MANAGEMENT OF BENEFICIAL MICROORGANISMS RESOURCES TO SUSTAINABLE AGRICULTURAL PRODUCTION

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Abstract

The sustainability and security of Romanian agricultural production is under threat from intensive production practices, global competition and climate change factors. Maintaining the sustainable production of land lies in striking a balance between socio-economic goals and responsible natural resource management. To rise to the challenge, an integrated approach making full use of all the major players involved – farmers, consumers, regulatory bodies and scientists – is necessary.

Trichoderma pseudokoningii Td85 antagonistic fungus was isolated from wheat seeds, Baragan agricultural region. Td85 beneficial strain has the following properties: a wide spectrum of action towards pathogenic microbiological contaminants in cereal crops, due to complex activity expressed by antagonism and competition for food and space colonization. Td85 beneficial strain provide reducing development of primary inoculum that survive in the soil and on the plant debris, due to high capacity for colonization and degradation of plant substrates.

Saccharomyces cerevisiae L30b yeast strain was isolated from grapes Chasselas D’ore variety, RDIPP Bucharest vineyard. Research of L30b beneficial yeast was sidelined on strawberry crop, focusing on biological control of Botrytis cinerea fungus. The efficacy of L30b formulations was assessed on Favette (early), Cardinal (middle) and Pandora (late ripening) variety, achieving a reduced disease incidence and severity of grey mould on fruit and providing high efficacy against the pathogen.

Key words: sustainable production, beneficial fungi, Trichoderma pseudokoningii Td85, Saccharomyces cerevisiae L30b.

INTRODUCTION

Microorganisms of the genus Trichoderma are some of the most common naturally occurring fungi. Most strains are beneficial and have ability to colonize plant roots easily, without damage them. Close relationship between plants and Trichoderma fungi, gives an excellent biocontrol agent qualities. These microbial biofungicide may compete for food and space with pathogenic fungi, may stimulate plant host defenses and increased positive effect for root system. In addition, antagonistic fungi have capacity to attack and parazitize agents of agricultural plant pest in certain environmental conditions (Sesan et al., 2012). Fungi of the genus Trichoderma ssp. possess innate resistance to to most agricultural chemicals, including fungicides, although individual strains of microorganisms differ in terms of their resistance to plant protection products (PPPs). Some strains of antagonistic fungi were selected or modified to achieve specific agricultural chemical resistance. Most owners of strains of the genus Trichoderma ssp, hold extensive lists of their sensitivity or resistance to a wide range of crop protection products, for biological control agents of pest. In nature, some organisms favors the development of others, forming associations that allow coevolution in the agriculture system, while others are exclude each other by mechanisms of antagonism. Microorganisms with importance in biological control of of plant diseases have a complex action. Antagonism of biological control agents against pathogens is due to the action of secondary metabolites and to direct destruction by micoparazitism. Reducing Botrytis cinerea pathogens may be due to the mechanism that destroy microsclerotium or limit mycelium growth by antagonistic yeasts of Saccharomyces and Metschnikowia genus.
MATERIALS AND METHODS

*Trichoderma pseudokoningii* Td85 strain, beneficial microorganism, isolated from wheat seeds collected from Baraganul de Sud (Figure 1).

Identification of *Trichoderma* Td85 strain to the species level was based on morphological and physiological characteristics, assigned to the species *Trichoderma pseudokoningii*.

Td85 colony habitat: colony on oatflake-agar at 25°C filling the Petri-dish within 4 days, mycelium hialine, sporulating areas tuffed, green. Colony reverse colour unchanged. No odour. No growth on PDA at 40°C; 50mm radius at 40°C.

Td85 morphology: conidiophores tree-like, branched at right angles; length of branches increasing to the basis; no sterile appendages. Phialides flask shaped, straight or bent, arranged mostly in groups of three of the end of branches. Conidia ellipsoidal, smooth-walled, 5x3 μm.

Lyophilized *Trichoderma pseudokoningii* Td85 strain Figure 2).

Condition for storage of beneficial microorganisms Td85 are lyophilization (freeze-drying cell) and for cultivation PDA medium.

*Saccharomyces cerevisiae* L30b strain, beneficial microorganism, isolated from *Chasselas* D’ore grapes variety, RDIPP vineyard (Figure 3, 4 and 5).

Identification of *L30b* yeast at the species *Saccharomyces cerevisiae* was performed by sequence analysis of 26S rDNA D1/D2. Identification species: *Saccharomyces cerevisiae* (100% sequence D1/D2).

The culture medium used for isolation: glucose 5%, 2% agar, 0.5% tartaric acid and pH 2.5 and for cultivation: YPGA medium (glucose 20g, peptone 10g, 5g yeast extract, 20g agar; sterile distilled water (1000 ml).

The criterion used for the proposed taxonomic designation for L30b yeast strain is the key to the species of *Saccharomyces* adapted from Stelling-Dekker.

Morphology of *Saccharomyces* L30b strain surface on agar: shiny; texture on agar: smooth.

Lyophilized *Saccharomyces cerevisiae* L30b strain stored into international collection of
microorganisms DSMZ Braunschweig – Germany, Figure 6).
Mineralization capacity of Td85 strain on plant debris was assessed by Strathox respirometer. Pest incidence (% infected fruit) and pest severity (% infected area) of Botrytis cinerea was assessed on 100 fruit per plot. The results were interpreted statistically by ANOVA and Newman & Keuls 5% test.

Figure 6. Vials of L30b yeast strain as lyophilized spores

RESULTS AND DISCUSSIONS
Experiments conducted in this research allowed the definition of optimized conditions (composition of media, cultivation parameters—temperature, aeration) and conditioning formula that provides optimum performance in growing and multiplication replicates antagonistic strains to phytopathogenic microorganisms. Modern crop protection products must fulfill the requirements increasingly higher efficiency requirements in the field, but with minimal impact on the environment and the highest possible level of safety for both, the consumer and food products. Eliminate risks to health and environment of plant protection products is a major imperative for any company producing PPPs. Product behavior in soil, water and air and their effects on the fauna and flora are thorough tested in greenhouses and in the field, following international regulations for sustainable development.

BIOPRODUCTS BASED ON Trichoderma Pseuderokonigii Td85 TO PROTECT CROPS
Different conditioning formulas and modes of actions: controlled release granules (F1 left) microgranules with curative action (F2 midl.) powder conditioning of seed treated with preventive actions (F3 left) (Figure 7). Active ingredient: Trichoderma pseudokoningii Td85 strain (1x10^8 spores/ml).

Figure 7. Bioproducts based on Trichoderma pseudokoningii Td85 strain with different conditioning formulas

Microgranules viability of Td85 spores was 100% after 12 months of conditioning and more than 90% viable spores, demonstrating the high level of conditioning formulas in yield (Figure 8 and 9).

Figure 8. Viable conidia of Td85 strain after 4 (left) and 8 (right) days from conditioning as controlled release granules (F1)

Figure 9. Viability of bioproducts based on Td85 strain embedded in alginic acid (F1) and sodium alginate (F2) after 12 month from conditioning

For testing the viability, embedded Td85 spores are transplanting on PDA medium and growned for 5 days at 22°C. Alternatively, a pure fungal suspension from a tube is tested to quantify microscopic spore viability (hemacytometer+methylviolet/methylene blue), considering spores viable, if more than 90% of the spores is not colored. Biological activity of strain Td85 is differentiated according to the formula of beneficial microencapsulation. (Figure 10 and 11). Our research publicated in Patent application a 2010 01161 demonstrated that controlled slowly releasing of Td85 spores (F1) are applicable in a conservative agriculture system.
The selection criteria of beneficial microorganism *Trichoderma pseudokoningii* Td85 are: antagonism towards different agents of damages; ensure reducing primary inoculum developed on plant debris from agricultural crops; high capacity for colonization and degradation of plant substrates; a complex action expressed by: antagonism, competition for food and space, high capacity of sporulation, submerged cultivation, in order to obtain biomass because it is an industrial profitable technique readily taken up.

To define optimal industrial parameters of *Trichoderma pseudokoningii* Td85 strain, the following composition was used: KH₂PO₄ 5 g/l, (NH₄)₂SO₄ 5 g/l, yeast extract 2 g/l, MgSO₄·7H₂O 0.3 g/l, CaCl₂·2H₂O 0.3 g/l, whey powder 5 g/l, pulp corn to produce bioethanol by dry milling about 5 g/l, pH 5.6. The results based on our research published in Patent application a 2010 01161 and 127293 demonstrated that *Trichoderma pseudokoningii* Td85 has antagonistic action against pathogens from *Fusarium*, *Botrytis* and *Sclerotinia* genus. Also, Td85 strain is producing hydrolases that degrades cellulose, chitinase and beta-glucanase; oxidase which degrade lignin and has high mineralization capacity of plant debris (Figure 12).

**BIOPRODUCTS BASED ON YEAST Saccharomyces cerevisiae** L30b TO PROTECT CROPS:

Different conditioning formulas and modes of actions: soluble granules into sodium alginate (F1); soluble granules into sodium bicarbonate (F2); mixed bioproduct based on *Saccharomyces* and *Metschnikowia* yeasts (Figure 13).

To obtained biomass of L30b yeast, the following biosynthesys composition was used (all amounts are per litre) : 30 g of plasmolizate syrup, 5g yeast plasmolizates (supplemented with iron), 2g of (NH₄)₃PO₄, 1g of (NH₄)₂SO₄, 0.5 g of MgSO₄.7H₂O (Figure 14).

For the industrial parameters, plasmolizate syrup and yeast were determined following the optimum growing of L30b strain: 33°C temperature and an aeration rate of 0.75 l/air/l medium/min (Oancea F. et. all., Patent 01382 and 01383, 2012). Design, analysis and reporting of the strawberry trials in accordance with the European and Mediterranean Plant Protection Organization (*EPPO*) guidelines 152 and 181, regarding quality assurance program for agricultural testing in the field, in greenhouses and in laboratories condition.
Protocol of research and reporting in accordance with Good Experimental Practice and Directive 93/71/EEC.

by preventive treatments included: tested Efficacy study of Saccharomyces cerevisiae L30b yeast against Botrytis cinerea (grey mold) bioproducts Saccharapolvin 25 PU with yeast cell at different dilution rates (2x10⁶ and 6x10⁶ cell/ml); reference products (thiophanate methyl 0.07%) and an untreated control.

The efficacy of L30b formulation was assessed on Favette (early), Cardinal (middle) and Pandora (late ripening) variety on areas of 100 m²/plot with 3 replicates.

During strawberry vegetation period, 2 treatments were applied in the following phenophases:

T₁: After flowering fruit-binding (when there are a number of physical and chemical processes that lead to the creation of a favorable environment for the development B. cinerea fungus.)

T₂: with 2-3 weeks before harvest to protect the fruit from lately attack of B. cinerea.

Observations were made on strawberry fruit before harvest.

L30b strain used as biological control agent of preharvest strawberry diseases has protection activity against Botrytis cinerea pathogen, the causal agent of gray mould (Figure 16).

Saccharomyces cerevisiae L30b yeast was effective against gray mold (Botrytis cinerea) during strawberry vegetation period, applied as Saccharopolvin 25 PU bioproduct with cell suspension at different dilution rates (2x10⁶ cell/ml and 6x10⁶ cell/ml).

Bioproduct Saccharapolvin 25 PU (6x10⁶ spores/ml), in field conditions, recorded highest efficacy (E = 86.8 to 90.2%) compared to the efficacy of chemical standard thiophanate methyl (E = 89.1 to 95.7%) for Favette, respectively, Pandora variety (ANOVA and Newman & Keuls 5% test).

The attack level during efficacy trial, ranged between 19.0 to 47.0% for Favette respectively, Pandora variety.

Bioproduct Saccharapolvin 25 PU maintain strawberry fruit quality properties and does not affect the organoleptic properties when applied two treatments in vegetation (Based on our research published in patent no. 125071).

The economic implications of bioproducts: an alternative to chemical treatments avoiding the formation of breeds resistant to fungicides, compatibility with integrated control systems, reducing quantitative and qualitative losses through high efficacy, not necessary breaks.

CONCLUSIONS

Trichoderma pseudokoningii Td85 strain is a solution applicable in a conservative agriculture system.

Td85 strain has high mineralization capacity of plant debris based on our reasearch published in patent no. 127293 and antagonistic properties against soil-borne pathogens, reducing the primary inoculum level of microbiological pest agents.

Saccharomyces cerevisiae strain has biological activity against Botrytis cinerea pathogen, achieving a reduced disease incidence and severity of grey mould on strawberry and providing high efficacy against the fungus.

The both beneficial microorganisms in agricultural crops, owned by RDIPP Bucharest are stored into International collection of
microorganisms *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) in Braunschweig, Germany.


**ACKNOWLEDGEMENTS**

This research work was carried out with the support of: Dr. Oancea Florin, project manager of MAKIS and BIOTECH 4630; Agricultural Research and Development Station Caracal, experimental field of Td85 strain; Research and Development Station for Fruit Tree Growing Baneasa, Bucharest, experimental field of L30b yeast.

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