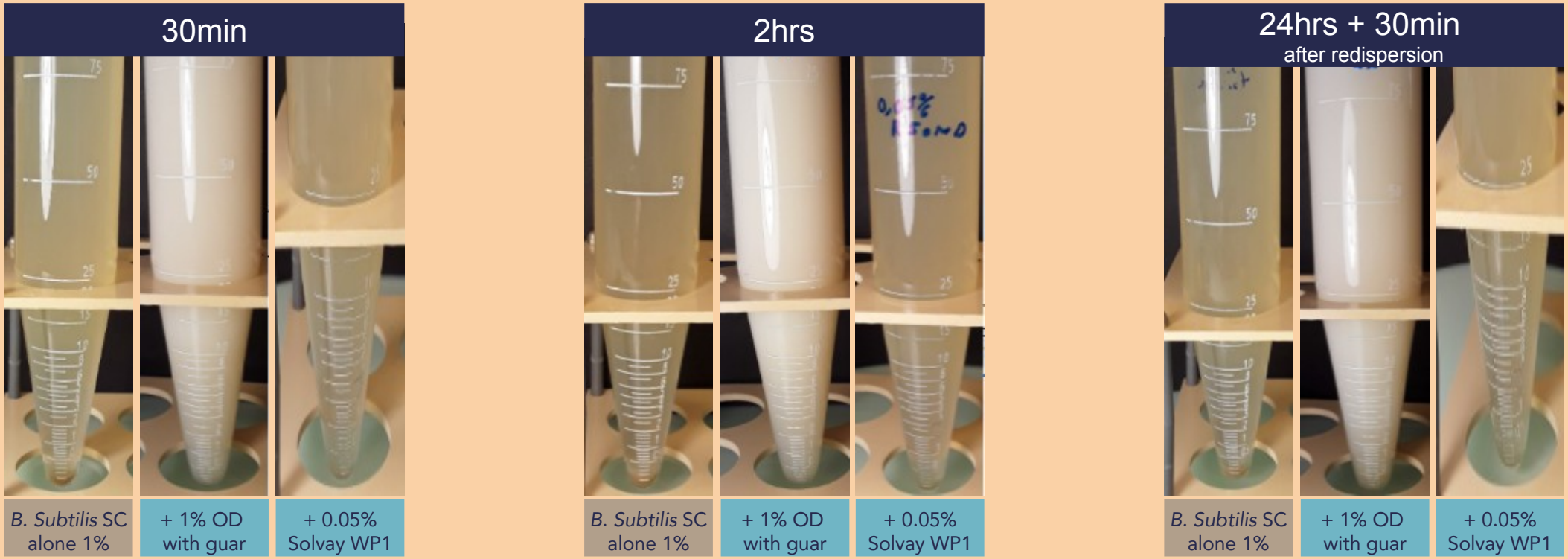
COMMERCIAL *BACILLUS THURIGIENSIS* WDG FORMULATION



Resolving dispersion challenges with Solvay solution

Strong improvement of *B. Subtilis* suspensibility with solvay WP1 (Built-in) and OD guar (Tank Mix) solutions

COMMERCIAL *BACILLUS SUBTILIS* SC FORMULATION



On-Seed application of biologicals

Microplastic-Free Binder Solutions

AgRHO BIOBINDER

- ✔ **Compatible with microorganisms**
- ✔ Can be used in **Organic Farming** EU for Seed Treatment according to EC834/2007
- ✔ **Readily biodegradable** (OECD 301F),
- ✔ Good dust reduction performance for some biological slurries
- ✔ No negative impact on germination

Synergistic combination Biostimulants / Biologicals

AgRHO S-Boost Range

- ✔ Stronger root architecture development
- ✔ Improved nutrients & water uptake
- ✔ Symbiotic interaction with soil microorganisms
- ✔ Synergy with microbial biopesticides applied on seeds

Enhanced crop yield, with mitigation of adverse drought conditions

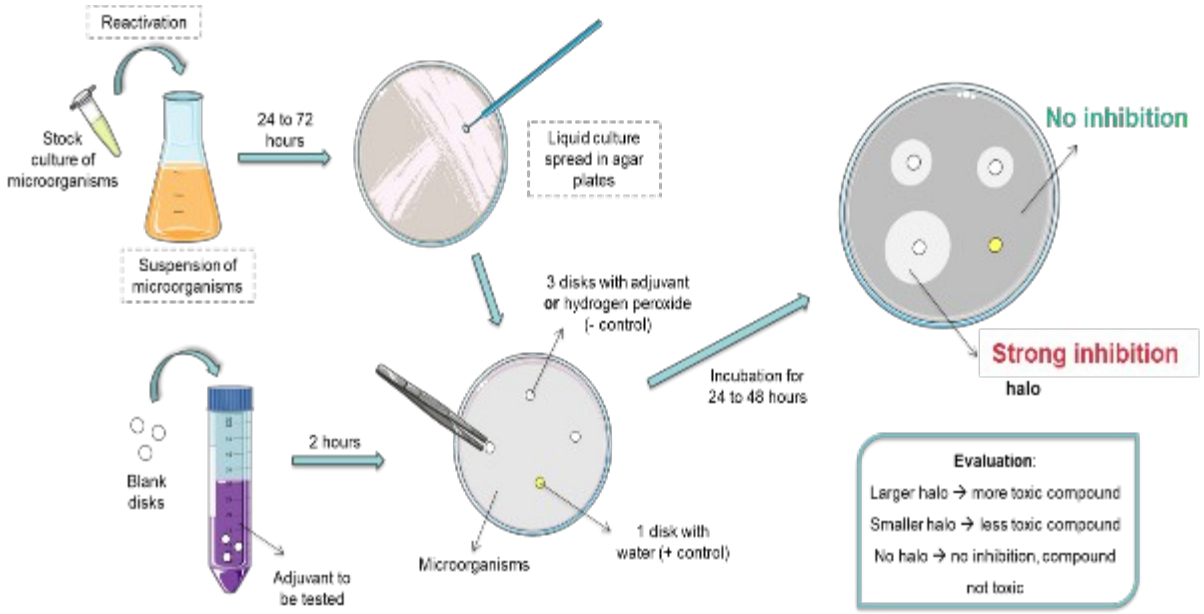
Flowable Suspensions and Slurries

Solvay co-formulant portfolio compatible with microbials :

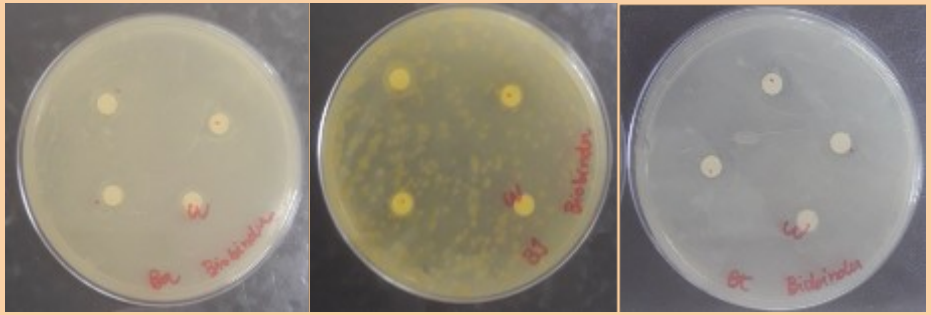
- ✔ Wetting agents
- ✔ Dispersant agents for high load flowables
- ✔ Rheological agents

AgRHO Biobinder compatibility with microorganisms

Zone of inhibition ("Halo") Test

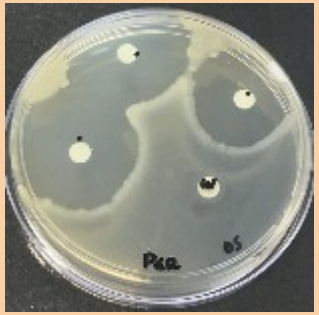


AgRHO BioBinder



B. Amylo-liquefaciens ***B. Japonicum*** ***B. Thuriengiensis***

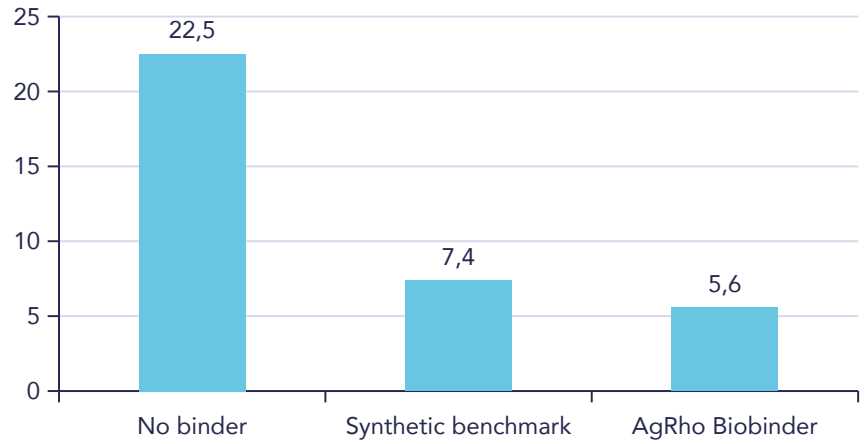
Negative control Peroxide



- AgRho Biobinder does not trigger any inhibition of microorganism growth for the three bacteria species tested.
- **AgRho Biobinder can be safely co-applied with slurry based on microorganisms for biocontrol or inoculant purpose.**

AgRHO Biobinder dust reduction performance

Dust Reduction Performance, in biological slurry*



** Seed treatment: biological inoculant based on a fungus species applied on corn seeds at a dose rate of 500 g/qt as a dry powder. The binder formulations are co-applied on seeds with an application rate of 585 g/qt.*
***ESTA standard test** (100gr seed, 30RPM, air flow 20L/min, rotation 120 sec)*

Germination Impact

Germination tests to **assess the innocuousness of AgRho® Biobinder:**

Treatment	% normal plant
Control	89
AgRho Biobinder 175 mL/qt	88

Test was conducted in wet sand (9% humidity) during 7 days with 8 hours of light per day and alternative temperature 20/30°C.

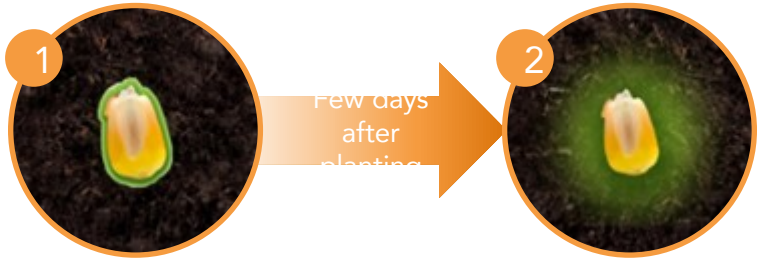
For biological slurry based on microorganism dispersions, **AgRho Biobinder offers higher dust reduction performances than the synthetic benchmark, and is safe for application on seeds**, no sign of phytotoxicity observed

AgRho® S-Boost Biostimulant Technology at a Glance

AgRHO S-Boost, Architect of stronger roots

- S-Boost is a bio-based macromolecule, initially apply on the seed

S-Boost physically shapes the soil into water & nutrients attractor



S-Boost enhances plant's interactions with soil microorganisms

SECURE EARLY STAGE

- ✔ Boost seed germination
- ✔ Enhance plant vigor
- ✔ Maximize plant establishment

IMPROVE ABIOTIC STRESS RESISTANCE

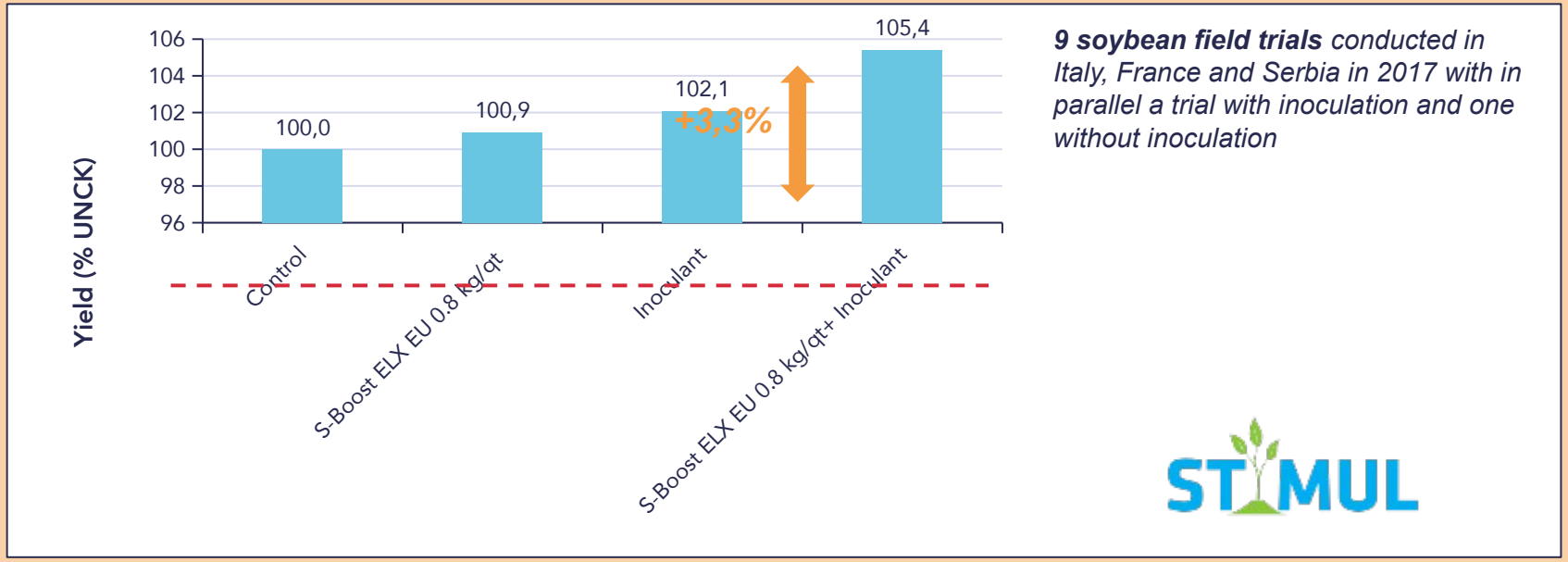
- ✔ Stronger root architecture
- ✔ Optimized water & nutriments uptake
- ✔ Symbiotic interaction with soil micro-organisms

OPTIMIZE CROP YIELD

- ✔ Positive yield response on multiple crops
- ✔ Mitigation of adverse drought conditions

FEATURE PRODUCTS #3

Synergy of S-Boost with Bradyrhizobium Japonicum on Soybean

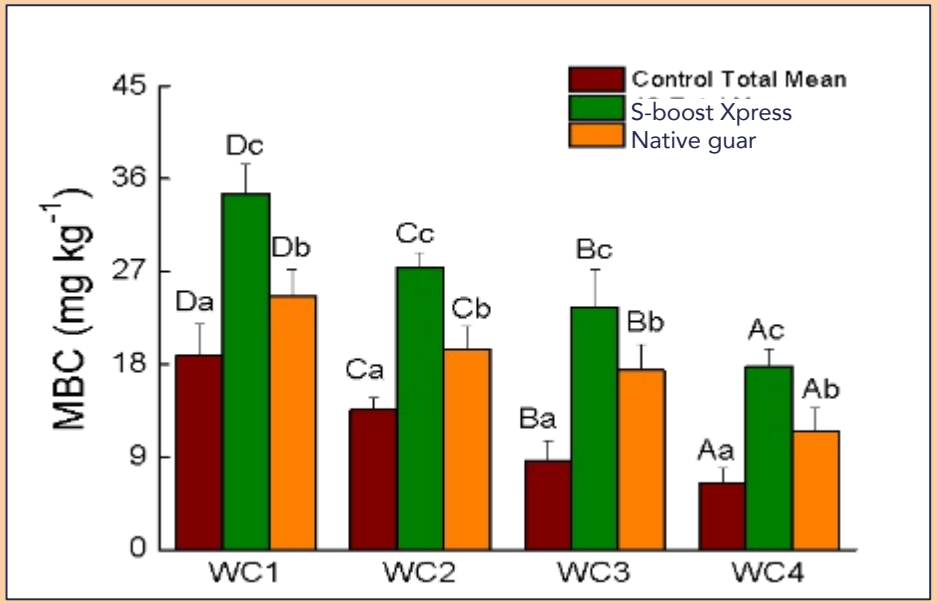


***9 soybean field trials** conducted in Italy, France and Serbia in 2017 with in parallel a trial with inoculation and one without inoculation*

*S-Boost ELX is compatible with the principal species of microorganisms used in seed treatment such as **Bradyrhizobium Japonicum and Bacillus Subtilis***

A higher yield increase with S-Boost™ ELX technology is observed in presence of rhizobium bacteria: + 3,3% (+ 1qt/ha)

AgRho® S-Boost™ impact on soil microbial content



Bacterial abundance measured with "Microbial Biomass Content" measured in several decreasing soil water content (from WC1 to WC4). Data collected in cinnamon soil collected from Linfen City, Shanxi Province .

S-Boost™ technology increases Microbial Biomass Content in soil

Conclusion

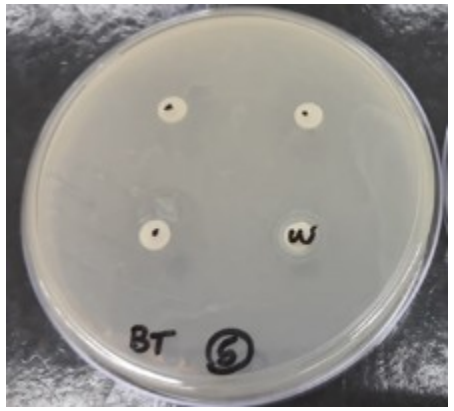
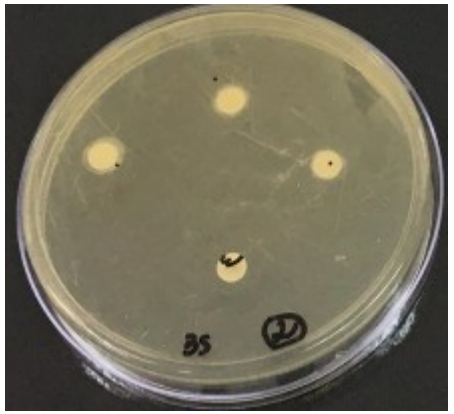
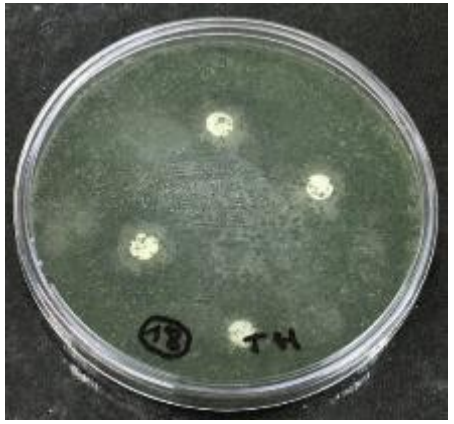
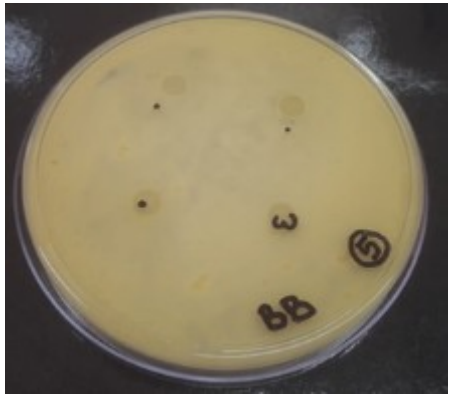
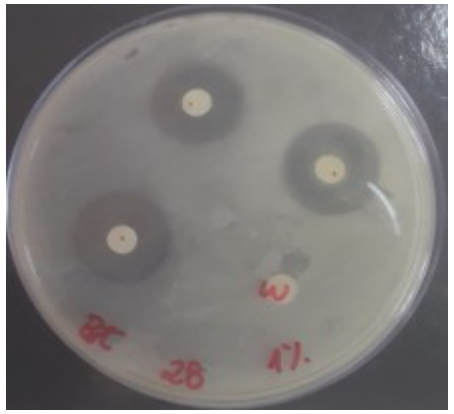
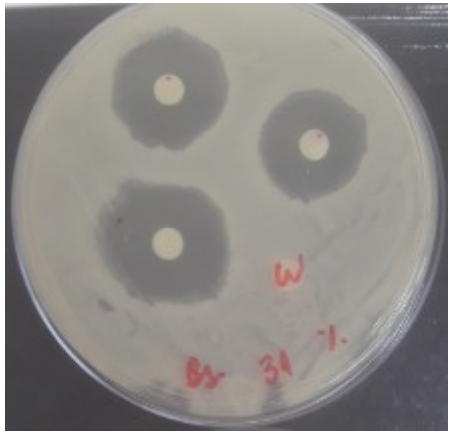
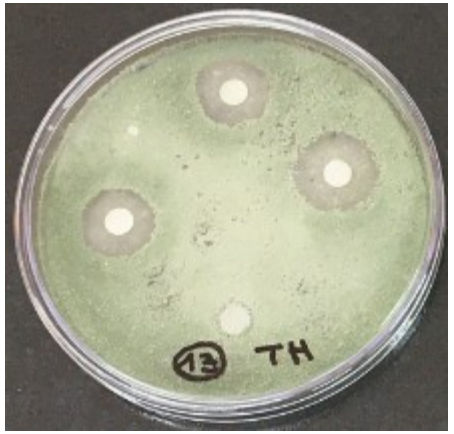
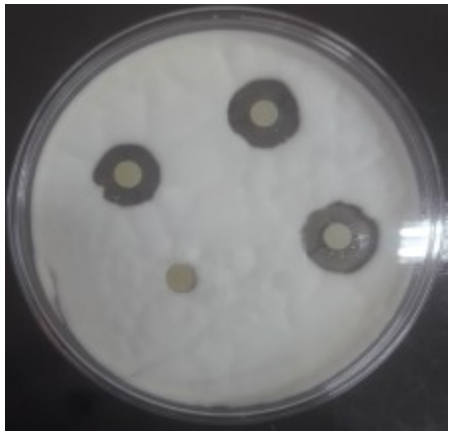
- Solvay has mobilized its **agro formulation know-how** coupled with **microbiology expertise, phys-chem & high throughput capabilities** to develop solutions for formulators of microbial pesticides for **foliar or seed applications**:
    - Inert toolbox adapted to standard dry or liquid biocontrol formulation
    - Feature sustainable solutions designed specifically to answer the most demanding challenges of biocontrol formulations: retention/rainfastness, desagglomaration, and overall bioefficacy enhancement
- Future developments will be focused on microbial **shelf-life enhancement**, **UV stability** & improvement of microorganisms **compatibility with conventional actives** through innovative **encapsulation technologies**

We would be happy to collaborate on your projects, develop new partnerships and support your Biocontrol formulation developments !

Thank You!

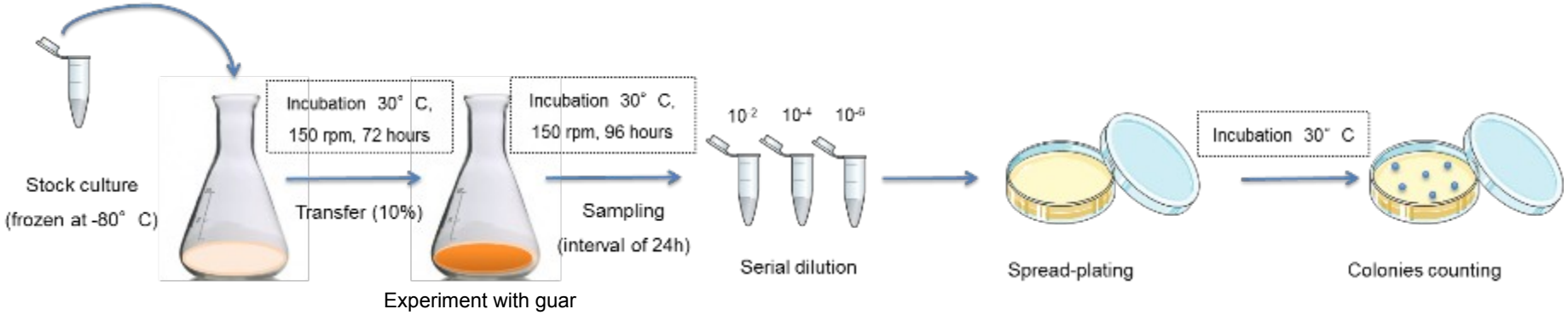
Progress beyond

Zone Inhibition Tests

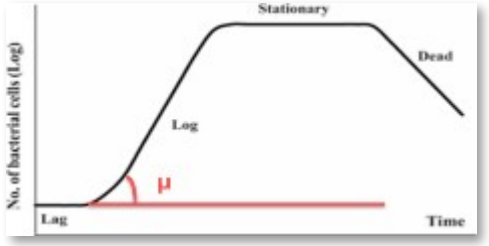
	B. thuringiensis	*B. subtilis*	*T. harzianum*	*B. bassiana*
No inhibition				
Significant inhibition				

Methodology, microorganism compatibility with adjuvant

METHODOLOGY FOR BACTERIAL INCUBATION AND GROWTH MEASUREMENT



Experiment with guar



Bacterial growth rate = μ

Exponential growth represented by

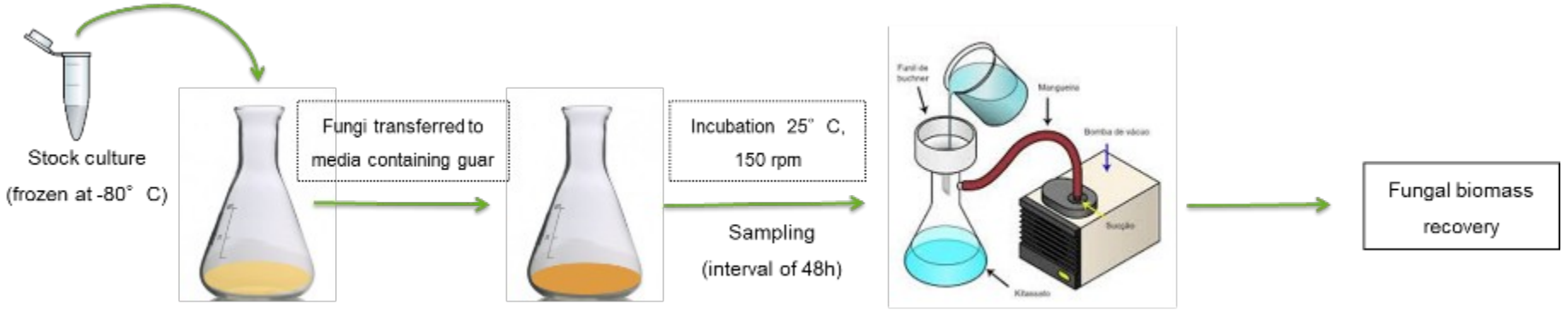
$$N_t = N_0 \cdot e^{\mu t}$$

or

$$lnN_t = lnN_0 + \mu\, t$$

➡ **Evaluation of the influence of guar on bacteria growth (μ value)**

METHODOLOGY FOR FUNGI INCUBATION AND GROWTH MEASUREMENT



Experiment with guar

Vacuum filtration

Formulation needs for some Bacillus spores

Microorganism	Mode of Action	Products characteristics & form	Key Formulation & application needs
Bacillus Subtilis / Amyloliquefaciens MBI600, QST713, D747 etc. **Biofungicide**	*B. Subtilis* + their lipopeptides metabolites (eg surfactins, iturins, agrastatins/plipastatins for QST 713) - Lipopeptides synergize each other to inhibit germ tubes, mycelium and disrupt fungal pathogen cell membranes - Lipopeptides prevent pathogen growth - Colonization of *B. subtilis* spores on leaf surface & root systems, competition/site exclusion for pathogens, nutrient competition. **Pathogen targets:** Botrytis, Powdery Mildews, sour rot, rust, sclerotinia, white mold etc.	Good resistance of Bacillus spores, to heat, UV, draught, chemical stress. **Typically WP/WDG & SC/FS form**  SC WP FS	✔ Better **dispersibility / desagglomeration** characteristics upon dilution ✔ Better **bioavailability, solubilization efficacy of lipopeptides** ✔ For foliar application, better **adhesion/retention & rainfastness** to avoid environmental loss of key metabolites and spores.
Bacillus thuringiensis (Bt ssp. Aizawaii & Bt ssp. Kurstaki) **Bioinsecticide**	During sporulation of *Bt*, crystal aggregates of proteins are produced. These crystals are taken up via ingestion and will interact with the midgut (specific interaction of crystal-insect depending on *Bt*) causing death of the insect. **Targets:** +30 species of insects, incl. tent caterpillar and various bagworms, looper, tobacco budworms and armyworms	**Typically WP/WDG & SC form**  	✔ Increased **UV stability** ✔ Better **adhesion/retention & rainfastness** (Bt & protein crystals aggregates) ✔ Increased **stickiness** (insect cuticles) ✔ Better **dispersibility/desagglomeration** characteristics upon dilution

Formulation needs for some conidial species

Microorganism	Mode of Action	Products characteristics & form	Key Formulation & application needs
Beauveria bassiana (PPRI 5339, ATCC74040) *Bioinsecticide*	Fungal conidial spores contact pathogen, acting as microbiological insecticide. - adheres to the insect cuticle (interaction spore wall/epicuticle lipids) - germinates, and penetrates the cuticle (enzymes) - Replicates in the insect body and destroys the internal structures of the host insect.	No protective barrier so low resistance to temperature, chemicals, UV, low shelf-life Low stability in water (easy reactivation in water) **Water-free media: WP/WDG, OD** 	✔ Increased **shelf-life** ✔ Increased **temperature & UV stability** ✔ Better **adhesion/retention & rainfastness** ✔ Increased **stickiness** (acarids/insect cuticles) ✔ **OD preferred** vs WP (oil increases the adhesion of spores to insect cuticles, through hydrophobic interaction between spores & cuticle surface) ✔ Better **dispersibility/desagglomeration** characteristics upon dilution
Trichoderma (harzianum T-22, asperellum ICC012, atroviride I 1237 etc.) *Biofungicide*	Biofungicides used against damping-off diseases. - colonizes the soil and roots of the host plant - competes with plant pathogenic fungi for space and nutrients. - attacks the cell walls of pathogens with enzymes. - promotes plant growth, increases the uptake and availability of nutrients	High sensitivity to chemicals, low shelf-life (<6 months), low temperature & UV stability, low water activity required **Typically WP/WDG , OD**  	✔ Increased **shelf-life** ✔ Increased **temperature & UV stability** ✔ Better **adhesion/retention & rainfastness** ✔ Improve **germination under cold conditions** ✔ Better **dispersibility/desagglomeration** characteristics upon dilution

Biotechnology and Microbiology Facilities in Brazil

- Located at Solvay's **R&I Center in Paulínia** (SP)
- Capabilities in **Biotechnology, Environmental analysis and Microbiology**
- **Microbiology labs:** biosafety level 2 Biosafety Certificate **Genetic Modified Microorganisms** (GMM - biosafety level 1)

Team of experts in:

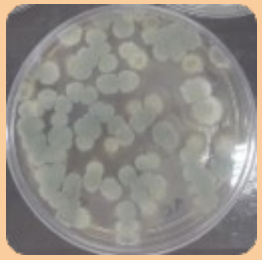
- ✔ Biology
- ✔ Organic Chemistry
- ✔ Chemical Engineering
- ✔ Process Engineering
- ✔ Renewable Chemistry
- ✔ Environmental Engineering

Main competencies:

- ✔ Microbiology assays
- ✔ Enzymatic processes
- ✔ Fermentation
- ✔ Biomass Processing and Valorization
- ✔ Ecotoxicity evaluation

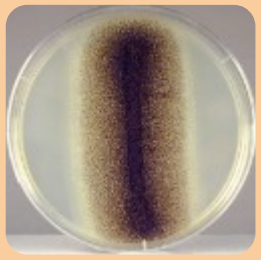
Partnerships with start-ups and Universities

MICROORGANISMS LIBRARY



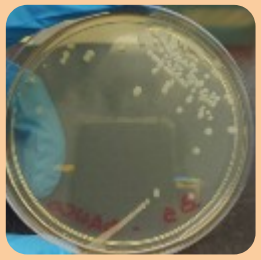
T. harzianum

B. bassiana

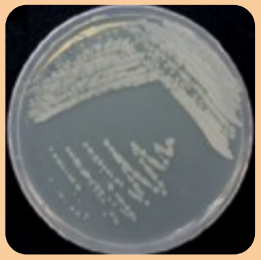


A. brasiliensis

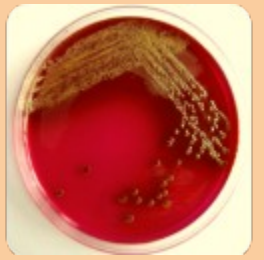
B. thuringiensis



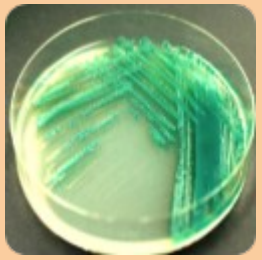
B. subtilis



B. amyloliquefaciens



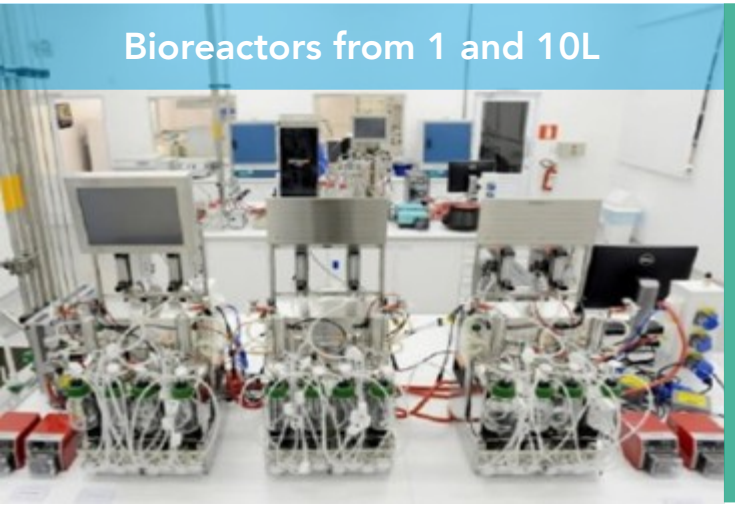
E. coli



P. aeruginosa

And others…

Biotechnology and Microbiology Facilities in Brazil



Bioreactors from 1 and 10L

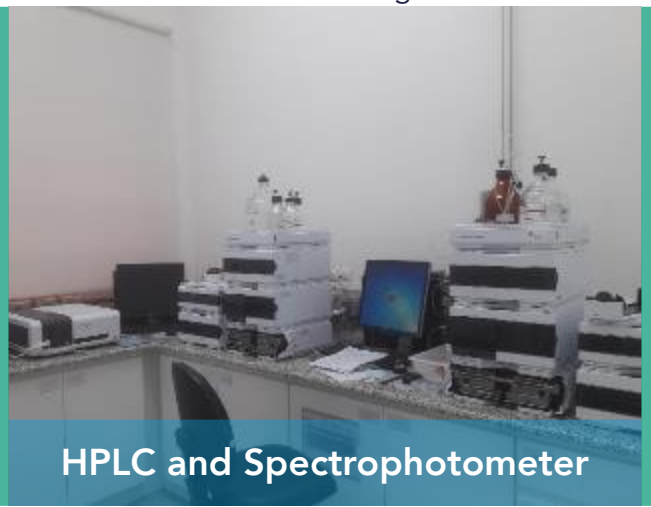
- Production of microorganisms biomass
- Fermentation process to obtain valuable products

Shakers

Anaerobic hood and laminar flow chamber

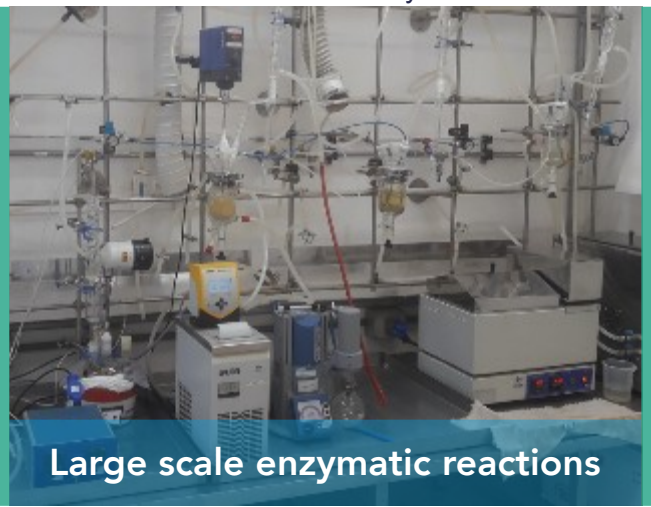
- Sterile manipulation of aerobic and anaerobic strains
- Stock of microorganisms at -80°C

- Fermentation products analysis
- Growth curve for microorganisms



HPLC and Spectrophotometer

- Enzymatic assays up to 2L
- Free and Immobilized enzymes



Large scale enzymatic reactions

- Culture media preparation
- Decontamination of materials

Sterilization room

Capabilities Agro formulation labs

High throughput robotic platform (solubility studies, dispersion etc.)

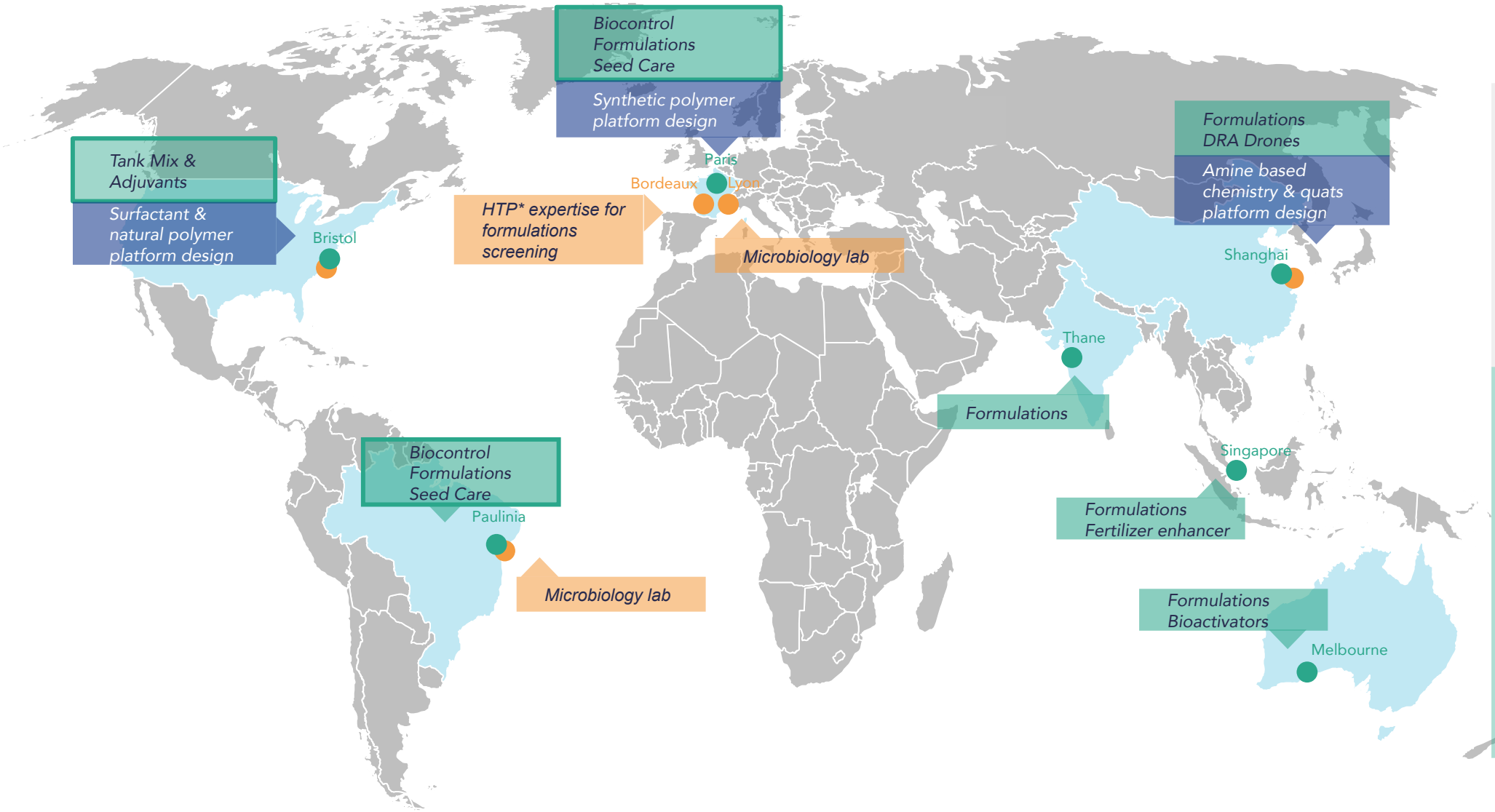
Spray chamber with Sympatec laser for droplet size distribution assessment

Rheometer (AR-G2 / TA)

Optical microscopy

Horiba Granulometer

BioControl: A Key platform in Solvay's Agro R&I organization



- Agro Lab
- Technologies development centres
- Agro application labs
- Biocontrol application lab
- Upstream labs

- **5 Major R&D Centers with phys-chem, synthesis, analytical and process/scale-up labs**
- **3 application labs working for our Biocontrol innovation platform**
- **2 Upstream & Technology labs dedicated to Microbiology and Biotechnologies**