Microbiological Control of Bacterial Soft Rot Caused by *Bacillus pumilus* Od23 on Potato

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Research Article

ABSTRACT

Potato soft rot, caused by *Bacillus pumilus* Od23, greatly affect potato tuber quality in storage and cultivars of Mali and indicated that it can affect all potato cultivars. Bacteria isolated from soil rhizosphere samples of healthy Malian indigenous trees were screened for their antagonistic effect against this pathogen. Three actinomycetes isolates (RoN, G1P, and N1F) were the most effective microbioagents in suppressing the growth of the pathogen. The biological control essay showed the possibility of controlling potato soft rot by these three actinomycetes isolates under conservation conditions. These treatments significantly decreased soft rot compared with the untreated potato tuber slices. The microbiological control results of this study suggest that the actinomycetes isolates RoN, G1P and and J1N are effective microbioagents in controlling soft rot of potato and could be considered as promising alternative to chemical products.

Keywords: Microbiological control; Bacillus pumilus; potato; soft rot; actinomycetes; Mali.

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1. INTRODUCTION

In Africa, potatoes represent an important source of food for people living in cities and urban area. Therefore it contributes to reducing worldwide food shortages (Han et al., 2005). However, potato tubers are susceptible to devastation by various diseases, such as soft rot caused by bacteria from *Erwinia* genus and other pathogen bacteria (Strömberg, 1999; Altin and Bora, 2001). In Mali, farmers lose more than 30% of their potato production because of soft rot caused by *Bacillus pumilus* (Coulibaly, 2002). This pathogen can greatly affect tuber quality in storage, and, therefore, can severely reduce its market value (Bathily et *al.,* 2010). Unfortunately, all the commonly grown potato cultivars are susceptible to *B. pumilus* Od23, although some are less susceptible than others (Bathily, 2007). Control of this pathogen is difficult because the pathogens survive for many years as spores in soil (Rai et al., 2006). The control of soft rot diseases in storage has commonly relied on conservation practices and on the use of chemicals. However, conservation practices alone are not efficient and, at the present time, no effective pesticides are available. Although some chemicals are recommended (Olivier et al., 1999), their use is limited by their prices and their adverse effects on human health and environment (Margosan et al., 1997). Currenlly, the use of bioagents would be an alternative, as well as ecological as durable, for protecting plants against pathogens (Gesheva, 2001; Aysan et al., 2003; Karabulut et al., 2003). Antagonistic plant-associated bacteria and actinomycetes are another important group of beneficial microorganisms for the control of soil-borne plant pathogens (Crawford et. al., 1993; Toussaint et al., 1997; Ozaktan et al., 1999; Rangajaran et al., 2003; Ryan et al., 2004; Ran et al., 2005; Nonoh et al., 2010). Therefore, the present investigation was designed to investigate the potential of tubers treatment with microbioagents, from rhizospheric soils of Malian indigenous trees, to reduce potato soft rot in storage.

2. MATERIALS AND METHODS

2.1 Pathogen

Bacillus pumilus Od23 was previously isolated from infected potato tubers, and identified as the principal pathogen of potato soft rot in storage in Mali (Bathily et al. 2010). *B. pumulis* cultures were maintained on potato dextrose agar (PDA) slants at 4°C. The spores suspension was prepared from 3-day-old cultures grown at 30°C in nutrient broth medium. After the incubation period the medium was centrifuged at 3000 rpm for 20 min and the pellet was collected in sterile distilled water (which was considered as bacterial spore and cell suspension) and diluted to a concentration of $6x10^6$ spores/ml.

2.2 Screening and Isolation of Biocontrol Agents

Four diverse rhizospheric soils of indigenous Malian trees were selected for the screening and isolation of the *B. pumilus* Od23 antagonists streptomycetes. The trees used in this study are: *Acacia albida* (Balanzan), *Parkia biglobosa*, *Vitelaria paradoxa* and *Jatropha gossypiifolia,* were chosen because of their capacity to survive in soils highly infested by plant pathogens. Three samples from each of the rhizospheric soils were taken (up to 10 cm depth) after removing approximately 3 cm of the soil surface. Samples were placed in sterile polyethylene bags, closed tightly and stored in the refrigerator at 4°C until use. Samples of each rhizospheric soil were first mixed, suspended in sterile distilled water (1 g in 100 ml) and shaken on rotatory shaker (200 rev/min, 30 min). All treated samples were serially diluted up to 10^6 and spread (0.1 ml) over the surface of nutrient agar (Difco, USA) and soil extract agar (Barakate et al., 2002). To increase the selectivity of the soil extract agar, this medium was supplemented with glycerol (5 g/l) as a carbon source, nalidixic acid (10 mg/l)

which inhibits the bacteria capable of swarming without affecting the growth of actinomycetes (Bulina et al., 1997), and cycloheximide (40 mg/l) to inhibit fungal growth. Plates were incubated at 28°C and the number of colonies was determined after 7 days for total bacteria and after 21 days for Streptomycetes. After 21 days of incubation, morphologically different colonies on soil extract agar were selected as candidate antagonists. Streptomycetes colonies were recognized on the basis of morphological and physiological characteristics following directions given by the standard protocol of the International Streptomyces project, which fixed the harmonized methods for actnimycetes identification (Shirling et Gottlieb, 1966).

2.3 In Vitro Antimicrobial Activity on Petri Dishes

The antimicrobial activities of the bacterial isolates were determined by the plate diffusion method (DeFrank and Putnam, 1985) against *Bacillus pumilus* Od23. Isolates were grown on the Bennett medium (beef extract (Merck, Germany) 1 g/l; glucose (Merck) 10 g/l; peptone (Merck) 2 g/l; yeast extract (Merck) 1 g/l and agar (Difco) 15 g/l) for 14 days and three discs (10 mm in diameter) were cut and placed on Muller–Hinton agar which were seeded with *B. pumilus* Od23. Plates were first kept in a refrigerator (4°C) for at least 2 h to allow the diffusion of any antibiotics produced, then incubated at 30°C. Antagonistic activities were evaluated by measuring (as mm) the widths of the clear zones surrounding the spot cultures (Xue et al., 2009).

2.4 Suppression of Soft Rot Development on Potato Slices

Antagonistic effect of all candidate antagonists for *B. pumilus* Od23 was studied by using potato slices. All treatments in this study were done as three replicates. Potato (*Solanum tuberosum* L.) tubers free from cuts and bruises were selected and washed for 10 min in running tap water and then surface sterilized with 1% NaOCl solution for 10 minutes. Then tubers were again washed in running tap water, air-dried at ambient temperature (30±2°C) and diametrically divided into two parts. A wound cavity was produced in each slice by inserting a sterile cork borer to remove the core of tissue (figure 1).
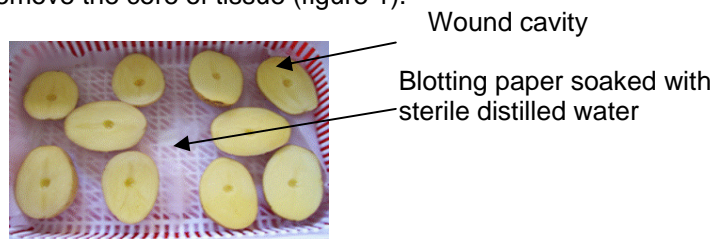

Fig. 1. Potato tuber slices with wound cavity

Treatments were performed as follow : (i) simultaneously 100 μl of *B. pumilus* Od23 (10^6 ufc/ml) and 100 μl of the supernatant of the antagonist culture suspension (T0), or (ii) 100 μl of *B. pumilus* Od23 followed by the inoculation of 100 μl of the supernatant of the antagonist culture suspension 12 h later (T12). Slices inoculated only with antagonists culture supernatant served as controls. The treated and untreated slices were kept at room temperature (30±2°C) in loosely tied polyethylene bags. After two days of incubation, the rotted tissues of every slice was recovered and weighed.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Screening and isolation of antagonists

As shown in Table 1, the pH of the soils ranged from 5,78 to 6. Total microbial counts ranged from 0,1 to $3,89 \times 10^8$ ufc/g of rhizospheric soil.

**Table 1. Number of total bacteria and streptomycetes and soil pH
of sampling sites**

Sampling sites	Total bacteria count ($\times 10^8$ cfu/g)	Streptomycetes count ($\times 10^7$ cfu/g)[b]	Percentage streptomycètes	pH
Acacia albida	0,1	0,1	10	5,80
Parkia biglobosa	10,4	35,9	35	5,90
Vitelaria paradoxa	1,56	53,4	34	6,00
Jatropha gossypiifolia	3,89	18,4	47	5,78

Important variations in streptomycete counts were observed between studied habitats; they ranged from $0,1 \times 10^7$ to $53,4 \times 10^7$ cfu/g dry soil. In relation to the total bacteria population, streptomycete counts ranged from 10 to 47% in the rhizospheric soil samples investigated.

3.1.2 Identification of microbioagents

The initial screening of more than 27 bacterial and 12 actinomycetes isolates originated from different rhizosphere-soil samples of healthy Malian indigenous trees, resulted in the isolation of 3 bacterial and 5 Streptomyces isolates exhibiting obvious antagonistic action on plates against *Bacillus pumilus* Od23, a new pathogen on potato in Mali (Bathily et *al.,* 2010). The *in vitro* antagonists showed that three isolates identified as *Streptomyces* spp. RoN*, Streptomyces* spp. G1P and *Streptomyces* spp. N1F were the most effective microbioagents in suppressing the potato cells rot caused by *B. pumilus* Od23 (Figure 2).
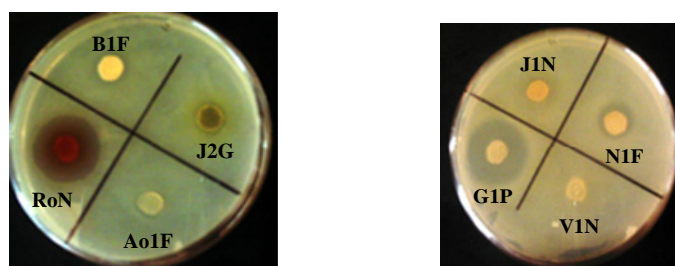

**Fig. 2. Growth inhibition of *Bacillus pumilus* Od23 by *Streptomyces* spp. RoN,
Streptomyces spp. G1P and *Streptomyces* spp. N1F**

3.1.3 Microbiological control of potato soft rot in storage

Three out of the eight antagonists selected exhibited their efficacy to control the soft rot of potato cv. Pamina (the most cultivated and appreciate potato cultivar in Mali) under the storage conditions. Data presented in figure (3) show that if the pathogen is simultaneously inoculated with the microbioagents, the soft rot of the potato tuber slices was significantly decreased by the *Streptomyces* spp. RoN and the *Streptomyces* spp.G1P, compared with the tuber slices treated only by the pathogen *B. pumilus* Od23.
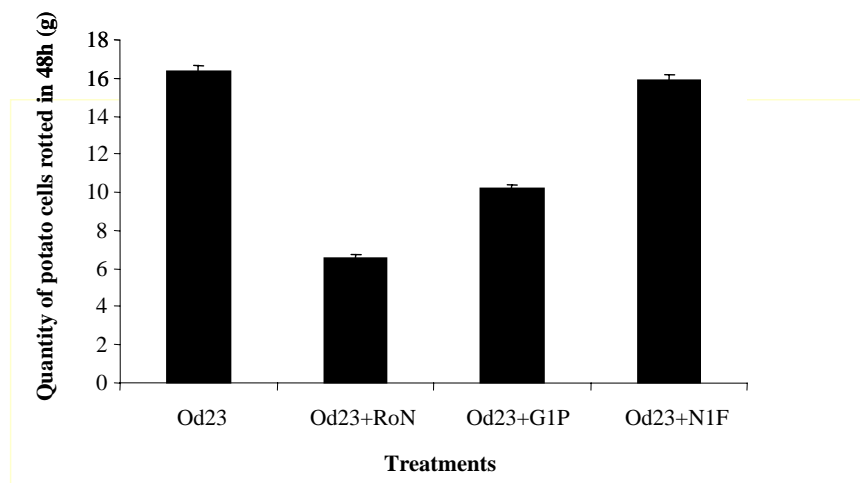

**Fig. 3. Effect of simultaneously inoculation with Od23 and microbioagents
on potato soft rot in storage**

On the other hand, potato slices inoculated with microbioagents 12h after the inoculation with *B. pumilus* Od23, the soft rot of potato tuber slices was significantly decreased by the *Streptomyces* spp. N1F compared to the treatment with the pathogen only (Figure 4).
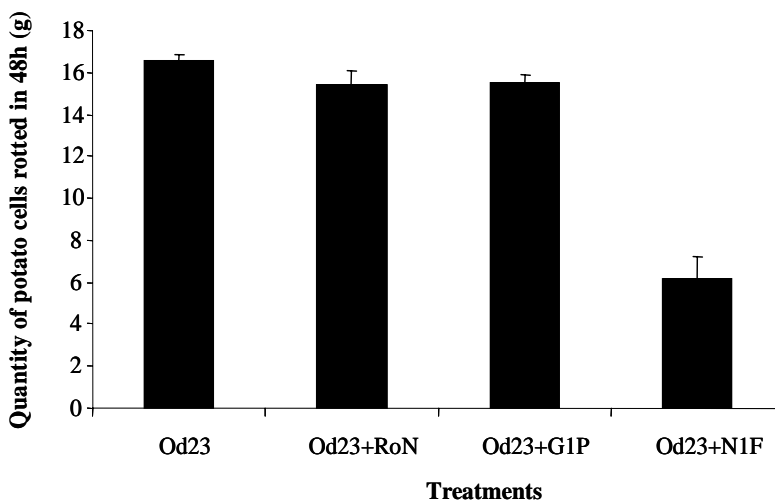

**Fig. 4. Effect of inoculation with Od23 12 hours after the inoculation with the
microbioagents on potato soft rot in storage**

3.2 Discussion

In this work, the streptomycete counts ranged from 10 to 47% in the rhizospheric soil samples investigated. Contrary, Barakate et al. (2002) studied the distribution of streptomycetes and their antimicrobial activities from Moroccan habitats and found a different proportion of actinomycetes in the bacterial population (0.36–8.63%). Moreover, Crawford and al. (1993) found that rhizosphere-associated soils gave almost twice as many actinomycete isolates as the non-rhizosphere-associated soils. All together, these results suggest that rhizospheric soils, compared to the soils, offer more nutrients needed for bacterial growth, owing to root exudates production by plants. Several studies showed that the number of streptomycetes was related to type of soil and edaphic conditions (Jordan, and Xue et al., 1996; Crawford et al., 1993; Saadoun et al., 1998). The presence of a small number of microorganisms in sandy soils can be explained by the fact that sandy soils are less favourable to bacterial growth; because it stores less humidity and contains little organic matter. In fact, Saadoun et al. (1998) reported that streptomycetes accounted for about 1–20% of total bacterial count in soil samples collected from northern Jordan, and Xue et al. (1996) reported actinomycete populations in cool areas of China where streptomycetes constituted up to 83% of total count.

Streptomyces spp. RoN and *Streptomyces* spp. G1P significantly controlled potato soft rot caused by Od23 if inoculated simultaneously with the pathogen. They were ineffective if inoculated 12h after the pathogen. Contrary, *Streptomyces* spp. J1N significantly controlled the soft rot of potato slices if inoculated 12h after the inoculation with the pathogen. These different results can be explained in the light of results recorded by Fatope et al. (2000) who stated that microorganisms work through different mechanisms such as, toxins, proteins, hormones, vitamins and amino acids for controlling diseases. RoN and G1P, probably require the presence of *Bacillus pumilus* Od23 to synthesize the antimicrobial compounds which in this case reduce the pathogen growth. N1F, unlike RoN and G1P, have a low growth rate in relation with the pathogen. Therefore, it is unable to inhibit the pathogen growth when incubated simultaneously. However, N1F is effective when incubated 12H before it has been in contact with pathogen.

There is a growing interest in the use of these natural products from microorganisms, because they are readily biodegradable, specific and generally have low toxicity (Newman et al., 2003). Also, attempts had been successfully carried out using antagonists to control soil-borne pathogens on potato tuber plants (Abussaoud and Saadoun, 1988; Toussaint et al., 1997; Ndonde and Semu, 2000; Rangajaran et al., 2003; Ryan et al., 2004; Ran et al., 2005). The antagonistic activity of these used microbioagents was recognized by many investigators (Xue et al., 1996; Crawford et al., 1993; Saadoun et al., 1998). Many species of genus are known to be potent producers of many antibiotics against soil-borne pathogens (Crawford et al., 1993; Ndonde, 2000; Nonoh et al., 2010). The obtained results of this study suggest the tested microorganisms proved to be an effective microbioagents in controlling the tested potato pathogenic bacteria and could be considered an alternative to chemical products and hence it can reduce the environmental pollution resulting from using pesticides in controlling post harvest diseases.

4. CONCLUSION

This work shows that rhizopheric soils of Malian indigenous trees lodges a great number of actinomycetes. The microbiological control realised with three streptomycetes isolates proved the effectiveness of the tested microbioagents in controlling the potato pathogenic bacteria, *Bacillus pumilus* Od23 and suggest that the use of these microbioagents could be

considered as an alternative to chemical products to reduce the environmental pollution resulting from using fungicides in controlling post harvest diseases.

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