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# Translocation and persistence of antibiotics produced by *Bacillus* and *Streptomyces* spp. in the bean plant

J. M. WAGACHA, E.W. MUTITU, J.W. MUTHOMI. & F.B. MWAURA[1]
Department of Crop Protection, University of Nairobi, P. O. Box 30197, Nairobi, Kenya.
[1]Department of Botany, University of Nairobi, P. O. Box 30197, Nairobi, Kenya.

**Abstract** Antibiotic culture filtrates produced by *Bacillus* (CA5) and *Streptomyces* spp. were tested for translocation and persistence when applied on snap beans inoculated with rust (*Uromyces appendiculatus*). The antibiotics were applied on the first trifoliolate leaves and translocation was assessed as the number of rust pustules on non-treated leaflets or trifoliolates, while persistence was assessed as the number of rust pustules on rust infected plants at different times after antibiotic treatment. The treatments were replicated three times, each replicate consisting of pots containing three plants. Antibiotics from both *Bacillus* and *Streptomyces* were found to have up to 100% trans-lamina and leaflet-to-leaflet translocation but no significant trifoliate-to-trifoliate translocation. Moreover, the antibiotic culture filtrates controlled rust infection for up to 10 days after application on the bean plant. However, no rust control was found on the plants after 16 days. The study indicated that the antibiotics produced by antagonistic *Bacillus* and *Streptomyces* species possess systemic activity that can persist for slightly over one week. These metabolites are potential bean rust control products and could be incorporated in integrated disease management programs for high value horticultural crops.

Key words: Antibiotics, Rust, Translocation, Persistence, Snap bean.

## Introduction

Snap bean is an important crop in Kenya; it has contributed about 62% of the total volume of vegetables exported from Kenya from 1996 to 2000. The volume of snap beans exported from Kenya rose from 58% in 1996 to 67% in 2000 (HCDA, 1999). Bean rust (*Uromyces appendiculatus*) is a common and serious disease of beans with a worldwide distribution (Robert, 1991). Yield losses ranging 25 - 100% depending on stage of infection and the prevailing weather conditions, have been reported (Venette & Jones, 1982; Stavely, 1984; Baker *et al*., 1985; Robert, 1991; Mwangi, 1998; Schwartz *et al*., 2001 ). Severe rust infection results in defoliation, stunted growth leading to reduced yields while infected pods are of low market value (Venette & Lamey, 1998; Partridge, 1997). Although chlorothalonil fungicides have been effective in the control of bean rust, various problems have arisen including; pesticide residues on produce, environmental pollution, development of new physiological races and the prohibitive cost (Jassen *et al*., 1992; Ken *et al*., 1987). Additionally, they have negative effects on human health (Jassen *et al*., 1992) and kill antagonists (Juan and Pedro, 1992). In Kenya, the problem of pesticide residues is very important because the main European markets are increasingly becoming intolerant to residues in the horticultural produce (Mulandi, 1998). Biological control using antagonistic microrganism is an alternative option. However, absorption and translocation of antibiotics are markedly affected by the nature of both the antibiotics and the plant tested. The selective toxicity of antibiotics is exploited in full only when they act systemically (Miller, 2001). Some antibiotics will penetrate the plant but fail to be translocated; such antibiotics will not act systemically or behave as surface protectants but assume an intermediate position (Dernoeden, 2002). This study was carried out to investigate the possibility of translocation and level of persistence of antibiotics from *Bacillus* and *Streptomyces* species in snap beans towards the control of bean rust under greenhouse conditions.

## Materials and methods

**Production and assay of antibiotics in liquid culture filtrates**
*Bacillus* sp. (CA5) and *Streptomyces* sp. (CS35) were cultured on nutrient agar (NA) and Czapexs Dox agar, respectively, to produce inocula for liquid fermentation cultures. The test culture filtrates were produced by growing *Bacillus* isolate CA5 in Tschen's liquid medium consisting of glucose 15g, glycerol 15mls, soybean meal 15g, $NH_4SO_4$

5g, yeast extract 1g, Nacl 5g, $Caco_3$ 5g, distilled water 1000ml, pH 7.5 (Tschen & Kou, 1984) and *Streptomyces* isolate CS35 in glucose-soyabean medium containing 30g soybean meal, 20g commercial glucose, and 1000ml of distilled water (Loeffler *et al*., 1986). The cultures were incubated at room temparature on a circulatory shaker at 125 rpm for 7 days.

Culture filtrates were harvested by centrifugation and concentrated to 30% by evaporation of water under vaccum using a rotary evaporator. The filtrates were assayed for antibiotic activity using the paper disc method (Abdel & Sinclair, 1984). *Fusarium oxysporum* and *Pythium* sp. were the test pathogens for filtrates from CA5 and CS35, respectively. Sterilized nutrient agar and potato dextrose agar cooled to $40^{O}$ C seeded with 10% (v/v) $10^6$ *Fusarium* and *Pythium* $10^5$ propagules/ml, respectively were dispensed in sterile petri-dishes and allowed to cool. Sterile filter paper discs measuring 9mm diameter dipped in the culture filtrates were placed at equidistant points on the solidified seeded media. Antibiotic activity was determined by the diameter of clear zones of inhibition formed around the paper discs after 48 hours of incubation at room temparature.

**Determination of translocation and persistence of antibiotic activity**

The experiments were carried out in the greenhouse using snap bean variety Samantha grown on 2:1:1 (v/v) soil; sand; manure potting medium. Three weeks after germination, the plants were treated with antibiotic culture filtrates from *Bacillus* (CA5) and *Streptomyces* (CS35). For persistence nine plants per treatment were sprayed and followed for data collection. For translocation, there were six plants per treatment; for each, the leaflets or trifoliates were dipped in the culture filtrates. Plants were inoculated with urediospore suspension at a concentration of $2x10^6$ spores/ml containing a few drops of tween 80. Inoculation was done two days after application of culture filtrates for the translocation experiments and at 0, 4, 8, 12 and 16 days for the persistence experiments. The experiment was laid out in a completely randomized design (CRD) with 3 replications each containing 3 plants.

Three aspects of translocation were investigated (i) trans-lamina where the filtrate was applied on the upper surface of the first trifoliate leaf and rust infection observed on the lower surface (ii) leaflet-to-leaflet where filtrate was applied on the first leaflet of the first trifoliate and infection observed on the other leaflets of the same trifoliate (iii) trifoliate-to-trifoliate where filtrate was applied on the first trifoliate and infection observed on the other trifoliate leaves. Persistence of antibiotic activity was determined by assessing rust severity on plants inoculated at 4 days interval upto $16^{th}$ day after application of the culture filtrates. Disease assessment started with the first appearance of rust symptoms and was based on severity. Three uniform sized plants per pot were tagged and the number of rust pustules per leaf counted every other day until 24 and 28 days after inoculation for translocation and persistence experiments, respectively. Data was analysed using Genstat$^R$ 3.0 statistical software.

## Results

**In vitro antibiotic activity of culture filtrates**

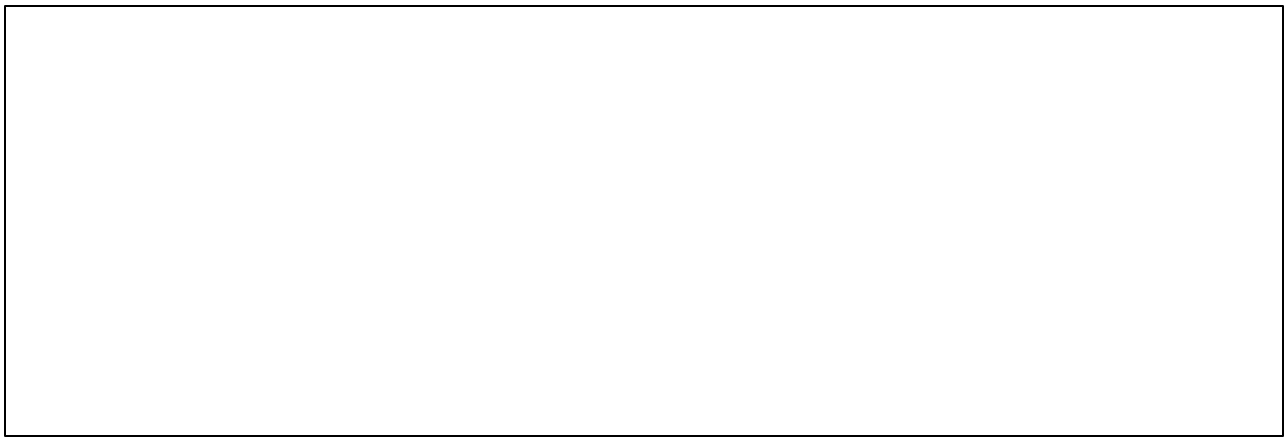
Both culture filtrates from *Bacillus* (CA5) and *Streptomyces* (CS35) inhibited the growth of *Fusarium oxysporum* and *Pythium* spp in culture. Culture filtrate CA5 gave the largest zones of inhibition of mean diameter 14.1mm on *Fusarium oxysporum*, whereas CS35 produced the largest zones of mean diameter 15.5mm on *Pythium* spp. (Table 1). Combining the culture filtrates of *Bacillus* sp. and *Streptomyces* sp. (CA5 + CS35) did not result in increased antibiotic activity, and gave similar diameters inihibition levels.

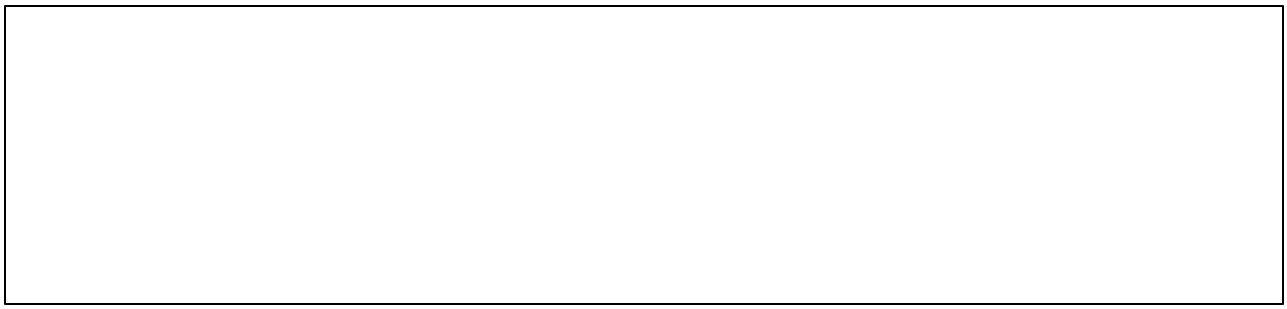
**Translocation and persistence of antibiotic activity**

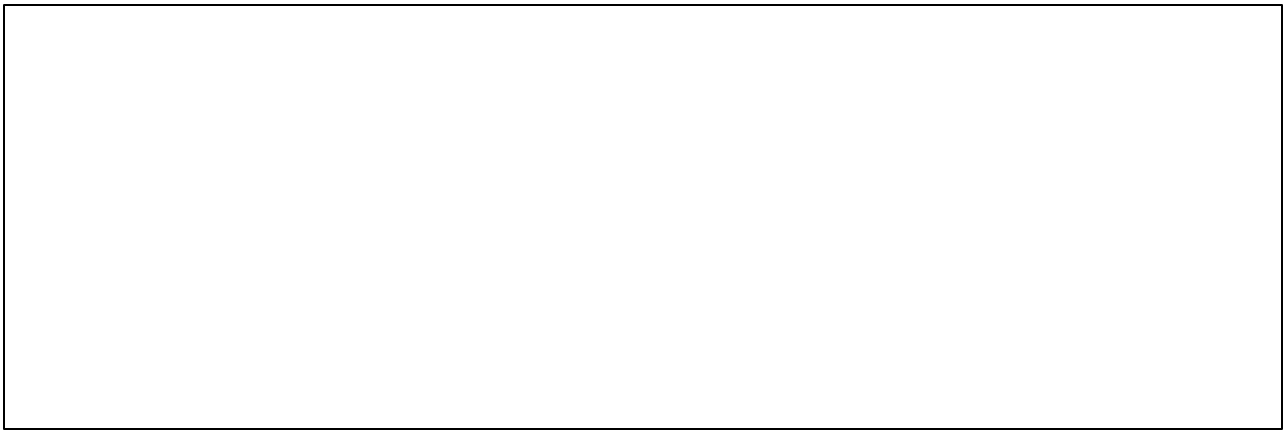
The antibiotics produced by *Bacillus* CA5 and *Streptomyces* CS35 were translocated from upper to lower leaf surface and within leaflets of the same trifoliate leaf. Rust infection was completely inhibited on the treated leaves (Table 2). No translocation was observed from one trifoliate leaf to other leaves within the plant. However, the treated leaves exhibited some phytotoxicity, which was expressed as thickening, scotching and early senescence of the leaves. No antibiotic activity was observed when inoculation was done 16 days after application of both culture filtrates (Table 3). Culture filtrates CA5 and CS35 gave an overall reduction in rust severity of 47.2% and 27.8%, respectively.

## Discussion

The results of this study showed that culture filtrates of *Bacillus* and *Streptomyces* possess antibiotic activity against different fungal pathogen. The filtrates inhibited the growth of *Fusarium oxysporum* and *Pythium in vitro* and bean rust on inoculated plants. The effectiveness of antibiotics produced by a given antagonist depends on the test pathogen and the type of antibiotic produced (Muiru, 2000; Yeo and Hol, 1997; Muthomi, 1992; Campbell, 1989; Loefler *et al*., 1986). The antibiotics produced by *Bacillus* CA5 and *Streptomyces* CS35 possess limited systemic action as indicated by their limited translocation within the treated bean leaves. Lack of significant upward translocation could be attributed to dilution effect of weakly active compounds (Dernoeden, 2002). Its therefore necessary that efforts be made to increase the antibiotic efficacy. Elsewhere,







absorption and translocation of antibiotics can be enhanced by dissolving the active compounds in free oil (Moss, 1989). The antibiotic produced by *Bacillus* CA5 was more persistent than that of *Streptomyces* CS35. Persistence of antibiotic activity of commercial formulations of antagonistic bacteria has been reported elsewhere (Kiewniek and Jacobsen, 1998). The systemic action and persistence of the antibiotics is of great significance in plant disease control. This is because the active metabolites get absorbed into the plant cells where they are retained and protect the plant from new infections for about 10 days. Indeed, prolonged persistence of chemicals on food crops is not recommended due to current international regulations on maximum residue levels and pre-harvest interval of treated produce (Wandiembe & Adipala, 2001; Mulandi, 1998; Watson & Koon, 1997). Therefore, antibiotics that are highly effective and of limited resudual effect, can be useful disease control options in the horticulral industry.

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