ECOLETTER

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Nematicidal activity of protein hydrolysates of plant origin.

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Introduction

The general objective of ENZYTECH project was to design the bases to develop a biotechnological process that would allow finding bioactive molecules (L-amino acids and low molecular weight peptides) with low risk biostimulant or phytosanitary activity, from the protein fraction of different industry by-products of plant origin.

Therefore, 4 out of 29 possible plant sources were selected and characterized in detail, determining amino acid profile, lipid content, humidity, content of inorganic matter, carbohydrates, and heavy metals as well as their microbiological analysis. The 4 sources complied with fertilizer legislation (Annex V of Royal Decree 506/2013).

From the 4 selected plant sources, 16 preliminary prototypes were prepared varying the conditions of extraction and enzymatic hydrolysis at laboratory scale (in 2L bioreactors).

The prototypes were evaluated in different bioassays to determine their (possible) Biostimulant activity (promoter of plant growth and / or anti hydric or saline stress) and Phytosanitary (anti-microbial and / or nematicide).

The objective of the present study was to determine whether the nematicidal activity previously observed with some of the hydrolysates from the 2L bioreactor (LEITAT Technological Centre) was maintained when they were produced in 14L and 130L bioreactors (FUTURECO BIOSCIENCE).

Materials and Methods

Initially 2 *in vitro* bioassays were performed to evaluate the nematicidal activity (against eggs or juveniles of *Meloidogyne* nematode) of different hydrolysates produced in 14L bioreactor.

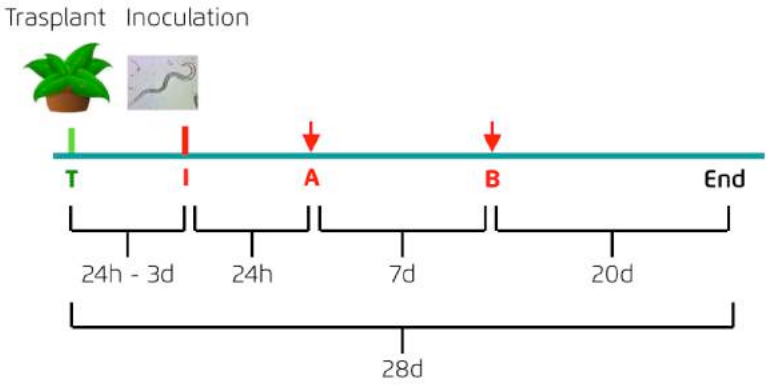
Table 1. Prototypes of different hydrolysates evaluated in the different bioassays *in vitro* and *in vivo*.

Hidrolysate	Composition	Bioreactor 14L	Bioreactor 130L
M3	Source 1 + SND B25	√	
M7	Source 2 + cocktail of commercial enzymes	√	
M11	Source 2 + SND B25	√	
M13	Source 3+SND B25+cocktail of commercial enzymes	√	
COMBI	Source 1 + Source 2 + Source 3 +Source 4+SND B25		√

Sources 1, 2, 3, and 4: Confidential (patent pending). SND B25: Supernatant of the fermentation in Bioreactor of Lysobacter enzymogenes strain B25 (patented by Futureco Bioscience)

Subsequently, the effect of the different hydrolysates on the penetration capacity (2 tests in the climatic chamber, Figure 1) and reproduction (3 trials on the greenhouse, Figure 2) in two crops: tomato and cucumber, was evaluated.

Figure 1. Experimental design of *in vivo* bioassays for penetration evaluation.



2016-2018
ENZYTECH
Design of Enzymatic Technologies of plant origin to obtain low risk bioactive molecules with biostimulant or phytosanitary activity.

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Nematodes of the genus Meloidogyne inhabit almost all warm and temperate regions of the world, and are internal parasites of plant roots, many of them of agricultural crops, so their infection is considered an economic risk. They are inductors of galls that affect the absorption of nutrients and the life cycle of plants. Above: eggs and juvenile of Meloidogyne. Middle: Gall with three egg masses. Below: root system of tomato with galls caused by Meloidogyne.

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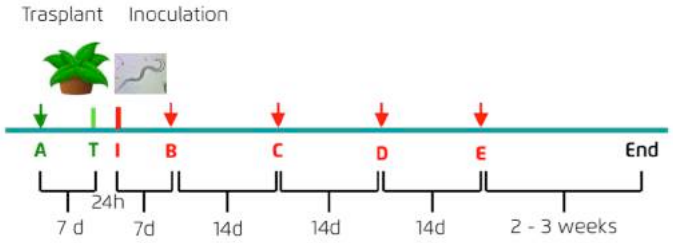
Figure 2. Experimental design of *in vivo* bioassays for evaluation of reproduction.



Results and Discussion

Table 2. Summary of the efficacies reached in the 7 bioassays

	IN VITRO		*IN VIVO* Penetration assays		*IN VIVO* Reproduction assays		
Assay	N180515	N180515	180604 CC	180627 CC	180417 IN	180531 IN	180605 IN
Parameter	Efficacy		Penetration		Reproduction		
Stage/crop	Eggs	Juveniles	Cucumber	Tomato	Tomato	Cucumber	Tomato
M3	**37.8 c**	**87.3 a**	**72.5 a**	**25.3 b**	**16.8 c**	**70.6 a**	**45.7 b**
M7	**43.1 bc**	**96.4 a**	**54.3 ab**	**73.8 a**	**60.7 ab**	**89.5 a**	**48.4 b**
M11	**54.3 b**	**95.9 a**	**53.6 ab**	**74.1 a**	**39.9 bc**	**81.9 a**	
M13							**58.0 b**
COMBI							**56.5 b**
Biological 1			**9.0 b**	**70.7 a**			
Chemical 1	**75.9 a**	**89.5 a**	**91.8 a**	**87.2 a**		**25.6 b**	
Chemical 2					**84.1 a**		**83.8 a**

	Level of Infection in Control Plants				
	180604 CC	180627 CC	180417 IN	180531 IN	180605 IN
	Penetration		Reproduction		
	Cucumber	Tomato	Tomato	Cucumber	Tomato
Control Eggs/Plant	**138,67**	**14,0**	**473.998**	**13.542**	**3.079.001**
Control Eggs/g root	**53,01**	**11,67**	**26.019**	**2.358**	**208.210**

The data in a column followed by the same letter are not significantly different (P<0,01). The Reference Products (Chemical 1, Chemical 2 and Biological 1) are commercial nematicidal products.

The three hydrolysates evaluated under *in vitro* conditions (M3, M7 and M11) showed efficacy on the hatching of eggs (between 37.8% and 54.3%) and especially on the survival of juveniles of the gall nematode, achieving efficacies between 87% and 96% comparable with the reference chemical standard (Table 2, column 2-3).

The 3 hydrolysates evaluated reduced significantly the penetration of the nematode in the plant, generally at the same level as the reference chemical nematicide and even much better than the commercial biological nematicide in the case of cucumber crop (Table 2, column 3-4).

Finally, the effectiveness on the reproduction inhibition of the nematode in cucumber plants was very high (between 70.6 and 89.5%), being much higher than the reference chemical (25.6%). In the case of tomato bioassays, although the efficiencies compared with the commercial chemical were lower, in general they were notable (with the exception of M3 in one of the trials), especially considering the very high population level of the nematode (Table 2, columns 5, 6 and 7).

Conclusions

The 5 preliminary prototypes based on different protein hydrolysates had a high effect on the hatching of eggs of the phytoparasite nematode *Meloidogyne*, as well as on juvenile survival, the penetration of these into tomato and cucumber plants, and the reproduction of the nematode in these crops.

Although they are preliminary prototypes and next steps will be needed, such as optimization of their production at pilot and industrial scale in order to reduce the dose and production costs, the results presented in this ECOLETTER are promising.