SEARCHING NEW BIO-PROTECTIVE MICROBIAL AGENTS FROM DIFFERENT TYPES OF COMPOSTS

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1 INTRODUCTION

Compost is widely used as a soil amendment to provide nutrients and organic matter, and to improve the physical and biological properties of soils. In addition, some types of compost also suppress soilborne plant pathogens, although the exact mode of action is not clear. Several mechanisms are involved, however, many studies have been developed to identify the compost microbiota able to reduce the activity of plant pathogens and additionally, able to favour the plant growth by the production of phytohormones, vitamins or/and amino acids (Murcia *et al.*, 1997; Gravel *et al.*, 2007; Suárez-Estrella *et al.,* 2007).

Since the control of several plant pathogens is considered a pressing need of current agriculture, the main aim of this work was to select and test those microorganisms whose antagonistic capacity were detected towards phytopathogenic bacteria and fungi. From selected microbiota, production of the most important plant growth promoting substances such as phytohormones, vitamins and amino acids was also investigated.

2 MATERIALS AND METHODS

The raw materials for the mixtures of composting processes were vegetable wastes (VW), urban solid wastes (USW) and sewage sludges (SS). Piles were periodically turned and aerated while fluctuations in temperature were observed (approximately during 70 days). Compost samples were collected at different stages of the composting process (initial, early thermophile, thermophile, cooling and maturation phases). The most representative actinomycetes, bacteria and fungi were recovered from compost samples on specific culture media and colonies growing on plates were selected, isolated and kept in the same source media.

Microbial antagonist capacity (not directly from compost) was detected by *in vitro* and *in vivo* test. One hundred and thirty five strains were *in vitro* tested against six phytopathogenic agents: *Fusarium oxysporum* f.sp. *melonis* (FOM), *Pythium ultimun* (PU), *Rhizoctonia solani* (RS), *Pectobacterium carotovora* subsp. c*arotovora* (PCC), *Pseudomonas syringae* subsp. *syringae* (PSS) and *Xanthomonas campestris* (XC). Methods applied in this case were those based on Petri plate confrontation between phytopathogenic and antagonistic agents and previously described by Landa *et al.* (1997), de Boer *et al.* (1999) and Rojas-Rosas (2000). Several of them, selected on the basis of the best inhibitory capacity, were investigated in relation to their capacity to produce several phytohormones or precursors such as indol acetic acid (IAA), zeatin (Ze), Abscisic acid (ABA), 1-aminociclopropane 1-carboxylate (ACC), jasmonate (JA) and salicylic acid (SA). Besides, production of vitamins and amino acids such as niacin (NIA), thiamine (THI), biotin (BIO), vitamin B12, pantothenic acid (PAN) and cystine (CYS) was also investigated by chromatographic and spectrophotometric methods.

Finally, five of the fourteen strains selected were *in vivo* tested to induce systemic resistance in melon and tomato plants towards the pathogenic fungus *Fusarium oxsyporum* f.sp. *melonis* and the pathogenic bacterium *Xanthomonas campestris*. Methods applied in this case were previously described by Larkin and Fravel (1998).

3 RESULTS AND DISCUSSION

3.1 Isolation of potential antagonistic strains

One hundred and thirty five strains were isolated from compost samples obtained at different stages of the composting process. Due to viability problems related to the strains isolated, only one hundred and six out of the total were finally tested against phytopathogenic bacteria and fungi previously cited (39 bacteria, 39 actinomycetes and 28 fungi). Methods and references are indicated in the paragraph of “Material and Methods”.

Twenty eight (9 bacteria, 14 actinomycetes and 5 fungi) out of the total strains isolated showed antagonistic activity against at least one of the pathogenic bacteria assayed (approximately 21% from the total of strains tested). In relation to results obtained against the pathogenic fungi tested, forty six (6 bacteria, 24 actinomycetes and 16 fungi) out of the total strains showed antagonistic activity against at least one of them (approximately 34% from the total of strains tested).

The ratio of strains antagonistic found in this work is satisfactory in relation to data previously obtained. Landa *et al*., (1997) showed that approximately 32% of 74 bacterial isolates from the chickpea rhizosphere inhibited *in vitro* growth of *F. oxysporum* f.sp. *ciceris* in dual cultures. In contrast, Myatt *et al.,* (1992) obtained a lower proportion of antagonistic bacteria toward *Phytophthora megasperma* Dreschsler f.sp. *medicaginis* from a similar environment. Therefore, the degree of disease suppression depends on the strength of both antagonistic microorganism and host, as well as the inoculum concentration of the pathogen (Landa *et al*., 1997).

On the basis of the best inhibitory capacity, fourteen strains were finally selected (Table1). The main criterion to achieve this selection was to show antagonistic activity against at least two of the six phytopathogenic microorganisms assayed (Table 1). Origin and behaviour of the fourteen strains selected is showed in Table 1. Strains named as RSU312, RV413 and RV523 were capable to inhibit to four or five phytopathogen agent.

TABLE 1 ***In vitro* selected strains on the basis of the best inhibitory capacity: origin and behaviour and antagonistic capacity**

Code	Type	Raw Material	Composting Phase	PSS	XC	PCC	RS	PU	FOM
L233	Actinomycete	SS	Termophile Phase	+	-	-	-	+	+
L312	Fungus	SS	Termophile Phase	-	+	+	-	-	-
L333	Actinomycete	SS	Termophile Phase	-	-	-	-	+	+
L512	Fungus	SS	Maturation	-	-	-	-	+	+
RSU123	Actinomycete	USW	Beginning	-	-	-	+	+	+
RSU231	Bacterium	USW	Termophile Phase	-	+	-	+	+	-
RSU233	Actinomycete	USW	Termophile Phase	-	-	-	-	+	+
RSU312	**Fungus**	**USW**	**Termophile Phase**	**-**	**+**	**-**	**+**	**+**	**+**
RSU522	Fungus	USW	Maturation	-	-	-	-	+	+
RV312	Fungus	VW	Termophile Phase	-	+	+	-	-	-
RV413	**Actinomycete**	**VW**	**Cooling Phase**	**+**	**+**	**+**	**-**	**+**	**+**
RV422	Fungus	VW	Cooling Phase	-	-	-	+	+	+
RV523	**Actinomycete**	**VW**	**Maturation**	**+**	**+**	**+**	**+**	**+**	**-**
RV532	Fungus	VW	Maturation	-	+	+	-	-	-

3.2 Production of agricultural interesting substances

All strains indicated in Table 1 were tested in relation to its capacity to produce agricultural interesting substances such as vitamins, amino acids and phytohormones.

In this sense, strains named L512, RSU522 and RV422 (fungi), showed satisfactory results respect to both thiamine, niacin and panthotenic acid production, while strain RV413 (actinomycete) emphasized with respect to thiamine, niacin and vitamin B12 production. In relation to phytohormones production, IAA was detected only from strain named L333 (actinomycete), while the strains L512, RSU522 and RV413 were pointed up in relation to the rest of phytohormones. Microbial strains producing amino acids were not detected in any case.

On the basis of results previously described by others authors respecting to niacin, thiamine and pantothenic acid production from several species of *Azotobacter* spp., our results could be satisfactory and encouraging (Murcia *et al.*, 1997). Although in general, results related to phytohormones production have not been comparable to those previously described, several strains have shown an interesting capacity to produce different substances at the same time.

A summary related to the production of agricultural interesting substances is shown in Table 2. Strains L512, RSU522, RV422 (fungi), RSU233 and RV413 were characterized as the most interesting producing microorganisms.

TABLE 2 **Production of agricultural interesting substances from selected strains**

Code	NIA	THI	BIO	B12	PAN	CYS	IAA	Ze	ABA	ACC	JA	SA
L233	+	+	++	+	+	-	+	+	+	+	+	+
L312	+++	+	+	+	+	-	+	+	+	++	+	++
L333	+	+	+	+	-	-	+++	+	+	++	+	++
L512	**+++**	**+++**	**+**	**+**	**+++**	**-**	**-**	**+++**	**+++**	**+++**	**+**	**++**
RSU123	-	+	+	+	-	-	+	+	+	++	+	+
RSU231	++	+	+	+	+	-	+	+	+	++	+	++
RSU233	**+++**	**+**	**+**	**+**	**+++**	**-**	**+**	**++**	**+**	**++**	**+**	**+++**
RSU312	+	+	+++	+	++	-	+	+	+	++	+	++
RSU522	**+++**	**++**	**+**	**+**	**++**	**-**	**-**	**++**	**+++**	**++**	**+**	**++**
RV312	++	+	+	+	+	-	-	+	+	+	+	+
RV413	**++**	**++**	**+**	**++**	**+**	**-**	**+**	**++**	**++**	**++**	**+++**	**+++**
RV422	**+++**	**+++**	**+**	**+**	**+++**	**-**	**-**	**+**	**+++**	**++**	**+**	**++**
RV523	++	+	+	++	+	-	nt*	nt	nt	nt	nt	nt
RV532	++	+	++	+	+	-	+	+	++	+	+	+++

+: low level ++: medium level +++: high level *: non tested

3.3 *In planta* evaluation of antagonistic activity of selected strains

Five strains (RSU123, RSU312, RV523, RSU231 and RV532) were finally *in vivo* tested to induce systemic resistance in melon and tomato plants against the fungus FOM and the bacterium XC. In the first case a low suppressive capacity was detected in plants infected with FOM. A reduction range of the fungal isolation between 10-15 % was observed. However, plants exhibited an enhanced defensive capacity against the pathogenic bacterium as compared with control plants non inoculated with inducing agents. This capacity was higher in the case of the strains named RSU312 (fungus) and RSU231 (bacterium). These biocontrol agents showed a reduction range of XC isolation higher than 60%, from inoculated plants.

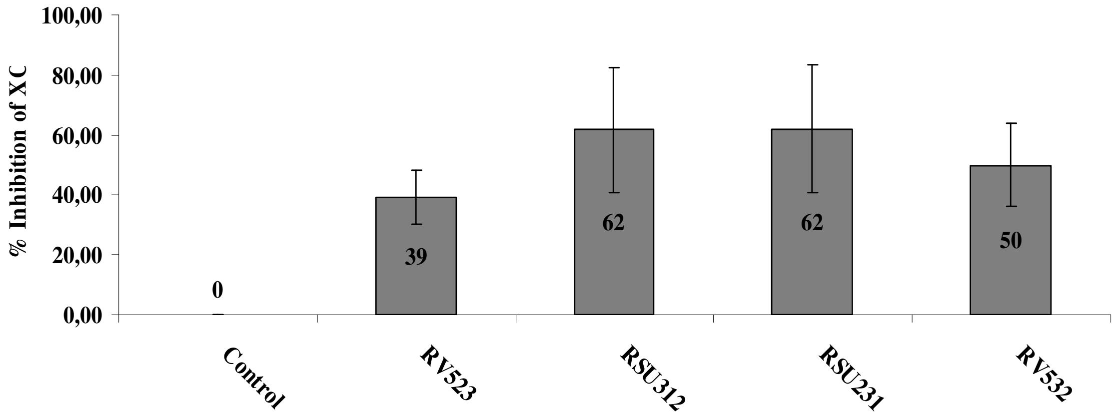

FIGURE 1 **Microbial inhibitory capacity towards FOM-infected plant treated with antagonist strains**

Numerous microbes are antagonistic to soilborne plant pathogens but few of them have been commercialized as biocontrol agents due to problems such as inconsistent performance in the field, lack of broad-spectrum disease suppression activity, or slower or less complete suppression when compared with chemical pesticides. In this work, several of the isolated strains showed an *in vitro* antagonistic broad spectrum and could be applied individually for the suppression of soilborne diseases.

4 CONCLUSIONS

One of the most interesting aspects derived from this extensive work was the discovery of microbial agents able to show antagonistic capacity against a broad-spectrum of phytopathogenic microorganisms as well as a potential to produce plant growth promoting substances. The combination of both capacities could be of interest from an agronomical point of view.

The isolation of a high percentage of potential antagonistic microorganisms has been possible from compost manufactured from vegetable wastes, urban solid wastes and sewage sludges. Antagonistic bacteria and actinomycetes were mainly isolated from vegetable based-compost while antagonistic fungi were more important from urban and sludge based-composts. In relation to the composting phase, most of antagonistic agents were isolated between thermophile and maturation phase, while a very low number of antagonistic microorganisms were isolated at the initial phase of the process.

Several of these strains have shown an *in vitro* broad-spectrum disease antagonistic activity, and four of them have shown an interesting suppressive capacity *in planta* against the pathogenic bacterium *Xanthomonas campestris*. These strains are RV523, RSU312, RSU231 and RV532.

A broad-spectrum of agricultural interesting substances is produced from strains L512, RSU233, RSU522, RV413 and RV422. However, additional research is required in order to perform *in vivo* tests leading to ascertain the real role these strains can play as effective plant growth promoters.

Bearing in mind the results here shown, it has been therefore corroborated that the suppressive capacity of different types of composts as well as their fertilizing capacity are mainly caused by the presence of bio-protective microorganisms.

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