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Antagonistic interactions among cultivable actinomycetes isolated from agricultural soil amended with organic residues

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The present work focuses on the antagonistic interactions among cultivable actinomycetes isolated from agricultural soil and organic amendments (farmyard manure and municipal solid waste compost). Antagonistic interactions, assayed by the double-layer agar method, were checked among isolates obtained from (i) the same treatment (ii) control soil (unamended) against those from amendments and (iii) each treatment against tow phytopatogenic bacteria (*Agrobacterium tumefaciens* B6 and C58). A high suppressive interaction ratio (≥ 50%) was registered some either the treatment soil. It was found that amendments application decreased this suppressive interaction ratio between *actinomycetales*. But, it increases the ratio of the antagonistic *actinomycetales* from soil against *Agrobacterium tumefaciens* confirming the role of these organic residues as fertilizers. It was also shown, based on the phylogenetic affiliation of bacteria, that the antagonism can play a significant role in structuring bacterial communities in soil.

Key words: Antagonism, *Actinomycetales*, agricultural soil, manure, compost.

INTRODUCTION

Because of their important role on the ecosystem, numerous ecological studies of *actinomycetale* were conducted in marine habitats: beach sands (Suzuki et al., 1994), sub-glacial ice of Antarctica (Priscu et al., 1999), underground caves (Groth et al., 1999), Antarctic marine sponge (Mangano et al., 2009); agricultural habitats: orchards (Lee and Hwang, 2002), grasslands (Lee and Hwang, 2002), rice-paddies (Hayakawa et al., 1988), rhizosphere (Gesheva, 2002), tomato plants (Fialho de Oliveira et al., 2010), composted Eucalyptus bark (Hardy and Sivasithamparam, 1995), forest soils (Jayasinghe and Parkinson, 2008) and wastewater treatment (Bensultana et al., 2010). It was shown that *actinomycetales* constitute a significant component of the microbial population in each environment, especially in soil. They are a main member of soil decomposer community; they play an important role in recycling complex organic materials such as lignocelluloses and chitin (Epstein, 1997; Li et al., 2010; Tiquia et al., 2002).

In previous works on the effect of organic amendments application to agricultural soil on the distribution of the *actinomycetales* community, it was shown that the bacteriological and physico-chemical composition of amendments strongly affect the bacterial diversity. This was either by contribution, stimulation or inhibition (Mokni-Tlili et al., 2011a). Nevertheless, other phenol-mena can involve on the community structure of bacteria. The antagonism is one of the most important phenomena as shown by several workers (Grossart al., 2004; Hentschel et al., 2001; Lo Giudice et al., 2007; Long and Azam, 2001; Mangano et al., 2009). The interactions among bacteria inhabiting the same niche represent an in-

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interesting evolutionary strategy, conferring a selective advantage in competition for nutrients and space in the environment and acting as an effective control of microbial populations (Hentschel et al., 2001). Moreover, it was suggested that the antagonistic effect, studied for phylogenetically different bacterial groups, is a widespread trait in several habitats (Bhattarai et al., 2006; Grossart et al., 2004; Long and Azam, 2001).

On the other hand, bacteria interactions can result on the production of biocontrol agents by microorganisms. These agents can play a primordial role in reducing pesticide use in the treatment of phytopathogenic diseases (Fialho de Oliveira et al., 2010), knowing that agrochemical treatment may result in environmental impact and pose a threat to humans and animals. *Actinomycetales* have been considered among potential biocontrol agents for plant diseases producing antibiotic (Igarashi, 2004) and enzymes such as cellulases, hemicellulases, chitinases, amylases, and glucanases (Mokni-Tlili et al., 2010; 2011b; Yuan and Crawford, 1995).

The present work aims mainly to better understand the effect of organic amendments application on the diversity of actinomycetes in agricultural soil through an antagonistic interactions study. Amendments were farmyard manure (FM) and municipal solid waste compost (MSWC). Antagonistic interactions were performed among isolates obtained (i) from the same treatment (ii) from control soil (unamended) against those isolated from amendments and (iii) from each treatment against tow phytopatogenic bacteria (*Agrobacterium tumefaciens B6 and C58*) using the double-layer agar method.

MATERIALS AND METHODS

Actinomycetales collection

Studied bacteria are actinomycetes already isolated from soil untreated and treated with farmyard manure (FM) and municipal solid waste compost (MSWC). Soil samples were collected from an open field in the experimental farm of the Agronomic National Institute of Tunis (INAT). The field is located in northern Tunisia and belongs to the semi-arid superior bioclimatic stage (Mokni-Tlili et al., 2009). We recall here briefly that soil, which has a clayey–loamy texture, was treated with MSWC applied at 40 t ha^{-1} (C40), 80 t ha^{-1} (C80), 120 t ha^{-1} (C120), FM at 40 t ha^{-1} (F40) and 120 t ha^{-1} (F120), respectively. The main physicochemical characteristics of soil and amendments were reported previously in Mokni-Tlili et al. (2011a).

The isolation, enumeration and identification of actinomycetes were based on a morphological and a molecular study using PCR amplification and restriction analysis of 16S rRNA genes from bacterial isolates (Amplified rDNA Restriction Analysis, or ARDRA; Vaneechoutte et al., 1992). On the basis of restriction patterns obtained, the isolates were clustered into Operational Taxonomic Units (OTUs), assuming that each OTU (assigned to a number) included strains belonging to the same species. For each OTU, one to three representative strains showing the identical ARDRA pattern were randomly selected for 16S rDNA sequencing. Each sequence was then used as a query in a BLASTn (Altschul et al., 1997) search and further aligned to the most similar sequences retrieved from the database using the program ClustalW (Thompson et al., 1994). The representative bacteria sequences of the *actinomycetales* collection, composed of 281 isolates, were submitted to GenBank and assigned the following accession numbers: FJ941916 to FJ941954. This was shown three dominant *Actinomycetales* families: *Streptomycetaceae* (72%), *Pseudonocardiaceae* (23%) and *Nocardioidaceae* (5%) (Table 1).

In the present work, the three families (*Streptomycetaceae* represent the majority of tested bacteria) were considered for the antagonism tests (i) among bacteria from the same treatment (20 isolates from each soil treatment) and (ii) among bacteria from untreated soil against those from amendments (20, 17 and 35 isolates from soil T, MSWC and FM, respectively).

Screening for antagonistic interactions among bacterial isolates

To evaluate the antimicrobial activity, all isolates were screened for antagonistic interactions. For this, bacteria were spot-inoculated onto SCA medium - composed of 10.0 g starch, 0.3 g casein, 2.0 g K_2HPO_4, 2.0 g NaCl, 2.0 g KNO_3, 0.05 g $MgSO_4$, $7H_2O$, 0.01 g $Fe_2(SO4)_3.6H_2O$ and 15.0 g agar - and incubated at 30°C for 14 days. Then, the antagonism between bacteria was evaluated using the double-layer agar method. It consists to add 10 ml Mueller-Hinton overlay agar medium inoculated with 10^6 spores/ml of isolate. All plates were incubated at 30°C during 48 to 72 h. All experiments were carried out in duplicate. Hereafter, bacterial isolates tested for inhibitory activity will be termed ‘‘tester’’ strains, whereas those used as targets will be called ‘‘target’’ strains. Tester strains were streaked on SCA medium and the target strains were inoculated in Mueller-Hinton overlay agar medium.

The antagonistic effect was indicated by failure of the target strain to grow in the confluence area. Bacterial isolates were then operationally distinguished into three different interactivity clusters, termed: (1) active, if they were able to inhibit growth of at least one bacterial target; (2) sensitive, if their growth was inhibited by at least one isolate used as a tester; and (3) resistant if their growth was never inhibited by tester strains. It must be noted that an individual strain could be included in one or two interactivity clusters.

Screening for antagonistic interactions against tow phytopathogen bacteria

Antagonism test was performed as above. The Mueller-Hinton overlay agar medium was added and inoculated with 10^9 cells/ml of target bacteria: *A. tumefaciens* B6 and C58. All plates were incubated at 30°C during 24 h and experiments were carried out in duplicate.

RESULTS AND DISCUSSION

Antagonistic interaction among bacteria isolated from the same treatment

Figure 1 represents the inhibitory interaction ratio among bacteria obtained from each soil treatment and amendments. It shows that the active isolates varied between 50 to 95% of the total bacteria number. The highest rate of producer strains, registered in all treatments, can be attributed to the bioactive secondary metabolites synthesis, known propriety for actinomycetes

Table 1. 16S rRNA gene sequence affiliation, with their closest phylogenetic neighbors, of soil bacterial isolates representing each OTU obtained by ARDRA.

Phylogenetic group	OTU	Soil T	Soil F	Soil C	FM	MSWC	Representative isolate	Accession number	Most related species	Sequence homology (%)
	7	4	3	-	-	-	T1	FJ941954	EF063499, *S. venezuelae*	99
	14	8	5	-	1	-	T2	FJ941922	DQ026634, *S. globisporus subsp. globis.*	99
	48	17	12	15	4	-	T3	FJ941923	AB249953, *S. albidochromogenes*	99
	1	1	-	-	-	-	T5	FJ941929	AB249935, *S. flavofuscus*	99
	1	1	-	-	-	-	T8	FJ941936	AB184157, *S. narbonensis*	99
	19	10	9	-	-	-	T9	FJ941938	AB249921, *S. spiroverticillatus*	99
	13	8	5	-	-	-	T10	FJ941937	DQ026647, *S. exfoliatus*	99
	7	-	6	-	1	-	M1	FJ941916	DQ026670, *S. clavifer*	99
	1	-	1	-	-	-	M2	FJ941917	AB184068, *S. fradiae*	99
	1	-	1	-	-	-	M3	FJ941953	DQ487016, *S. sp. B267*	98
	7	-	7	-	-	-	M4	FJ941918	AJ308577, *S. sp. Nm5*	99
	9	-	7	-	2	-	M5	FJ941920	AB184800, *S. coelicolor*	99
	9	-	8	-	1	-	M6	FJ941921	DQ663179, *S. sp. 3004*	98
	1	-	1	-	-	-	M7	FJ941926	EF119843, *S. sp. AHW3*	98
	20	-	6	13	1	-	M9	FJ941931	EF063493, *S. sp. P3562*	98
	17	-	1	12	1	3	C1	FJ941919	AB184220, *S. longisporoflavus*	99
Streptomycetaceae	1	-	-	1	-	-	C3	FJ941925	DQ026671, *S. cavourensis subsp. Wash.*	99
	4	-	3	1	-	-	C22	FJ941930	DQ250003, *S. sp. L42*	98
	3	-	-	-	3	-	FM3	FJ941946	DQ445791, *S. cavourensis subsp. C.*	100
	5	-	-	-	5	-	FM5	FJ941947	AY999837, *S. californicus*	100
	2	-	-	-	-	2	MSWC1	FJ791059	EF371429, *S. aureus*	99
	3	-	-	-	1	2	MSWC2	FJ941949	EU257256, *S. sp. A528*	99
	3	-	-	-	2	1	MSWC3	FJ941945	EU273552, *S. collinus*	100
	6	-	-	-	2	4	MSWC5	FJ941942	AF306660, *S. sacchari*	99
	4	-	-	-	2	2	MSWC8	FJ941951	AF026081, *S. sp. CHR28*	99
	2	-	-	-	1	1	MSWC9	FJ941952	DQ086264, *S. sp. AB654*	99
	1	-	-	-	-	1	MSWC11	FJ941940	AB184676, *S. griseoaurantiacus*	99
	1	-	-	-	-	1	MSWC12	FJ941941	EF178674, *S. azureus*	100
	15	-	9	-	6	-	M8	FJ941928	AF005005, *Nocardioides albus*	98
Nocardioidaceae	2	-	-	-	2	-	FM9	FJ941939	EU284126, *Amycolatopsis sp. WX001*	99
Pseudonocardiaceae	20	13	7	-	-	-	T7	FJ941934	AY561610, *Actinobacterium RG-51*	98
	1	-	1	-	-	-	M11	FJ941935	DQ125918, *Uncultured bacterium*	99
Unidentified	33	10	7	13	2	1				

[a] Accession number of isolates.
MSWC: municipal solid waste compost; FM: farmyard manure; soil T: control soil; soil F: samples amended with 40 t ha^{-1} or 120 t ha^{-1} of farmyard manure; soil C: soil amended with 40 t ha^{-1}, 80 t ha^{-1} or 120 t ha^{-1} of MSWC.

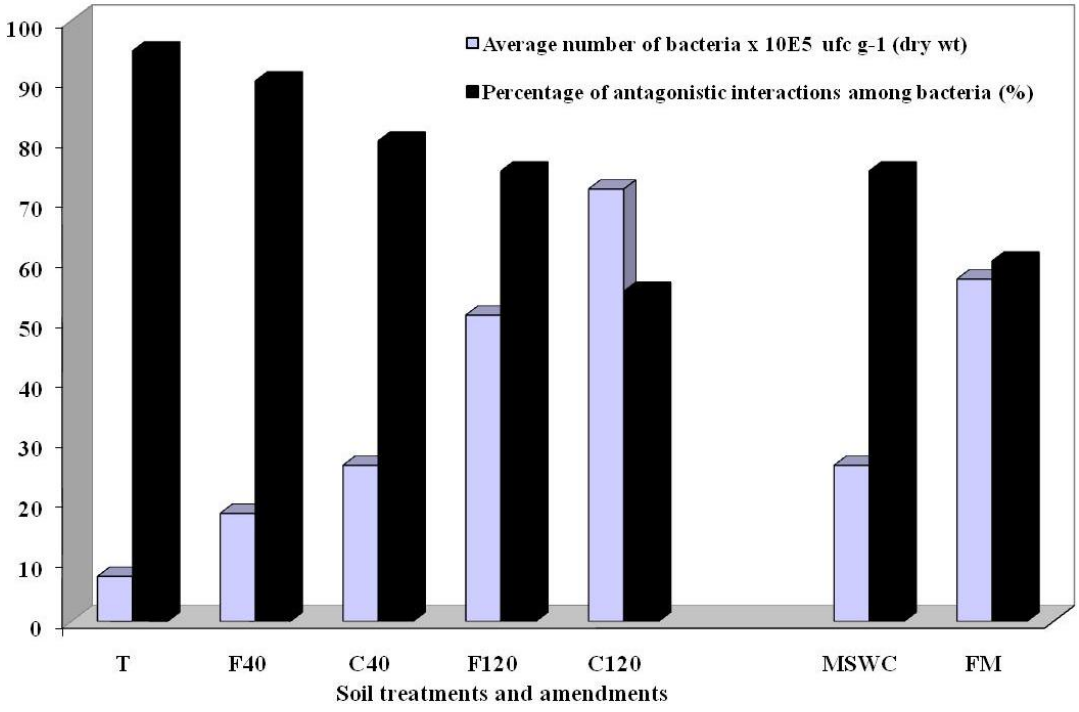

Figure 1. Plate counts (ufc g^{-1} dry weight) of *Actinomycetales* content in soil and percentage of antagonistic interactions among tested isolates according to the type of amendment

originated from environment (Duraipandiyan et al., 2010; Fialho de Oliveira et al., 2010; Fourati-Ben Fguira et al., 2005; Mangano et al., 2009). Indeed, they produce about 75% of commercially and medically useful antibiotics (Miyadoh, 1993) and approximately 60% of antibiotics which have been developed for agricultural use (Tanaka and Mura, 1993).

On the other hand, it was clearly shown that amendments strongly affect the bacteria behavior regarding the suppressive interactions between studied isolates. The higher antagonistic interaction rate, registered for control soil T (95%), decreases with amendment application. This effect depends not only on the amendment nature but also on its applied amount. e.g. for treated soil with FM, the active isolates rate decreases to 90 and 75 when it was applied at 40 (F40) and 120 t/ha of FM (F120), respectively. This decrease can reach lower values, for the same applied amount, about 55% (C120) in the case of MSWC application (120 t/ha). This can be explained based on the physico-chemical analyses which show that amendments are richer in nutrient (carbon, nitrogen and organic matter) than soil T (Mokni-Tlili et al., 2011a). Indeed, the stress due to the shortage of nutrients can activate the metabolic mechanisms for microorganisms as founded by several workers (Celar, 2003; Lumsden et al., 1990; Meidute et al., 2008). Especially, for the study elaborated by Celar (2003) on the competition for nitrogen forms between some phytopathogenic and antagonistic soil fungi, where it was shown that nutrients have a direct and indirect influence on growth, morphogenesis, and organogenesis of antagonistic.

However, amendments can stimulate the antagonistic interactions. Indeed, previous works show that the addition of organic matter stimulates the activities of microorganisms such as actinomycetes (Mokni-Tlili et al., 2011b), bacteria, fungi, algae, and other (Akhtar and Malik, 2000) by enhancing their enzymatic synthesis. Seeing that the amendment application play a double opposite roles by decreasing the stress in nutriments and then the antagonism and by enhancing the enzymatic synthesis and then stimulate the antagonism, it seems that the first effect outweighs the last one for the present study. Knowing that FM is richer in nutrient than MSWC, this above discussion is not suitable to explain obtained results for amended soil where the antagonistic interactions are more important in soil F than soil C (e.g. F120: 75 and C120: 55% of antagonists bacteria). This shows that the effect of the other chemical composition, apart the nutriments, of the amendments must not be neglected. Indeed, MSWC presents the particularity to be richer in heavy metals (Cu^{2+}: 337 mg kg^{-1}, Zn^{2+}: 290.25 mg kg^{-1}, Ni^{2+}: 90.8 mg kg^{-1}, Cr^{3+}: 78.9 mg kg^{-1}, Pb^{2+}: 80.1 mg kg^{-1}). It was reported that such elements are toxic and can decreases the bacterial activity (Mokni-Tlili et al., 2010; Rajapaksha et al., 2004; Tobor-Kaplon et al., 2005).

In Figure 1, the superposition of antagonism and numbering results shows that the antagonistic bacteria ratio

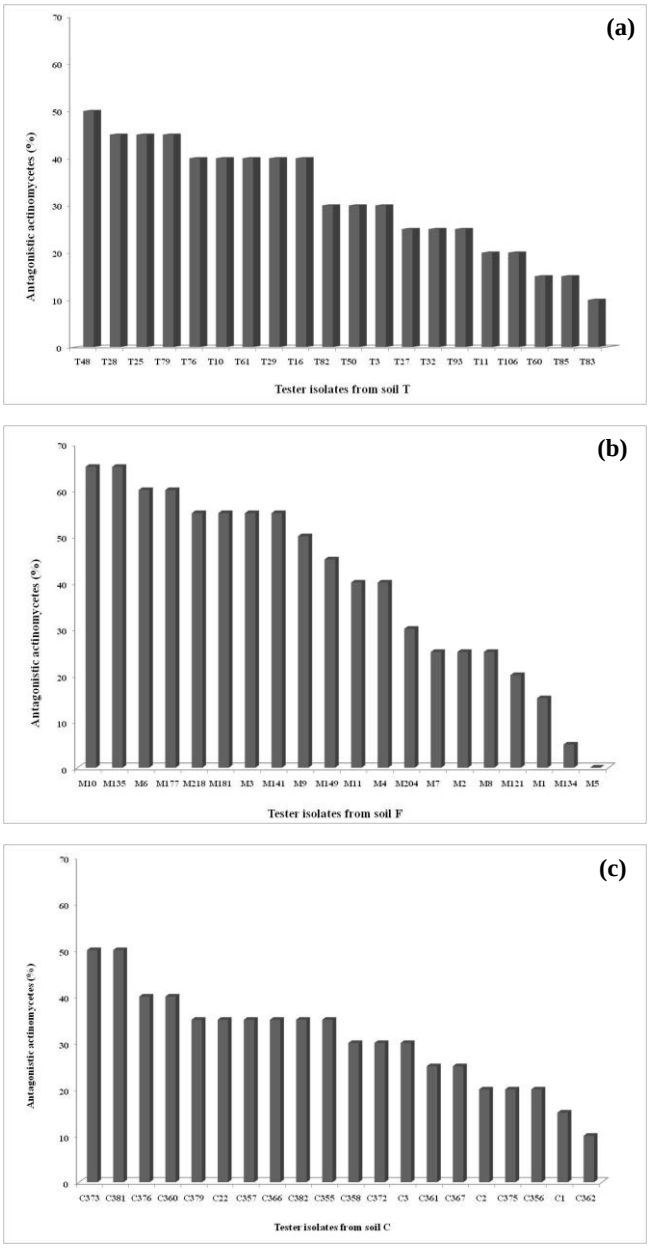

Figure 2. Antagonistic interactions among *actinomycetales* isolated from control soil (a), soil F (b) and soil C (c)

increases inversely with the number of bacteria in ufc g^{-1} dry matter of soil. This increase of bacteria number in amended soil was previously attributed to the amendment contribution of bacteria and nutriments, which stimulate the bacteria of control soil (Mokni-Tlili et al., 2011a). Now, from founded results (Figure 1), it can be suggested that the antagonistic activities play also a primordial role. Indeed, amendments application inhibits the suppressive interactions.

Other workers which were interested on bacteria originnated from the rhizosphere soil of Citrus (Gesheva, 2002) and from the Antarctic sponges (Mangano et al., 2009), showed contradictory results: the bacteria number increases with the antagonistic activities. In the case of the rhizosphere soil of *Citrus*, the more loaded with *Streptomyces: C. sinensis*: 87% (against *C. limon*: 52%) presents the high rate of antagonistic actinomycetes: 72% of antagonistic cultures (against 43% for *C. limon*). The Antarctic sponge containing more loaded with cultivable bacteria: *Anoxycalyx joubini*: 5.2 10^5 cfu per gram of sponge tissues (against *Lissodendoryx nobilis*: 1.8 10^5) presents the high percentage of antagonistic bacteria: 90% (against *L. nobilis*: 62%). Therefore, this can shows that bacteria-origin is an important parameter on the antagonistic potentiality.

For more detail and specificity, antagonistic results were re-treated to show the percentage of active isolates against each target bacteria in each treatment. e.g. Figure 2 shows the results for isolates originated from soils T, F (F40) and C (C120). These histograms show that the sensitive isolates increase with amendments application, especially for FM. For example, considering the 20 tested strains originated from each treatment, it was founded that 9 strains are sensitive to more than 50% for soil F against 2 and only one strain for soils C and T, respectively. On the other hand, it could be noted that the most sensitive (M10 and M135 isolated from F40 and affiliated to *Streptomyces spectabilis* and *Sreptomyces coelicolor,* respectively) and resistant (M5: isolated from F40 and affiliated to *S. coelicolor* and M285: isolated from F120 and affiliated to *Nocardioides albus*) strains are founded in soil F (Table 2). Then, soil F presents more heterogeneity in inhibition patterns and rate than soil T and soil C. This can be attributed to the effect of FM on the bacteria diversity. Indeed, it was previously shown that FM application increases more the phylogenetically difference in soil than the MSWC (Mokni-Tlili et al., 2011a).

Relation between diversity and antagonistic potential

Tables 2 and 3 show the inhibitory activities and susceptibility of some isolates in relation to their phylogenetic affiliation following individual antagonistic tests. It was observed that the inhibition patterns varied for different bacterial isolates (Table 3), even if they were grouped in the same OTU and even belonging to the same species (e.g. M5, M135 and M204 affiliated to *S. coelicolor*) (Table 2). This finding was observed in all treatments and amendments (data not shown) and suggests that antagonism might be due to different inhibitory mechanisms within the same species. However, although possible, inhibitory interactions were rarely detected among isolates belonging to the same OTU (e.g. M141 and M181 affiliated to *Streptomyces longisporoflavus*) (Tables 2 and 3). This can be supported by the founded results of Grossart et al. (2004); Lo Giudice et al. (2007); Long and Azam (2001); Mangano et al. (2009). From

Table 2. Antagonistic interactions among bacteria isolated from soil F (F40).

Tester strain	Target strain																			
	M7	**M149**	**M218**	**M181**	**M11**	**M9**	**M10**	**M135**	**M3**	**M6**	**M141**	**M177**	**M2**	**M4**	**M134**	**M8**	**M5**	**M121**	**M204**	**M1**
M7	-	-	+	+	+	-	+	+	+++	+	+	+	-	-	-	-	-	-	+	+
M149	-	-	+	-	+	-	+	+	++	+	+	+	-	-	-	++	-	-	-	-
M218	-	+	-	+	+	+	+	+	+	-	+	-	+	-	-	+	-	-	-	-
M181	+	++	+	-	+	+	+	+	+	++	+	++	-	+	-	+	-	-	-	-
M11	-	+	+	+	-	+	-	+	+	++	+	++	++	+	-	-	-	-	++	+
M9	-	-	+	+	+	-	+	+	++	+	-	+	-	+	-	-	-	+	++	+
M10	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
M135	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-
M3	+	+	++	+	-	+	++	++	-	++	++	+	++	-	-	-	-	-	-	-
M6	-	-	+	-	-	-	+	++	-	-	-	-	-	+	-	-	-	-	++	-
M141	-	+	+	+	+	+	+	+	-	+	-	+	+	-	-	++	-	+	++	-
M177	-	+	++	++	-	++	++	+	++	+	+	-	++	+	-	-	-	-	-	-
M2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-
M4	++	++	-	++	++	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-
M134	-	+	-	++	-	+	+	-	+	++	+	+	-	-	-	+	-	-	+	-
M8	+	-	-	+	-	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-
M5	-	-	-	-	-	-	-	-	-	-	++	++	-	-	++	-	-	+	-	-
M121	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M204	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M1	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-

-: indicate no zone of inhibition; +: represents until 5 mm wide zone of inhibition, ++: represents 5-10 mm wide zone of inhibition and +++: represents > 10 mm wide zone of inhibition.

data relative to the individual antagonistic tests, an autoinhibition phenomenon was also founded: M10 from F40 (Table 3) and C340 isolated from C80 and affiliated to *S. spectabilis*, C356 and C371 isolated from C120 and affiliated to *Streptomyces albidochromogenes* (data not shown). This action mode suggests that the activity is due to bacteriocins; ribosomally synthesized polypeptides serving to selectively kill closely related species while the producer strain remains unharmed. This phenomenon acts as a controlling factor in the maintenance of species diversity (Baba and Schneewind, 1998; Hentschel et al., 2001; Mangano et al., 2009; Nair and Simidu, 1987).

Antagonistic interaction of soil bacteria against amendments bacteria

In vitro antagonistic interactions were investigated between bacteria isolated from control soil T (20 out of 72 isolates) and those isolated from amendments: FM (35 out 37 isolates) and MSWC (17 out 18 isolates). Results are illustrated in Tables 4 and 5.

Case of bacteria T and FM

Table 4 shows that all T-isolates are active and can inhibit the growth of 1 to 30 (~85%) FM-actinomycetes; some of them, such as T16, T32, T61 and T83 were previously unable to inhibit the growth of bacteria retrieved from the same treatment T. Also, it was founded that FM-strains belonging to *Streptomycetaceae* and to

Table 2. Number of sensitive and active bacteria for each tester strain isolated from different soil treatments (soil T, soil F and soil C) in relation to their phylogenetic affiliation.

Phylogenetic group	Isolate	Related specie	Inhibition against (no. of sensitive isolates)	Inhibited by (no. of active isolates)
treptomycetaceae	T60	AY561610, *Actinobacterium RG-51*	18	3
	T27	AB249953, *S. albidochromogenes*	2	5
	T11	AB249953, *S. albidochromogenes*	6	4
	T28	EF063499, *S. venezuelae*	4	9
	T82	AB249953, *S. albidochromogenes*	11	6
	T76	AB249953, *S. albidochromogenes*	1	8
	T10	AB249953, *S. albidochromogenes*	2	8
	T61	AB249953, *S. albidochromogenes*	0	8
	T32	AB249953, *S. albidochromogenes*	0	5
	T85	AB249953, *S. albidochromogenes*	17	3
	T93	AB249953, *S. albidochromogenes*	2	5
	T48	AB249953, *S. albidochromogenes*	17	10
	T106	DQ026647, *S. exfoliatus*	0	4
	T29	AB249921, *S. spiroverticillatus*	13	8
	T79	AB249921, *S. spiroverticillatus*	14	9
	T3	DQ026634, *S. globisporus subsp. globis.*	1	6
	T83	DQ026647, *S. exfoliatus*	0	2
	T16	DQ026647, *S. exfoliatus*	0	8
Unidentified	T25		8	9
	T50		10	6

Table 2. Contd.

	M7	EF119843, *S. sp. AHW3*	11	5
	M149	AY561610, *Actinobacterium RG-51*	9	9
	M218	DQ250003, *S. sp. L42*	10	11
	M181	AB184220, *S. longisporoflavus*	13	11
	M11	AB184800, *S. coelicolor*	13	8
Streptomycetaceae	M9	EF063493, *S. sp. P3562*	12	10
	M10	AB184677, *S. spectabilis*	12	13
	M135	AB184800, *S. coelicolor*	3	13
	M3	DQ487016, *S. sp. B267*	11	11
	M6	DQ663179, *S. sp. 3004*	5	12
	M141	AB184220, *S. longisporoflavus*	13	11
	M177	AB249921, *S. spiroverticillatus*	11	12
	M2	AB184068, *S. fradiae*	1	5
	M4	AJ308577, *S. sp. Nm5*	9	8
	M134	AB249953, *S. albidochromogenes*	10	1
	M5	AB184800, *S. coelicolor*	4	0
	M121	DQ026647, *S. exfoliatus*	0	4
	M204	AB184800, *S. coelicolor*	1	6
	M1	DQ026670, *S. clavifer*	1	3
Nocardioidaceae	M276	AF005005, *Nocardioides albus*	2	1
	M286	AF005005, *Nocardioides albus*	7	1
	M288	AF005005, *Nocardioides albus*	0	4
	M243	AF005005, *Nocardioides albus*	4	2
	M244	AF005005, *Nocardioides albus*	0	1
	M285	AF005005, *Nocardioides albus*	3	0
	M283	AF005005, *Nocardioides albus*	1	3
	M234	AF005005, *Nocardioides albus*	0	2
	M287	AF005005, *Nocardioides albus*	0	2
	M275	AF005005, *Nocardioides albus*	0	1
	M8	AF005005, *Nocardioides albus*	9	5

Table 2. Contd.

	M285	AF005005, *Nocardioides albus*	3	0
	M283	AF005005, *Nocardioides albus*	1	3
	M234	AF005005, *Nocardioides albus*	0	2
	M287	AF005005, *Nocardioides albus*	0	2
	M275	AF005005, *Nocardioides albus*	0	1
	M8	AF005005, *Nocardioides albus*	9	5
	C310	AB184393, *S. spectabilis*	0	2
	C307	AB249953, *S. albidochromogenes*	0	2
	C299	AB249953, *S. albidochromogenes*	8	3
	C301	AB184220, *S. longisporoflavus*	1	5
	C292	AB184220, *S. longisporoflavus*	1	4
	C295	AB249953, *S. albidochromogenes*	5	2
	C291	AB249953, *S. albidochromogenes*	2	4
	C289	AB249953, *S. albidochromogenes*	0	2
	C326	AB184677, *S. spectabilis*	4	3
	C353	AB184220, *S. longisporoflavus*	6	3
	C340	AB184393, *S. spectabilis*	7	3
Streptomycetaceae	C343	AB249953, *S. albidochromogenes*	8	3
	C329	AB184220, *S. longisporoflavus*	2	1
	C336	AB184220, *S. longisporoflavus*	1	5
	C344	AB184220, *S. longisporoflavus*	0	4
	C337	AB184220, *S. longisporoflavus*	6	3
	C341	AB249953, *S. albidochromogenes*	0	4
	C332	AB184393, *S. spectabilis*	0	5
	C358	AB249953, *S. albidochromogenes*	16	6
	C379	AB249953, *S. albidochromogenes*	17	7
	C372	AB184393, *S. spectabilis*	0	6
	C22	DQ250003, *S. sp. L42*	17	7
	C357	AB249953, *S. albidochromogenes*	7	7
	C362	AB249953, *S. albidochromogenes*	19	2
	C2	AB184393, *S. spectabilis*	0	4

Table 2. Contd.

	C361	AB184393, *S. spectabilis*	1	5
Unidentified	C367	AB184393, *S. spectabilis*	0	5
	C356	AB249953, *S. albidochromogenes*	4	4
	C382	AB184220, *S. longisporoflavus*	0	7
Streptomycetaceae	C3	DQ026671, *S. cavourensis subsp. Wash.*	5	6
	C370	AB249953, *S. albidochromogenes*	11	3
	C376	AB184220, *S. longisporoflavus*	11	8
	C373	AB184220, *S. longisporoflavus*	4	10
	C371	AB249953, *S. albidochromogenes*	5	8
	C355	AB249953, *S. albidochromogenes*	3	7
Unidentified	C298		1	2
	C311		9	1
	C375		8	4
	C366		1	7
	C381		3	10
	MSWC1	EF371429, *S. aureus*	20	3
	MSWC2	EU257256, *S. sp. A528*	12	1
	MSWC3	EU273552, *S. collinus*	4	3
	MSWC4	AB184220, *S. longisporoflavus*	15	1
	MSWC5	AF306660, *S. sacchari*	16	2
	MSWC6	AF306660, *S. sacchari*	18	0
	MSWC7	AF306660, *S. sacchari*	11	2
	MSWC8	AF026081, *S. sp. CHR28*	15	3
	MSWC9	DQ086264, *S. sp. AB654*	15	1
	MSWC10	AB184220, *S. longisporoflavus*	19	3
	MSWC11	AB184676, *S. griseoaurantiacus*	14	8
	MSWC12	EF178674, *S. azureus*	14	3
	MSWC14	AF306660, *S. sacchari*	17	2
	MSWC15	AB184220, *S. longisporoflavus*	9	0
	MSWC16	EF371429, *S. aureus*	8	2
	MSWC17	AF026081, *S. sp. CHR28*	10	0
	MSWC18	EU257256, *S. sp. A528*	0	3
	MSWC13			

Table 2. Contd.

Streptomycetaceae	*FM1*	DQ026670, S. clavifer	0	1
	FM2	AB249953, S. albidochromogenes	1	2
	FM3	DQ445791, S. cavourensis subsp. C.	0	0
	FM4	AB249953, S. albidochromogenes	2	3
	FM5	AY999837, S. californicus	1	0
	FM7	DQ663179, S. sp. 3004	1	0
	FM8	AB184800, S. coelicolor	0	0
	FM10	AF306660, S. sacchari	4	0
	FM11	AB249953, S. albidochromogenes	0	2
	FM12	AY999837, S. californicus	3	5
	FM14	AB249953, S. albidochromogenes	0	0
	FM15	AB184677, S. spectabilis	2	5
	FM16	DQ026634, S. globisporus subsp. globis.	6	3
	FM17	AF306660, S. sacchari	0	3
	FM18	DQ445791, S. cavourensis subsp. C.	0	0
	FM19	AB184220, S. longisporoflavus	0	4
	FM20	DQ086264, S. sp. AB654	0	5
	FM22	AF026081, S. sp. CHR28	6	9
	FM23	AF026081, S. sp. CHR28	3	0
	FM24	EU257256, S. sp. A528	0	4
	FM25	EU273552, S. collinus	0	5
	FM27	EU273552, S. collinus	5	3
	FM28	AB184800, S. coelicolor	0	0
	FM29	DQ445791, S. cavourensis subsp. C.	0	0
	FM30	AY999837, S. californicus	0	2
	FM33	AY999837, S. californicus	0	2
	FM34	AY999837, S. californicus	1	4
Pseudonocardioidaceae	*FM9*	EU284126, Amycolatopsis sp. WX001	1	10
	FM21	EU284126, Amycolatopsis sp. WX001	1	11

Table 2. Contd.

Noardioidaceae	*FM6*	AF005005, Nocardioides albus	0	1
	FM13	AF005005, Nocardioides albus	0	0
	FM26	AF005005, Nocardioides albus	0	0
	FM31	AF005005, Nocardioides albus	1	0
	FM32	AF005005, Nocardioides albus	1	1
	FM37	AF005005, Nocardioides albus	0	0
Unidentified	*FM35*			
	FM36			

Table 3. Antagonistic interactions among bacteria isolated from soil F (F40).

Tester strain	Target strain																			
	M7	**M149**	**M218**	**M181**	**M11**	**M9**	**M10**	**M135**	**M3**	**M6**	**M141**	**M177**	**M2**	**M4**	**M134**	**M8**	**M5**	**M121**	**M204**	**M1**
M7	-	-	+	+	+	-	+	+	+++	+	+	+	-	-	-	-	-	-	+	+
M149	-	-	+	-	+	-	+	+	++	+	+	+	-	-	-	++	-	-	-	-
M218	-	+	-	+	+	+	+	+	+	-	+	-	+	-	-	+	-	-	-	-
M181	+	++	+	-	+	+	+	+	+	++	+	++	-	+	-	+	-	-	-	-
M11	-	+	+	+	-	+	-	+	+	++	+	++	++	+	-	-	-	-	++	+
M9	-	-	+	+	+	-	+	+	++	+	-	+	-	+	-	-	-	+	++	+
M10	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
M135	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-
M3	+	+	++	+	-	+	++	++	-	++	++	+	++	-	-	-	-	-	-	-
M6	-	-	+	-	-	-	+	++	-	-	-	-	-	+	-	-	-	-	++	-
M141	-	+	+	+	+	+	+	+	-	+	-	+	+	-	-	++	-	+	++	-
M177	-	+	++	++	-	++	++	+	++	+	+	-	++	+	-	-	-	-	-	-
M2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-
M4	++	++	-	++	++	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-
M134	-	+	-	++	-	+	+	-	+	++	+	+	-	-	-	+	-	-	+	-
M8	+	-	-	+	-	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-
M5	-	-	-	-	-	-	-	-	-	-	++	++	-	-	++	-	-	+	-	-
M121	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M204	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M1	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-

-: indicate no zone of inhibition; +: represents until 5 mm wide zone of inhibition, ++: represents 5-10 mm wide zone of inhibition and +++: represents > 10 mm wide zone of inhibition.

Table 4. Antagonistic interactions among bacteria isolated from soil T and those isolated from FM.

Tester T isolate	Target FM isolate																																						
	***S.* FM**																											***P.***					***N.***						
S.	1*	2	3	4	5	7	8	10	11	12	14	15	16	17	18	19	20	22	23	24	25	27	28	29	30	33	34	9	21	6	13	26	31	32	37				
T60	+	+		+					+								+	+		+	+				+		+	+	+	+									
T27																		+																					
T11				+					+	+						+					+																	
T28																										+													
T82																	+	+										+	+										
T76												+																	+										
T10				+								+								+									+										
T61												+				+												+	+										
T32													+					+																				+	
T85		+								+								+				+					+	+	+										
T93													+			+	+	+			+																			
T50														+																									
T25																												+										
T48																				+	+	+			+	+		+	+										
T106													+			+	+	+																					
T29																											+		+										
T79										+								+		+	+						+	+											
T3												+		+														+	+										
T83										+							+					+						+	+										
T16										+		+		+				+										+	+										

*: Number means FM number (for 1, FM1), *S.*: *Streptomycetaceae, P.*: *Pseudonocardioidaceae*, *N.*: *Noardioidaceae*

Pseudonocardiaceae were sensitive to 19 (95%) and 13 (65%) tester T-isolates, respectively. However, strains belonging to *Nocardioidaceae,* except FM6 and FM32, were resistant. For T-isolates grouped in the same OUT, they showed a slight similarity in the inhibition patterns (Table 4). This was consistent with previously founded results for antagonistic bacteria in the same treatment (data not shown). Finally, as it can be observed the majority (55%) of sensitive FM-isolates were grouped in the same OTU (e.g. FM15 (*S. spectabilis*), FM20 (*S. sp. AB654*), FM22 (*S. sp CHR28*), FM24 (*S. sp. A528*) and FM25 (*S. collinus*).

Case of bacteria T and MSWC

Table 5 shows that only 13 (65%) T-isolates were active and they inhibit the growth of 1 to 6 (~35%) targets; 4 from them (T3, T16, T32 and T83) were unable to inhibit the growth of bacteria retrieved from the same treatment. It was founded that 55% of MSWC-actinomycetes targets were sensitive (76%) and that they were grouped in different OTU (*e.g.* MSWC7 (*S. griseoaurantiacus*), MSWC11 (*S. sacchari*)). However, T- isolates clustered in the same OTU have a difference in the inhibition patterns.

In the same way, suppressive interactions of strains isolated from FM and MSWC against T-actinomycetes were also done. It was founded that MSWC-actinomycetes inhibit a more high number of target bacteria (~95%) than FM isolates

Table 5. Antagonistic interactions among bacteria isolated from soil T and those isolated from MSWC.

	Target MSWC strains																
Tester T isolates	***Streptomycetaceae* MSWC**																
S.	1*	2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18
T60	+							+		+							+
T27											+						
T11												+					
T28			+		+		+				+		+		+		
T82			+		+		+						+		+		
T76																	
T10																	
T61																	
T32		+	+	+							+						
T85																	
T93																	
T50											+						
T25																	
T48	+										+	+					+
T106	+							+		+		+					+
T29																	
T79									+								
T3											+						
T83											+						
T16								+		+	+						

*: Number means MSWC number (for 1, MSWC1), *S*.: *Streptomycetaceae,*

shown). Above presented results show clearly that strains isolated from MSWC were more resistant and more active than those isolated from FM. Besides, it can be concluded that the amendment application can stimulat suppressive interactions of inactive bacteria of soil: e.g. T3, T16, T32 and T83. These potential antibacterial interactions of MSWC-isolates can be attributed to their high metabolic activities. Indeed, it confirms the results on assessment of enzymatic activities of *actinomycetales* performed in previously study (Mokni-Tlili et al., 2010; 2011b) showing that MSWC was a stimulator of enzymatic activities in soil and a potential source ofenzyme-producing bacteria. This finding was corroborated by several reports (Crecchio et al., 2004; Pascual et al. 1998; Serra-Wittling et al., 1996). Therefore, the addition of mature MSWC to soil improves soil quality, promotes plant development and reduces by antagonistic bacteria the number of diseases caused by pathogens in soil (Cotxarrera et al., 2002).

Previously (Mokni-Tlili et al., 2009), it was observed that FM was characterized by a highly *actinomycetales* diversity than MSWC. This was attributed to the physico-chemical properties of amendments. Now, the presented results permit to attribute also this diversity variation to the antagonistic interactions between the bacteria populations. Indeed, the clustering of sensitive FM-isolates in the same OTU reflected the maintaining of the *Streptomycetaceae* diversity in soil amended by FM but, the clustering of sensitive MSWC-isolates in different OTU reflected the decrease of genera types in soil amended by MSWC. This founded result was in agreement with those of several researchers indicating that the high genotypic and phenotypic diversity was estimated for antagonistic bacteria (Costa et al., 2006; Mangano et al., 2009). For example, Costa et al. (2006), in their investigation on the diversity and antagonistic potential of *Pseudomonas* ssp. in rhizosphere of maize cultivars, showed that antagonists having an enormous genotypic and functional diversity. However, homogeneous microorganism which co-inhabit in microenvironments, they have almost the similar metabolic activity which limit the antagonist reactions. Results suggest that antago-nism could play a significant role in shaping bacterial communities.

Antagonistic interactions between actinomycetes and tow phytopathogen bacteria

Two phytopatogenic bacterium *A. tumefaciens*: C58 and B6 were tested. Figure 3 illustrates the percentage of

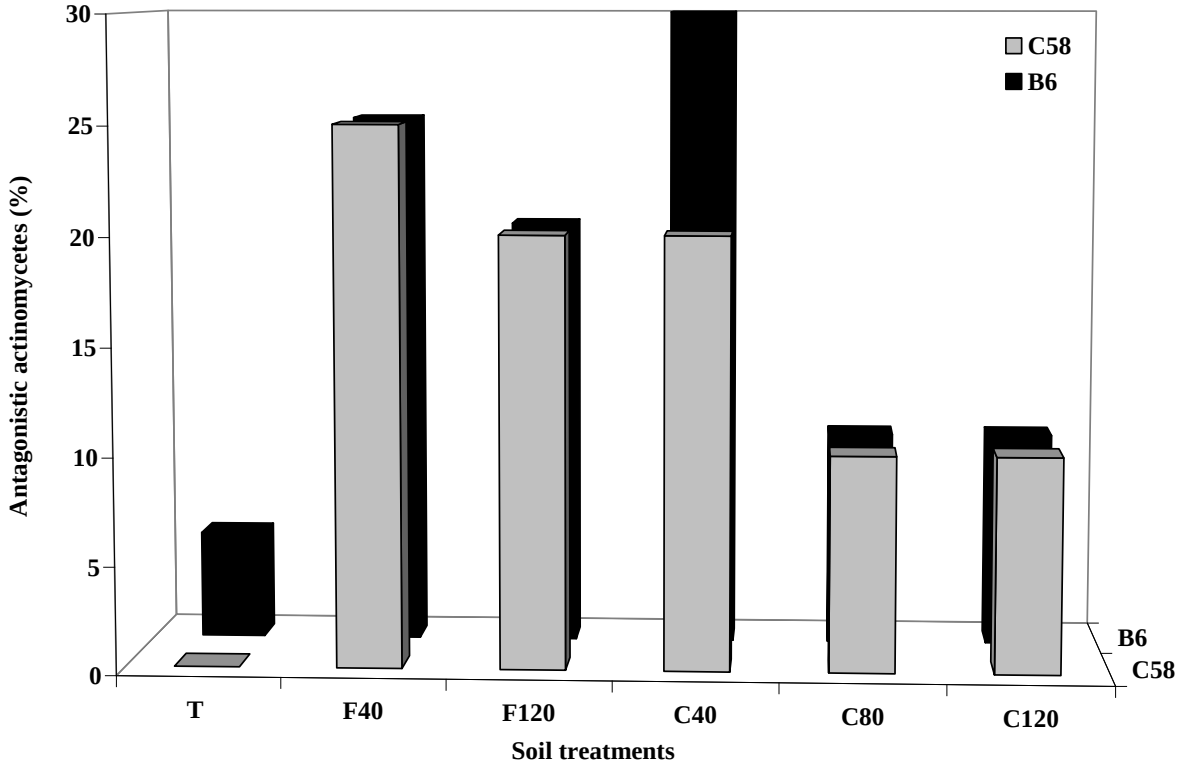

Figure 3. Antagonistic interactions among *actinomycetales* isolates and tow *Agrobacterium tumefaciens* B6 and C58.

active *actinomycetales* against these tow targets bacteria. They were inhibited by 10 to 30% of tester isolates. Therefore, *Actinomycetales* were considered among potential biocontrol agents for plant diseases producing antibiotics which can be developed for agricultural use (Tanaka and Mura, 1993). The high percentage of suppressive interactions was detected for bacteria isolated from soil F40, F120 and C40. The percentage of active bacteria was slightly decreased in soil C80 and C120. This decrease is due to the accumulation of toxic elements in these treatments (successive amendment application, one time/year for a period of 5 years) which inhibited the antibacterial activities (Mokni-Tlili et al., 2011b). For this purpose, it can be concluded that MSWC application in soil improves the soil fertility and structure.

Conclusion

At laboratory-scale, it is difficult to reproduce all biotic and abiotic features characterizing an environment, due to well-known biases arising from isolation and cultivation procedures. In fact, it must be pointed out that various environmental factors and specific soil biological and physico-chimical properties, as well as the occurrence of other kinds of microbial interactions (such as commensalism and symbiosis, which could also involve uncultivable bacteria), affect the true bacterial community composition. Nevertheless, results from inhibitory assays among cultivable bacteria in artificial systems could provide precious indications on bacterial interactions occurring in a natural environment, representing a baseline for further investigation of the ecological role of soil bacteria.

In this paper antagonistic interactions among *actinomycetales* in soil amended or not with FM or MSWC were investigated. Based on the experimental data, the following conclusions can be made:

1. All studied treatments present a higher rate of active bacteria (≥ 50%).
2. The amendment application plays a double opposite roles by decreasing the antagonism and by enhancing the enzymatic synthesis and then stimulates the antimicrobial interactions.
3. Following the antagonistic interactions of bacteria and their phylogenetic affiliation studies, it can be concluded that the antagonism could play a significant role in *Actinomycetales* diversification.
4. The percent of antagonistic actinomycetes against *A. tumefaciens* was high for strains isolated from amended soil; this indicates that studied amendments must improve the soil fertility and structure.
5. Results from inhibitory assays suggest that antagonism could play a significant role in structuring bacterial

communities in agricultural soil.

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