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Isolation and characterization of an antibiotic produced by the scab disease-suppressive *Streptomyces diastatochromogenes* strain PonSSII

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An antibiotic produced by the scab disease-suppressive *Streptomyces diastatochromogenes* strain PonSSII has been isolated and partially characterized. The antibiotic is produced throughout culture growth, with maximum amounts accumulating in the broth when the culture is in the early stationary phase of growth. The activity declines within about 30 h after the culture enters stationary phase. Purification techniques included chromatography on Amberlite XAD-2, DEAE Sephadex and SP Sephadex in addition to C18 HPLC with an average yield of 75%. This antibiotic only inhibits pathogenic strains of *S. scabies* that cause scab disease on potato and other tuberous vegetables and does not affect *S. griseus, S. venezuelae, Actinomyces bovis, Nocardia asteroides, Clostridium perfringens, Bacillus subtilis, Staphylococcus aureus, S. epidermidis, Enterococcus faecalis, Micrococcus luteus, Serratia marcescens* and *Escherichia coli*. The antibiotic has a molecular weight of 500 or less, and is stable for weeks at acidic pH but is very labile at alkaline pH conditions.

Keywords: antibiotics; biological control; scab disease; suppression; *Streptomyces scabies*

Introduction

Streptomycetes are Gram-positive, filamentous bacteria, the majority of which are soil saprophytes. *Streptomyces scabies* is one of the few pathogens in this genus, and is the major cause of potato scab disease [3,11,13,19,30], even though other species of *Streptomyces* have been identified that also cause scab [6,8,12,18,19,26]. This disease is characterized by the presence of lesions on the surface of the potato tuber [1,14]. The lesions can appear as regions of raised, corky material on the surface of the tuber or as deep pits into the potato tuber [3,13,31]. Although scab disease has not been shown to affect crop yield, the disease will drastically affect the marketability of the crop [13]. For this reason, potato scab disease is a major problem for potato growers worldwide [33].

The most common method of control for this disease is the use of resistant potato varieties when planting into scab-conducive soil. Recently, however, there has been much interest in developing a biological control approach for this disease. Suppressive strains of *Streptomyces* have been isolated that are capable of significantly reducing the level of scab disease when added to scab-conducive soil at planting time [21,22]. These suppressive isolates have been found in soils which had been cropped to potatoes for over 20 successive seasons and had subsequently become naturally suppressive to scab disease [20,25,28]. All of the suppressive strains isolated so far belong to the genus *Streptomyces* and are non-pathogenic. *In vitro* assays demonstrate that these isolates produce antibiotics which inhibit pathogenic strains of *S. scabies* [20,22,24,26].

Organisms belonging to the genus *Streptomyces* are prolific producers of a variety of antibiotics with diverse structures such as polyketides, β-lactams and peptides in addition to a variety of other secondary metabolites that have anti-fungal, anti-tumor and immunosuppressive activities [5]. Streptomycetes are responsible for the production of over 70% of the known antibiotics, and most of these antibiotics have been isolated and characterized using a variety of techniques dependent on the chemical properties of the compound. The antibiotics produced by the suppressive streptomycete isolates may play an important role in scab disease inhibition that has occurred naturally in the disease-suppressive soils. Antibiosis as a mechanism of biological control of plant disease has been studied in other systems [4,17,27,32,34]. Characterizing the nature and diversity of the streptomycete antibiotics will be critical to a complete understanding of the mechanism of biological control of scab disease [2,9]. With this in mind, we have partially purified an antibiotic produced by *S. diastatochromogenes* strain PonSSII [7]. This strain can inhibit the pathogenic *S. scabies* strain RB4 in *in vitro* antibiotic assays [26]. In addition, strain PonSSII has been shown to significantly reduce potato scab disease in greenhouse and field studies [21]. Information concerning the structure and properties of this antibiotic may suggest its mode of action against the *S. scabies* pathogen. Additionally, the availability of purified antibiotics for application to scab-conducive soil may prove to be an effective way to potentially augment the activity of the biocontrol organisms.

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Materials and methods

Strains and growth conditions

S. diastatochromogenes strain PonSSII was isolated from the lenticel of a potato tuber grown in scab-suppressive soil in Grand Rapids, MN, USA [26]. *S. scabies* strain RB4 was isolated from a scab lesion on a tuber grown in Becker, MN, USA [26]. To produce spores for the storage of streptomycete strains, cultures were grown for a week at 30°C on oatmeal agar medium (OM) [26]. The spores were suspended in water, filtered through cotton, centrifuged, and resuspended in 20% glycerol for storage at –20°C [15].

For the production of antibiotic, *S. diastatochromogenes* strain PonSSII was grown in a modified version of minimal liquid medium (NMM) containing 15 mM $(NH_4)_2SO_4$, 2 mM $M_gSO_4 \cdot 7H_2O$, 15 mM NaH_2PO_4/K_2HPO_4 pH 6.8, 3.5 μM $ZnSO_4 \cdot 7H_2O$, 3.6 μM $FeSO_4 \cdot 7H_2O$, 5.1 μM $MnCl_2 \cdot 4H_2O$, 9 μM $CaCl_2$, 0.5% Casamino acids (Difco, Detroit, MI, USA), and 1% (w/v) glucose [15]. Cultures were grown in 200 ml of NMM medium in 2-L flasks fitted with stainless steel springs and milk filter caps (Kendal Agricultural Products, Ashland, OH, USA) to improve aeration. Each flask was inoculated with spores from half a plate of OM medium. The flasks were placed in an air shaker at 30°C and agitated at 250 rpm. Growth of the culture was monitored by absorbance at 650 nm.

Bioassay for antibiotic activity

Samples of culture filtrate and column effluent were tested for antibiotic activity by spotting material onto filter paper disks (6.5 mm, Schleicher & Schuell, Keene, NH, USA). These disks were allowed to dry before they were placed on petri plates containing 25 ml of OM medium which had been freshly seeded with the test strain. The plates were incubated at 30°C for 3 days before measuring the diameter of the zone of growth inhibition.

Column chromatography

Several chromatographic reagents were used in the purification of the antibiotic produced by *S. diastatochromogenes* strain PonSSII. The first step used Amberlite XAD-2 (Mallinckrodt, Phillipsburg, NJ, USA) resin. Approximately 500 ml of this material were packed into a 3×150 cm column and equilibrated with distilled water before the sample was added. The column was washed with water, and the antibiotic was eluted with increasing concentrations of methanol (MeOH). The second step utilized DEAE Sephadex A-50 (Sigma) beads that were washed with 1 M KH_2PO_4 to exchange ions and then equilibrated with 10 mM KH_2PO_4. Approximately 60 ml of beads were packed into a 2.5×50 cm column. Most of the antibiotic applied to this column was eluted with a 10 mM KH_2PO_4 wash; additional activity was not eluted by washing the column with 1 M KH_2PO_4. The third step used SP Sephadex C-25 (Sigma, St Louis, MO, USA) beads. These were equilibrated with 10 mM KH_2PO_4 after rehydration in water and washing with 0.2 M HCl to exchange ions. Approximately 50 ml of beads were packed into a 2.5×50 cm column. After applying the antibiotic, the column was washed initially with 10 mM KH_2PO_4 (pH 4.4) and then with a gradient of 10–100 mM KH_2PO_4 to elute the activity.

For HPLC analyses, a 5 μ (4.6 $\times$ 250 mm) Alltima C18 HPLC column (Alltech, Deerfield, IL, USA) or a 10 μ (4.6 $\times$ 250 mm) Alltech Econosil C18 HPLC were used in an HP1090 HPLC machine (Hewlett Packard, Palo Alto, CA, USA). The mobile phase for the first run was a gradient from 0–100% MeOH in 10 mM KH_2PO_4 (pH 4.4) over 60 min. The antibiotic eluted from the column when the gradient reached approximately 30% MeOH. The second and third runs used an isocratic mobile phase of 65% 10 mM KH_2PO_4, 35% MeOH. The antibiotic eluted from the column at 12–15 min. In all three runs the flow rate was set to 1 ml min^{-1}, and fractions were collected at 30-s intervals.

Thin layer chromatography techniques

Attempts were made to purify the compound using thin layer chromatography (TLC) using silica gel plates (K6F, Whatman, Clifton, NJ, USA), C18 reversed phase plates (Whatman $KC_{18}F$), and cellulose TLC plates (Whatman K2F). To localize the active material on the TLC plate, the whole plate was bioassayed by placing it in a sterile glass tray, covering it with a layer of OM agar medium, and inoculating the medium with a lawn of *S. scabies* strain RB4. To recover material from the TLC plates, bands potentially containing the active compound were scraped from the plates using a razor blade, the material was packed into a pasteur pipette, and the compound was eluted through glass wool. For C18 reversed phase TLC, the compound could be eluted from the chromatogram with a mixture of 60% MeOH, 40% 10 mM KH_2PO_4. For cellulose TLC, the compound could be eluted with a mixture of 70% MeOH, 30% 10 mM KH_2PO_4.

pH stability assays

To test the stability of the antibiotic produced by *S. diastatochromogenes* strain PonSSII at different pH conditions, material which had been partially purified on an XAD-2 column was resuspended in 100 mM potassium phosphate buffer at pH values of 4.4, 7.0, and 9.6. The samples were left at room temperature for several weeks, and were bioassayed against *S. scabies* strain RB4 at regular intervals.

Ultrafiltration

Diaflo Ultrafilters (Amicon, Beverly, MA, USA) were prepared and used according to the manufacturer's instructions. YM3 and YC05 filters were chosen for these experiments to determine the approximate size of the compound produced by *S. diastatochromogenes* strain PonSSII. The YM3 filter has a molecular weight cut-off of about 3000, and the YC05 filter excludes compounds that are larger than 500 Da.

Results and discussion

Production of the antibiotic by S. diastatochromogenes strain PonSSII

S. diastatochromogenes strain PonSSII produces an antibiotic capable of inhibiting *S. scabies* strain RB4 in NMM liquid medium. This antibiotic is produced during exponential growth, with the peak of activity coinciding

with the onset of stationary phase (Figure 1). This pattern of production was very reproducible, although the growth rate of the culture as it approached stationary phase could vary, with stationary phase beginning anywhere from 30 h to over 40 h after inoculation. The antibiotic consistently maintained activity during at least the first 10 h of stationary phase, but then slowly declined during the next 10–20 h of stationary phase. There was typically at least 80% loss of antibiotic activity within 30 h from the beginning of the culture's stationary phase. This loss in activity is most likely due to degradation of the antibiotic by the organism, since the active material appears to be relatively stable in isolated culture filtrates. Since the antibiotic activity is sensitive to pH (see below), loss of activity could also be due to an increase in the pH of the medium that may occur during prolonged stationary phase.

It was noted that cultures grown in medium made with tap water were about 30% more dense and produced about twice as much antibiotic as cultures grown in medium made with distilled water. Element mixtures containing zinc, iron, manganese and calcium (NMM medium) or in addition copper, boron, and molybdenum (R2 trace elements; 15) had no effect on the growth or antibiotic production of the culture (Figure 1). These elements were therefore not limiting in the medium made with distilled water, and suggest that there must be some other factor in the tap water which accounts for the increased growth and antibiotic production.

The volume of the culture was also important in achieving maximum antibiotic production. A culture volume that was 20% of the flask volume produced only about half as much antibiotic per ml as one which used 10% of the flask volume. Even though cultures were always grown with milk filter caps and springs in the flasks to improve aeration, the culture volume relative to the flask size was presumably an important factor in maintaining good aeration and ultimately for maximizing antibiotic production.

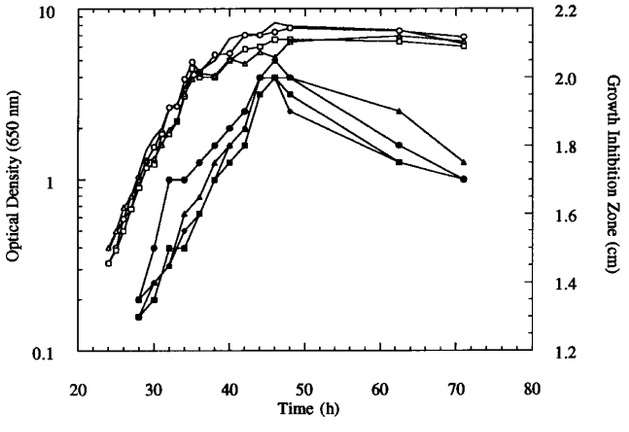

Figure 1 Growth and antibiotic production of *S. diastatochromogenes* strain PonSSII. An NMM liquid medium culture was grown in a rotary shaker (250 rpm) at 30°C. The optical density (open symbols) was monitored at 650 nm. Antibiotic production (filled symbols) was measured by bioassaying 20 μl of aqueous culture supernatant which had been extracted with chloroform. The size of the zone of inhibition against *S. scabies* strain RB4 was recorded. ●, ■: NMM medium; ◆, ▲: NMM medium plus R2 trace elements (15).

The bioassay for antibiotic activity

The sensitivity of the antibiotic bioassay was assessed by testing different amounts of the antibiotic from *S. diastatochromogenes* strain PonSSII for the size of growth inhibition zones against a sensitive test strain. Approximately 1 ml of a *S. diastatochromogenes* strain PonSSII culture was placed in a microfuge tube, mixed with 500 μl chloroform, vortexed, and incubated at room temperature for 1 h. Since the active material could not be extracted with organic solvents such as chloroform, ethyl acetate or butanol, the aqueous phase was then tested for antibiotic activity against *S. scabies* strain RB4. The samples were assayed directly, concentrated 10-fold by lyophilization, or diluted 10-fold.

When the zone diameter was plotted against the relative antibiotic concentration, a log-linear relationship was observed (Figure 2). The diameter of the growth inhibition zone increased linearly as the log of relative antibiotic concentration increased, indicating that a small difference in zone size between two samples could actually be due to significantly large differences in the amount of antibiotic tested. The equation that describes this log-linear relationship was used to quantify the antibiotic throughout the purification process: $y = (9.4 \times 10^{-4})(10^{1.7x})$.

Antibiotic purification

To purify the antibiotic produced by *S. diastatochromogenes* strain PonSSII, NMM cultures were grown to stationary phase and harvested. The culture broth was centrifuged, filtered through Whatman No. 1 filter paper, and added to a column of Amberlite XAD-2 resin. Amberlite XAD-2 resin effectively bound all of the active material present in the culture filtrate, and no detectable activity was found in the initial material which came through the column, or in the water and 20% MeOH washes

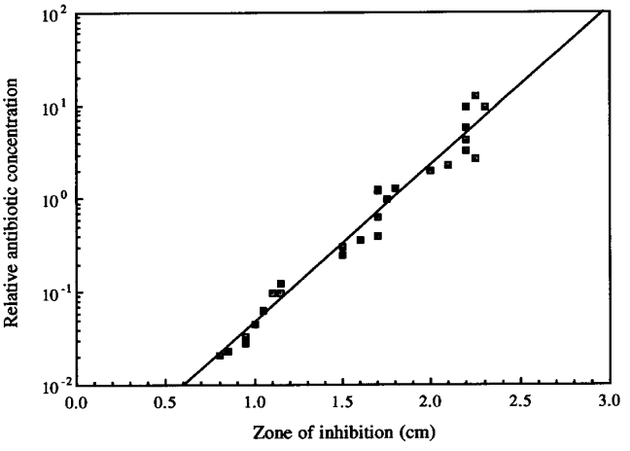

Figure 2 Size of growth inhibition zone as a function of relative antibiotic concentration. Culture filtrate from *S. diastatochromogenes* strain PonSSII was placed on a filter paper disc either directly, after 10-fold concentration, or after 10-fold dilution. The discs were placed on a freshly spread lawn of *S. scabies* strain RB4 on oatmeal agar, and the diameter of the resulting zones of growth inhibition was measured. The relative amount of antibiotic in each dilution was plotted against the resulting zone of inhibition. The results are from nine separate culture filtrate samples. The equation that describes this log-linear relationship is $y = (9.4 \times 10^{-4})(10^{1.7x})$ with an r^2 value of 0.97.

of the column. The antibiotic was eluted from the column with MeOH concentrations greater than 40%. Recovery from the XAD-2 column was nearly 100% in most cases. Fractions containing active material were dried in a rotary evaporator, resuspended in methanol, and dried again. Dried samples were stored at –20°C.

Active fractions from the XAD-2 column were resuspended in 10 mM KH_2PO_4 (pH 4.4) and applied to a column of DEAE Sephadex A-50. Under these conditions, DEAE Sephadex A-50 did not bind the antibiotic produced by *S. diastatochromogenes* strain PonSSII. Although the active material did not bind to the column, many of the visible contaminants did, leaving the column material darkly stained. This technique was used as a simple procedure to further purify the sample. Recovery of active material from the Sephadex A-50 column was about 79%.

Active material which was eluted from the DEAE Sephadex A-50 column was added directly to an SP Sephadex C-25 column. The antibiotic activity bound to the column in 10 mM KH_2PO_4, and most of the activity eluted from the column at 30 mM KH_2PO_4. Column fractions were stored at 4°C until results from a bioassay were obtained. Fractions containing the active material were pooled and dried, resuspended in MeOH, filtered through glass wool, dried, and stored at –20°C. Recovery was approximately 100% in this step.

The final purification step consisted of three passes through an Alltech Alltima C18 reversed phase HPLC column. About 50 peaks of material that absorbed at 210 nm could be observed in the chromatogram of the first column. The chromatogram of the third run showed at least five compounds that were detected at 210 nm which had copurified with the active material (data not shown). The samples were stored at –20°C until results from a bioassay were completed, and active fractions were pooled and dried. For each pass through the HPLC column, about 92% of the initial activity was recovered.

Additional purification methods

A number of TLC techniques were used in an attempt to improve the antibiotic purification protocol. On silica gel TLC plates, the active compound did not migrate from the origin using various mixtures of chloroform and MeOH in the solvent system. Recovery of active material from the origin was very inefficient (less than 10%). With C18 reversed phase TLC plates, the antibiotic migrated with an R_f of about 0.55 in a mobile phase of 60% 0.3 M NaCl, 40% MeOH, and only about 40% of the starting active material could be recovered. On cellulose TLC plates, the antibiotic migrated with an R_f of about 0.68 in a mobile phase of 50% chloroform, 40% MeOH, 10% water. Recovery of active material was about 40% of what was initially loaded onto the plate.

For all of these TLC techniques, the recovery of active material was too low to be useful for purification purposes. In addition, R_f values were not consistent from run to run, and the region of activity was often spread over 10% of the plate. Active material was not associated with a visible band on the TLC plate, nor could it be observed under UV light. Whether this was due to insufficient quantities or to the properties of the compound is unknown.

To counter these problems with TLC, an HPLC method was developed. Initially, an Alltech Econosil C18 reversed phase column (10 μ, 4.6 × 250 mm) was chosen. Using a gradient from 0–100% MeOH over 60 min, the compound eluted at 13–14 min, with a recovery of about 50% of the initial activity. To improve the retention time and recovery, 0.05% triethylamine was added to the solvent mixture. Phosphoric acid (0.02%) was also added to maintain a pH between 3.5 and 4.0. Using this system, active material eluted at about 8 min, with over 80% recovery. The Alltech Alltima C18 column that was eventually used in the purification scheme (Table 1) did not require the addition of triethylamine to obtain good recovery of active material.

The improvement in retention and recovery offered by the addition of triethylamine on C18 reversed phase HPLC may indicate the presence of amino groups on the antibiotic molecule. The C18 medium consists of a silica gel backbone bonded with octadecyl residues. Due to steric hindrances among these octadecylsilanes, there can be regions into which the sample molecules can penetrate and react with the silanol groups [10]. Triethylamine that is added to the mobile phase can also react with these sites and prevent potential interactions between the silanol groups and the sample molecules. Because amino groups react strongly with these silanol groups, it is possible that the antibiotic produced by *S. diastatochromogenes* strain PonSSII possesses at least one amino group. This would account for the poor retention time and recovery on HPLC using the C18 reversed phase column without triethylamine, as well as the wide bands of activity and poor recovery on TLC.

Table 1 lists the steps taken to purify the antibiotic produced by *S. diastatochromogenes* strain PonSSII and average recoveries for each step. In at least three separate purification trials, approximately 75% of the original starting activity was recovered. Although sufficient quantitites of purified antibiotic produced by *S. diastatochromogenes* strain PonSSII were not obtained to use for structural determination of the compound, a purification procedure has been developed which should eventually lead to the isolation of milligram quantities of purified antibiotic.

pH stability of the antibiotic

At pH 4.4, the antibiotic produced by *S. diastatochromogenes* strain PonSSII is quite stable, maintaining 100%

Table 1 Purification scheme for the antibiotic produced by *S. diastatochromogenes* strain PonSSII

Procedure	Recovery (%)[a]	Remaining (%)[b]
Amberlite XAD-2	115 ± 31	115
DEAE Sephadex A-50	79 ± 34	91
SP Sephadex C-25	105 ± 31	96
C18 HPLC – run No. 1	92 ± 18	88
C18 HPLC – run No. 2	92 ± 18	81
C18 HPLC – run No. 3	92 ± 18	75

[a]The percent recovery of antibiotic from each purification step was determined by a bioassay with *S. scabies* strain RB4. The average of at least three separate purification trials is shown with the standard deviation.
[b]100% is defined as the amount of antibiotic activity estimated to be in the original culture filtrate. The percent remaining was calculated from the average % recovery at each step of the purification.

activity for several weeks at room temperature. At pH 7.0, the compound retains activity for about a week, while at pH 9.6, there is no detectable activity after 2 h (Figure 3). Based on these results, the compound was resuspended in 10 mM KH_2PO_4 (pH 4.4) for all purification steps following the XAD-2 column.

At low pH, a molecule containing amino groups will be positively charged. In 10 mM KH_2PO_4 (pH 4.4), this compound did not bind to an anion exchange column (Sephadex A-50) but did bind to a cation exchange column (Sephadex C-25). These results are consistent with the presence of an amino group in this antibiotic, as also suggested by the HPLC column elution conditions discussed above. The instability of this compound under alkaline conditions was unfortunate as it might have allowed for an organic extraction of the antibiotic if it could have been maintained as an uncharged molecule.

The pH instability of this compound may have some implications for the effectiveness of *S. diastatochromogenes* strain PonSSII as a biocontrol agent. If the antibiotic plays an important role in suppressing the growth of the pathogen, strain PonSSII may not effectively control the disease in soils at neutral or alkaline pH. Additionally, if this antibiotic is used as a supplement to the application of a biocontrol agent by adding it directly to the soil, care must be taken to ensure that the pH of the carrier material or the soil is compatible with maintaining the stability of this compound.

Molecular weight estimation

Antibiotic activity which had been partially purified by HPLC was passed through Diaflo Ultrafilters in an attempt to determine the approximate size of this compound. The antibiotic was able to pass through both the YM3 (3000 Da cut-off) and the YC05 (500 Da cut-off) filters. These results suggest that the antibiotic produced by *S. diastatochromogenes* strain PonSSII has a molecular weight of about 500 or less.

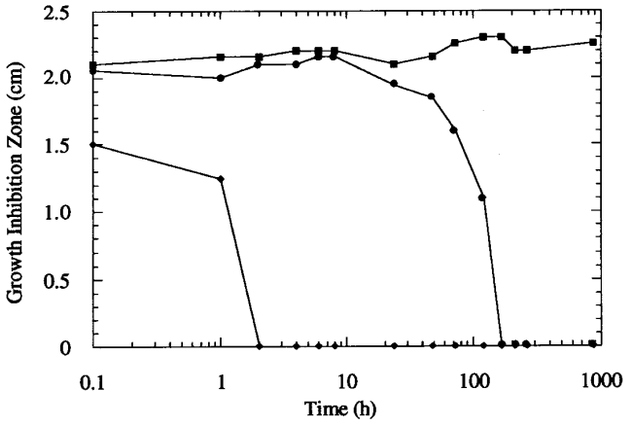

Figure 3 pH stability of the antibiotic isolated from *S. diastatochromogenes* strain PonSSII. Active material that had been partially purified on Amberlite XAD-2 was resuspended in 100 mM phosphate buffer at pH 4.4 (■), pH 7.0 (●), and pH 9.6 (◆). This material was left at room temperature and bioassayed against *S. scabies* strain RB4 over a 5-week period. The results shown are from one experiment. A separate experiment gave nearly identical results.

Use of strain PonSSII as a biocontrol agent

Considering the approximate size of this antibiotic and potential presence of an amino group, the antibiotic produced by strain PonSSII is similar to other known antibiotics produced by streptomycetes such as tetracycline [16] and the aminoglycosides, including streptomycin [35]. The PonSSII antibiotic, however, is quite different in its profile of susceptible target organisms. The antibiotic was tested against a variety of microorganisms, including *S. griseus*, *S. venezuelae*, *Actinomyces bovis*, *Nocardia asteroides*, *Clostridium perfringens*, *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *Micrococcus luteus*, *Serratia marcescens* and *Escherichia coli*. The only organisms that were sensitive to this compound were strains of *S. scabies*. This narrow pathogen inhibition profile strongly suggests that the PonSSII antibiotic has a unique structure compared to these other known antibiotics.

The narrow range of organisms susceptible to the PonSSII antibiotic could have implications for the use of this strain as a biocontrol agent, as well as insight into the evolution of this strain in a field which became suppressive to potato scab disease. If strain PonSSII is only able to inhibit *S. scabies*, its only use as a biocontrol agent will be to control potato scab disease. Other streptomycete strains that are capable of inhibiting other potato pathogens such as *Verticillium dahliae* (wilt), *Rhizoctonia solani* (stem canker), and *Helminthosporium solani* (silver scurf), in *in vitro* antibiotic assays, have been isolated [20] and may be able to control these potato diseases when they are inoculated into a potato field that is infested with these pathogens.

Overall, a number of suppressive streptomycetes have been identified that can be divided into several groups based on their pathogen inhibition profiles [20,29]. Some organisms inhibit a very narrow range of pathogens that include only *S. scabies* strains (such as *S. diastatochromogenes* strain PonSSII), whereas other isolates inhibit a broad range of fungal pathogens in addition to *S. scabies*. The suppressive strains that have a broad range of pathogen targets could be beneficial as biocontrol organisms if they are able to suppress not only diseases of potato but also diseases that affect other crops such as corn or soybean. The suppressive strains that inhibit a variety of fungal pathogens in addition to *S. scabies* may produce multiple antibiotics, each of which inhibit a subset of the target pathogens, or perhaps they produce one antibiotic with a mode of action that inhibits both prokaryotic and eukaryotic pathogens.

The suppressive strains, like *S. diastatochromogenes* strain PonSSII, that specifically inhibit the *S. scabies* pathogens may also have unique benefits as biocontrol organisms. With the production of fewer antibiotics or antibiotics that have a narrow range of susceptible targets, there may be a smaller chance of the suppressive strain inhibiting beneficial microorganisms such as *Rhizobium* and other soil saprophytes. As potato fields become suppressive to scab disease, the *S. scabies* pathogens are presumably eliminated from occupying the site of infection, which is the lenticel of the potato tuber. The disease-suppressive *S. diastatochromogenes* strain PonSSII was originally iso-

lated from a potato lenticel [26]. To control this site, the suppressive strain would either need to outcompete *S. scabies* for the limiting availale resources, or be able to directly inhibit the pathogen [23]. If inhibition is an important part of the suppressive mechanism, and the antibiotic produced by *S. diastatochromogenes* strain PonSSII plays a critical role in inhibition, then there may be no advantage to producing multiple antibiotics or an antibiotic which inhibits organisms other than *S. scabies*. The *S. scabies* pathogen may be the only organism with which the suppressive strain would compete for colonization of the lenticel.

The use of suppressive isolates of *Streptomyces* could be a simple, effective, and long-lasting solution to the problem of potato scab disease [23]. Because these isolates produce compounds which can inhibit pathogens *in vitro*, it is important to understand the nature of these antibiotic compounds. With this knowledge we will be able to further investigate the role of these compounds in the process of biological control.

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