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Granulate formulation protocol

In 1 collection

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Protocol status: Other
 The protocol was developed based on literature and has not been tested yet.

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ABSTRACT

Bioformulation is used in agriculture for various reasons like soil fertility, plant growth promotion, and suppression of phytopathogens. The bacterial inoculants are applied as a formulated product like powder, spray, or pellet. This is a green strategy that is being developed as a less harmful method to protect crops other than pesticides. In the paper published by *Alvarez et al, 2016*, they developed a talc-based powder formulation based on *Bacillus* B25 spores and evaluated some of its characteristics, like shelf life and efficacy against the pathogenic fungus *Fusarium verticillioides*.

CITATION

Martínez-Álvarez, J. C., Castro-Martínez, C., Sánchez-Peña, P., Gutiérrez-Dorado, R., & Maldonado-Mendoza, I. E. (2016). Development of a powder formulation based on *Bacillus cereus* sensu lato strain B25 spores for biological control of *Fusarium verticillioides* in maize plants.. *World Journal of Microbiology and Biotechnology*, 32(5), 75.

LINK

<https://doi.org/10.1007/s11274-015-2000-5>

Colony Forming Units (CFU) is a unit that is used in microbiology to estimate the number of viable bacteria or fungal cells in a sample. It also depends on their ability to multiply under controlled conditions. In the paper published by *El-Hassan and Gowen, 2006*, they analyzed various formulations of *Bacillus subtilis* by counting the CFU of *B. subtilis* present in every formulated product.

CITATION

S. A. El-Hassan and S. R. Gowen (2006). Formulation and Delivery of the Bacterial Antagonist *Bacillus subtilis* for Management of Lentil Vascular Wilt Caused by *Fusarium oxysporum* f. sp. *lentis*. *Journal of Phytopathology*, Volume 154, Issue 3.

LINK

<https://doi.org/10.1111/j.1439-0434.20>

Fatty acid analysis is an important means to characterize fats and oils and to determine the total fat content. The result can be used to identify the *Bacillus mycoides* strain in the soil and determine its survival with the granulate formulation.

CITATION

Friedrich von Wintzingerode, Frederick A. Rainey, Reiner M. Kroppenstedt, Erko Stackebrandt (1997). Identification of environmental strains of *Bacillus mycoides* by fatty acid analysis and species-specific 16S rDNA oligonucleotide probe. *FEMS Microbiology Ecology*, Volume 24, Issue 3.

LINK

<https://doi.org/10.1111/j.1574-6941.1997.tb00437.x>

Bacterium Inoculum

- 1 Grow a single colony of bacteria in an assay tube with 5 mL of Luria Broth (LB) medium
- 2 Incubate it in an orbital shaker at 200 rev.min⁻¹ at 30 °C for 18:00:00 .
- 3 After bacterial growth, take a 500 mL Erlenmeyer flask and add 100 mL of LB medium in it.

- 4 Add 1 mL of the culture (1 % (v/v)) in the flask and incubate at 30 °C and 200 rev.min⁻¹ for 24:00:00 , until an optical density of close to 1 is obtained.

Spore Production

- 5 Add 100 mL of Difco Sporulation Medium (DSM; 5gl⁻¹ peptone, 3gl⁻¹ yeast extract, 1gl⁻¹ KCl and 0.12 gl⁻¹ MgSO47H2O) in a 500 mL Erlenmeyer flask.
- 6 Sterilize this DSM medium at 121 °C and 1.5 psi for 00:15:00 .
- 7 Add 1 mL each of: 1 Molarity (M) Ca(NO₃)₂, 10 millimolar (mM) MnCl₂.4H₂O and 1 millimolar (mM) FeSO₄
- 8 Inoculate with 1X10⁶ c.f.u ml⁻¹ of the bacterial strain. Keep the culture conditions at 30 °C and 200 rev.min⁻¹ for 72:00:00 .

Powder Formulation

- 9 Mix talc (which will be the carrier) with, carboxy-methyl-cellulose (CMC; 1% w/w), CaCO₃ (15% w/w) and glucose (0.25% w/w) in powder form.
- 10 Autoclave the mixture at 121 °C and 15 psi for 00:15:00 .

- 11 Mix the same material with the bacterial spore suspension and dry at 55 °C for 36:00:00 .
- 12 Using sterile porcelain mortar and pestle pulverize the formulation.
- 13 Pack it in plastic bags and store it at room temperature.

CFU determination

- 14 Colony Forming Units (CFU) can be determined by estimating the OD of spore suspension using a tube-reading spectrophotometer adjusted at 1.978 [corresponding to $8.5 \cdot 10^{10}$ CFU/ml] at 600nm absorbance wavelength
- 15 The formulation will be placed on sterile aluminum foil in pans and air-dried for 24:00:00 with occasional stirring in a laminar airflow cabinet.
- 16 Dried formulations (35% moisture content) of *B. mycoides* will be passed through a 250µm mesh sieve to attain the desired particle size.
- 17 Pack in sterilized polypropylene bags, seal and store at Room temperature prior to use.
- 18 Count CFUs to estimate the number of viable propagules of *B. mycoides* using the standard dilution platin method.

STD dilution method

- 19 Take three 1 g aliquots of the dried powder and place in 99 mL sterile PBST solution (this will include PBS + 0.05 % (v/v) Tween 20). Stir magnetically at high speed for 00:15:00 . Now dilute this suspension with approximately and take 0.2 mL of this suspension and plate on Nutrient Agar (NA) media.

Fatty acid analysis

- 20 By performing saponification, methylation, and then extracting we can obtain fatty acid methylesters from wet biomass.
- 21 Next, separate the fatty acid methylester mixtures by using a microbial identification system. Peaks can be automatically integrated, and the Microbial ID will calculate the fatty acid names and percentages.