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Streptomyces noboritoensis isolated from rhizosphere soil and its use in controlling banana-tissue culture contaminants

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In this study, the role of *Streptomyces noboritoensis* (14) isolated from the rhizosphere of banana plant and having antagonistic activity against the bacterial- and fungal-tissue culture contaminants was determined *in vitro*. Results show that the filtrate was more effective against the fungal-tissue culture contaminants than the bacterial-tissue culture contaminants. This was indicated when jars were fungi-free and bacteria-free after one month and 21 days from incubation, respectively. Results of *in vitro* application show that the filtrate of *S. noboritoensis* (14) as a bio-control agent could be used for controlling the contaminants in banana. Data showed that treatment F (sterilized shoots treated with *Streptomyces* filtrate and cultivated on *Streptomyces*-inoculated medium) was the most effective followed by treatment E (sterilized shoots untreated with *Streptomyces* filtrate and cultivated on *Streptomyces*-inoculated medium). Therefore, the study suggests conducting further studies towards the use of streptomycetes in the biological control in a large scale production.

Key words: *Streptomyces*, tissue culture contaminants, antagonistic activities, bio-control.

INTRODUCTION

The nutrient media in which the plant tissue was cultivated is a good source of nutrient for microbial growth. These microbes compete adversely with plant tissue culture for nutrient. The presence of these microbes in these plant cultures usually resulted in increased culture mortality, the presence of latent infections can also resulted in variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting (Kane, 2003).

Studies on biological control of plant diseases, particularly using antibiotic metabolites of microbial origin, have been expanded and such materials may supplement to be an alternative to chemical disease control (Fischer et al., 1992). *Streptomyces* sp. is gram-positive filamentous bacteria that produce and secrete a wide array of biologically active compounds including antibiotics, hydrolytic enzymes and enzyme inhibitors. They are resistant to desiccation and nutrient stress, by their ability to produce spores (Dhanasekaran et al., 2005). These characteristics make streptomycetes attractive candidates for biological control agents against soil-borne plant pathogens (Samac and Kinkel, 2001; Hassan et al.,

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2011). Attempts have been made to develop *Streptomyces* species as fungal root disease control agents, since *Streptomyces* spp. are capable of producing a remarkably wide spectrum of antibiotics as secondary metabolites (Yuan and Crawford et al., 1995).

El-Tarabily et al. (2009) evaluated the potential of *Actinoplanes campanulatus*, *Micromonospora chalcea* and *Streptomyces spiralis* endophytic in cucumber roots, to promote plant growth and to protect seedlings and mature plants of cucumber from diseases caused by *Pythium aphanidermatum*, under greenhouse conditions. Sadeghi et al. (2009) studied the effectiveness of two disease-suppressive *Streptomyces* spp. to control sugar beet *Rhizoctonia solani* damping off under field conditions. *Streptomyces* seed treatments reduced seedling damping off in naturally and artificially infested soils. Evaluation of final harvest revealed that the root yield of the biocontrol agents increased compared to untreated control in these years. Soil-actinomycetes particularly *Streptomyces* spp. have antagonistic activity against a wide range of plant pathogens. In recent decades, they have attracted high interests as biological control agents (Zarandi et al., 2009). In this study, the role of *Streptomyces noboritoensis* (14) isolated from the rhizosphere of banana plant and having antagonistic activity against the bacterial- and fungal-tissue culture contaminants was determined *in vitro.*

MATERIALS AND METHODS

Source of *Streptomyces* strain

In this study, the *S. noboritoensis* 14 strain isolated from banana-rhizosphere and having antagonistic activities against five of each of fungal strains and bacterial isolates recorded as tissue-culture contaminants was used. The strain was obtained from Department of Biology, Female Branch, Faculty of Sciences, Taif University, KSA.

In vitro evaluation of the effective substances of the *S. noboritoensis* 14 to control the growth of microbial contaminants

Banana shoots were previously surface sterilized by immersion in a 2.5% (v:v) sodium hypochlorite solution containing 0.1% (v:v) and rinsed four times with $d.H_2O$ (Dornelas and Vieira, 1994). Sterilized plant materials were then placed in a clean box constructed from a clear 60 x 30 x 30 cm plastic box. All shoots were subcultured on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose, 5 mg L^{-1} 6-benzyl aminopurine (BAP) and 0.8% Difco-Bacto agar (Becerra et al., 2004). The jars were formulated to contain no antimicrobial agent, or contained the filtrate of *S. noboritoensis* 14 prepared as described by Mohamed et al. (2012) (1 ml for each box). The jars were incubated in a clean box at cabinet temperature (25°C) under constant illumination. Type and time of appearance of contaminants in tissue culture banana jars treated with the filtrate of *S. noboritoensis* 14 as a bio-control agent for controlling the contaminants in banana tissue cultures were measured up to 60 days using banana tissue culture shoots. Level and time of appearance of contaminants in tissue culture banana jars *via* six treatments (A, B, C, D, E and F) with the filtrate of *S. noboritoensis* 14, were also measured up to 19 weeks post incubation.

RESULTS AND DISCUSSION

Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. Additionally, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Among these alternatives are those referred to as biological controls (Pal and McSpadden Gardener, 2006).

Raudales and McSpadden Gardener (2008) reviewed examples of bactericides and fungicides for controlling some plant pathogens. *Streptomyces* strains are gram-positive, filamentous soil bacteria that are well known for their abilities to produce antibiotics and other secondary metabolites. These organisms have been implicated in the antagonism of a wide variety of plant pathogenic fungi, bacteria, and nematodes and are currently under investigation for their potential use as biological disease control agents (Ara et al., 2012). *Magnaporthe oryzae*, the causal agent of rice blast disease was *in vitro* suppressed with 100 *Streptomyces* isolates (Zarandi et al., 2009). They showed that soil-actinomycetes particularly *Streptomyces* spp. have attracted high interests as biological control agents. Results in Table 1 show the type and time of appearance of contaminants in control-tissue culture banana jars (untreated with *Streptomyces* filtrate). No bacterial contaminants were found 4 days post culturing while both of bacterial and fungal contaminants were increased by time of incubation, as the maximum growth of contaminants appeared after two months of incubation. Results are shown in Figure 1.

Type and time of appearance of contaminants in tissue culture banana jars treated with the filtrate of *S. noboritoensis* 14 as a bio-control agent for controlling the contaminants in banana tissue cultures are recorded in Table 2. Data appeared that the filtrate of the applied *Streptomyces* strain was effective in inhibiting the growth of fungal contaminants, as the jars were fungi-free and bacteria-free after one month and 21 days from incubation, respectively. In other words, the growth of fungal and bacterial contaminants was weak in the Jars of banana-tissue culture treated with *Streptomyces* filtrate till 60 days from incubation. As a conclusion, the filtrate was more effective against the fungal-tissue culture

Table 1. Type and time of appearance of contaminants in control-tissue culture banana jars (untreated with *Streptomyces* filtrate).

Type of contaminant	Jars of banana tissue culture untreated with *Streptomyces* filtrate						
	Days post incubation						
	4	6	8	10	15	30	60
Bacteria	-	+	++	++	+++	+++	++++
Fungi	+	++	++	++	+++	+++	++++

-, No growth; +, weak growth; ++, moderate growth; +++, good growth; ++++, abundant growth.

Figure 1. Type of contaminants in control-tissue culture banana jars (untreated with *Streptomyces* filtrate).

Table 2. Type and time of appearance of contaminants in tissue culture banana jars treated with the filtrate of *S. noboritoensis* 14 as a bio-control agent for controlling the contaminants in banana tissue cultures.

Type of contaminant	Jars of banana tissue culture untreated with *Streptomyces* filtrate						
	Days post incubation						
	4	6	8	10	15	30	60
Bacteria	-	-	-	±	+	+	+
Fungi	-	-	-	-	-	+	+

-, No growth; ±, in doubt; +, weak growth.

Table 3. Level and time of appearance of contaminants in tissue culture banana jars treated with the filtrate of *S. noboritoensis* 14 as a bio-control agent for controlling the contaminants in banana tissue cultures.

Week post inoculation																		
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Treatment A: Non-sterilized banana shoots cultivated on sterilized medium without *Streptomyces* filtrate																		
-	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Treatment B: Non-sterilized banana shoots cultivated on sterilized with *Streptomyces* filtrate																		
-	-	-	-	-	+	+	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Treatment C: Sterilized banana shoots cultivated on sterilized medium without *Streptomyces* filtrate																		
-	-	-	-	-	-	-	-	+	+	+	+	++	++	++	++	++	++	++
Treatment D: Sterilized banana shoots cultivated on sterilized medium with *Streptomyces* filtrate																		
-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	++	++	++
Treatment E: Banana shoots sterilized and dipped in *Streptomyces* filtrate then cultivated on sterilized medium without *Streptomyces* filtrate																		
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Treatment F: Banana shoots sterilized and dipped in *Streptomyces* filtrate then cultivated on sterilized medium with *Streptomyces* filtrate																		
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-, No contamination; +, weak; ++, moderate; +++, high.

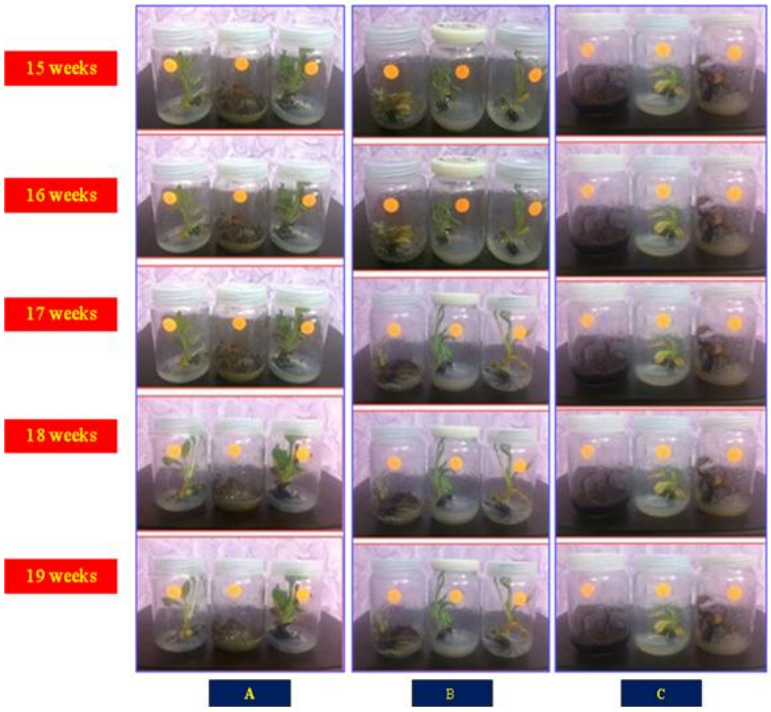

Figure 2. Level and time of appearance of contaminants in different tissue culture banana jar treatments (A, B and C) 15, 16, 17, 18 and 19 weeks post incubation (From top to bottom).

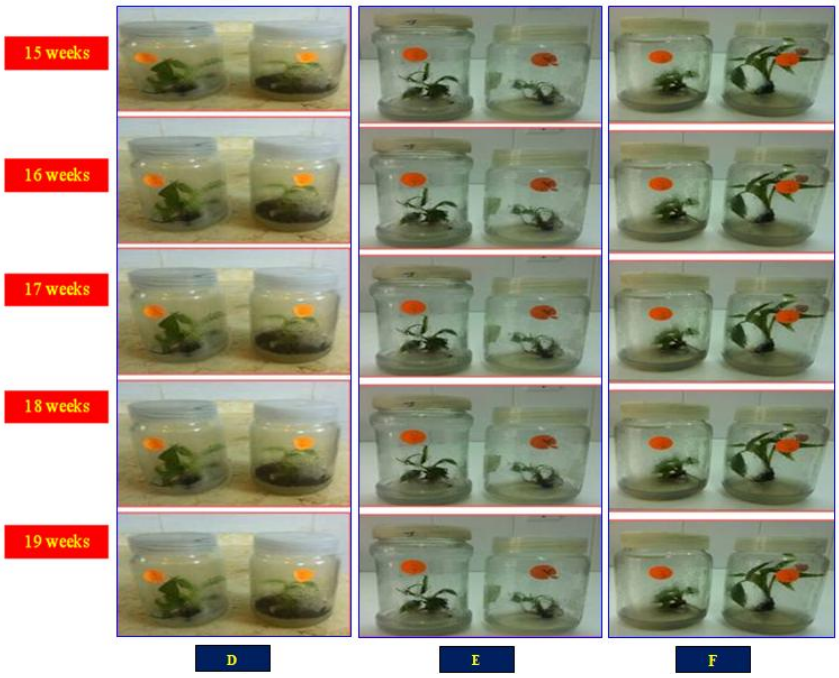

Figure 3. Level and time of appearance of contaminants in different tissue culture banana jar treatments (D, E and F) 15, 16, 17, 18 and 19 weeks post incubation (From top to bottom).

contaminants than the bacterial-tissue culture contaminants. These results are in harmony with that of Sabaratnam and Traquair (2002), who reported that formulations of a *Streptomyces* biological control agent for Rhizoctonia damping-off in tomato seedlings were developed for the first time from vegetative propagules obtained from actively growing, nonsporulating liquid cultures. Also, Strap and Crawford (2006) showed that rhizosphere-streptomycetes in soils could act as producers of bioactive metabolites and could be used commercially as inoculants bio-control agents primarily to control fungal root diseases. In Hungary, Dormanns-Simon (2007) reported that microbiological agents such as Mycostop based on *Streptomyces griseoviridis* K61 was used for the control of damping-off and Fusarium wilt. The MycostopReg was a commercial formulation of strain K61 of *S. griseoviridis*. Smith et al. (2012) showed that the Mycostop Reg could be used as a fungicidal biological control agent against blueberry blossom blight.

Results of *in vitro* application (Table 3, Figures 2 and 3) show that the filtrate of *S. noboritoensis* 14 as a bio-control agent could be used for controlling the contaminants in banana. Data showed that the treatment F (sterilized shoots treated with *Streptomyces* filtrate and cultivated on *Streptomyces*-inoculated medium) was the most effective one followed by treatment E (sterilized shoots untreated with *Streptomyces* filtrate and cultivated on *Streptomyces*-inoculated medium). This was clearly concluded by the absence of the microbial contamination in jars of treatment F up to 19 weeks post incubation compared to the control jars.

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