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PLANT MICROBE INTERACTIONS

Multiple, Novel Biologically Active Endophytic *Actinomycetes* Isolated from Upper Amazonian Rainforests

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Abstract Microbial biodiversity provides an increasingly important source of medically and industrially useful compounds. We have isolated 14 actinomycete species from a collection of approximately 300 plant stem samples from the upper Amazonian rainforest in Peru. All of the cultured isolates produce substances with inhibitory activity directed at a range of potential fungal and bacterial pathogens. For some organisms, this activity is very broad in spectrum while other organisms show specific activity against a limited number of organisms. Two of these organisms preferentially inhibit bacterial test organisms over eukaryotic organisms. rDNA sequence analysis indicates that these organisms are not equivalent to any other cultured deposits in GenBank. Our results provide evidence of the untapped biodiversity in the form of biologically active microbes present within the tissues of higher plants.

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Introduction

Virtually all plants possess a microbiota. Many microbes colonize the surface features of plants and survive as epiphytes. Another group of microbial colonizers are endophytes which take up residence in the inner tissues of plants and, by definition, cause no apparent damage to the tissues that they inhabit [4, 28, 32]. The simplest biological arrangement between these organisms is that the plant provides nutrition for the microbe and the microbe provides some form of protection for the plant [4, 15, 32]. Within the last decade, it has become clear that there is enormous microbiological diversity residing within the tissues of plants [2, 13, 15, 18, 24, 27]. The ecological role of the endophyte to the plant remains uncertain, although there are indications that certain endophytes confer heat tolerance [23, 25], salt tolerance [25], and protection to the plant from invading fungal pathogens [3].

The vast majority of microbes isolated as endophytes are fungi [4]. Only recently have biologically active endophytic actinomycetes been isolated and characterized (for example, [6–12, 14, 16, 26, 29, 31, 33, 34]). As a group, the actinomycetes provide nearly 80% of all of the world's antibiotics [4], though in all cases, organisms isolated from the soil were the source. The advent of drug-resistant bacterial pathogens and the increase in fungal infections has caused a resurgence of interest in finding other reserves of biologically active compounds from the streptomycetes [5, 21]. One biologically important niche that has been

overlooked as a source of novel streptomycetes is that endophytes may exist in various ecosystems around the world. This appears to be a reasonable source for new antibiotics given that endophytic streptomycetes are associated with a eukaryotic organism and may be less likely to produce compounds toxic to the eukaryotic host. Thus, one of the major concerns in drug discovery, relating to the toxicity of a drug candidate, may be reduced by dealing with endophytic streptomycetes and their biologically active products.

Many of the recently described endophytic streptomycetes possess unique 16S rDNA sequences, make totally novel biologically active products, possess unique hyphal structures, and have interesting and novel cultural characteristics [8–11, 14, 34]. Novel taxonomy may highlight novel chemistry providing prospects for finding new biologically active compounds for agriculture, medicine, and industry [28].

We took a collecting expedition into the Amazon—the largest and most biologically diverse terrestrial ecosystem in the world—in order to find novel endophytic actinomycetes. From this important ecosystem, only one endophytic actinomycete has been reported [11, 14]. It was suspected that such organisms must be present given its vast size and the enormous number of plant species that it supports. This report describes the isolation of several novel actinomycetes from the rainforests of the upper Amazon in Peru. Aspects of their classification and some characterization of their biological activities are also presented. An added feature of the work is that the sampling, processing, and discovery of these organisms was conducted by undergraduate students, each of whom had the experience and excitement of being involved in the discovery process.

Methods

Plant Sampling

Approximately 300 plants were sampled based primarily on ethnobotanical history. Plant specimens were obtained during the month of March 2007 in two locations of the Peruvian Amazon basin—near Lake Sandoval (12°36.377′ S to 12°36.344′ S longitude, 69°01.818′ W to 69°01.950′ W latitude) and along the Heath River near the Southeastern Peru–Bolivia border (12°39.716′ S to 12°40.760′ S longitude to 68°42.722′ W to 68°40.977′ W latitude). Stem clippings of fresh growth were collected into plastic bags and kept cold until they could be processed. Voucher specimens were collected in duplicate. One set of plants was placed in the herbarium at the Universidad Nacional San Antonio de Abad, Cusco, Peru and another at the Yale University Herbarium, New Haven, USA. Plant material was identified and authenticated by Percy Núñez Vargas.

Isolation of Actinomycetes

Stem samples were subjected to a 70% ethanol wash followed by brief flaming to remove surface-associated microorganisms. Using aseptic technique, the outer tissue was removed, and inner tissues were plated onto culture plates containing glycerol–arginine agar [19]. All plates were contained in plastic storage boxes at room temperature and checked every few days for microbe growth. As microbes became apparent over the course of a 6-week period, they were carefully transferred to plates containing either potato dextrose agar at one tenth the recommended concentration (1:10 PDA) or nutrient agar at one tenth the recommended concentration (1:10 NA). Media were used at a dilute concentration to avoid robust growth by the most vigorous organisms. Because both bacterial and fungal isolates were desired, no antimicrobial compounds were added to the media. All cultures were grown at room temperature. All organisms that appeared as actinomycetes and possessed an earthy odor were tagged as potential actinomycete candidates. These organisms were stored in 15% glycerol solution at −80°C. Permanent stocks were made by colonizing triply autoclaved barley seeds and the organisms were stored in the living culture collection at Montana State University at −70°C and each given an acquisition number (Table 1).

Genomic DNA Isolation and rDNA Analysis

Four-milliliter cultures were grown at room temperature (22°C) by shaking at 200 rpm in either nutrient broth medium or potato dextrose (PD) broth medium for approximately 2 days or until significant growth was observed. DNA was harvested using the Qiagen DNeasy Plant Mini Kit. Approximately 10 ng of genomic DNA was used as a template for amplification of a region of the 16S ribosomal DNA. Amplifications were performed using an initial denaturation step of 5 min at 95°C followed by 30 cycles each of 1 min at 95°C, 1 min at 55°C, 1.5 min at 72°C. The 30 cycles were followed by a 5-min extension at 72°C. One hundred microliter reaction mixtures contained approximately 10 ng genomic DNA; 1× GoTaq® Flexi Buffer (Promega, Madison, WI, USA); 200 µmol/L (each) dATP, dCTP, dGTP, and dTTP; 2.5 mM $MgCl_2$; 2.5U GoTaq® Flexi DNA Polymerase (Promega); 1.5 µM each primer. The primers used in all cases except for P801A were 63F (5′-GGG CGG WGT GTA CAA GGC-3′) and 1387R (5′-GGG CGG WGT GTA CAA GGC-3′) [22]. Primers used for P801A were 907R (5′-CCG TCA ATT CMT TTR AGT TT-3′) and 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) [20]. The amplified product was purified using the QIAquick polymerase chain reaction purification kit (Qiagen). DNA sequence analysis was carried out on an Applied Biosystems 3730 capillary instrument. rDNA

Table 1 Plant source, sequence and culture identification information, and morphological descriptions of the actinomycetes described in this study

Endophyte ID	Host plant genus	Host plant species	GenBank number	MSU acquisition number	Closest GenBank match (percent identity)	Mycelia	Colonies, reverse side	Secreted metabolite	Spore size
P201B	*Siparuna*	*crassifolia*	EU977265	MSU 2351	*Streptomyces kimningensis* (98%)	Off-white	Off-white turning brownish	Yellow turning orange	~1 µM
P503A	*Calycophyllum*	*acreanum*	FJ429652	MSU 2352	*Streptomyces atrolaccus* (99%)	white	Grayish green	None observed	~1 µM
P506A	*Capirona*	*decorticans*	EU977222	MSU 2353	*Streptomyces mucoflavus* (97%)	Off-white	Pale yellow to brown	None observed	Not known
P513A	*Ocotea*	*longifolia*	EU977235	MSU 2354	*Streptomyces paraguayensis* (98%)	Off white	Pale yellow to brown	Yellow turning brown	Not known
P801A	*Aspidosperma*	*sp.*	EU977286	MSU 2355	*Amycolatopsis kentuckyensis* (98%)	white	Pale yellow	None observed	~1 µM
P911A	*Palicourea*	*longifolia*	EU977303	MSU 2356	*Streptomyces kunmingensis* (98%)	Pale yellow	Yellow-orange	orange	~1 µM
P1207Bh	*Monstera*	*spruceana*	EU977223	MSU 2357	*Streptomyces thermosacchari* (99%)	Pinkish white	Pale grey-yellow	None observed	unknown
P1303B	*Croton*	*lechleri*	FJ429653	MSU 2358	*Streptomyces hygroscopicus* (98%)	Pale greenish yellow	Greenish yellow	Clear droplets on colony	~1 µM
P1318F	*Cantua*	*buxifolia*	FJ429657	MSU 2359	*Micromonospora arenae* (99%)	black	Orange-grey	None observed	unknown
P1400C	*Banisteriopsis*	*caapi*	FJ429655	MSU 2360	*Streptomyces hygroscopicus* (99%)	White turning dark grey	Yellow, turning greyish	Orange-brown	unknown
P1400D	*Banisteriopsis*	*caapi*	FJ429658	MSU 2361	*Streptomyces hygroscopicus* (99%)	white	Tan to reddish black	Not seen	unknown
P1403H	*Iryanthera*	*laevis*	FJ429656	MSU 2362	*Streptomyces hygroscopicus* (99%)	Light yellow	Bright yellow	Clear droplets on colony (NA)	unknown
P1519C	*Eucharis*	*cyaneosperma*	EU977285	MSU 2363	*Streptomyces recifensis* (99%)	Pale gray-yellow	Pale gray-yellow	None observed	~1 µM
P1801B	*Monstera*	*spruceana*	FJ429654	MSU 2364	*Streptomyces melanosporofaciens* (97%)	white	Pale yellow	Not seen	~1 µM

Description of mycelia, reverse side colony color and metabolite production refers to that observed after 2 weeks on PDA plates except the metabolite production for P1403H was observed after growth on NA

sequences were submitted to GenBank and accession numbers assigned (Table 1). Sequences were subjected to BLASTN analysis [1] with the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/) accessed on June 4, 2008. The closest named species match was recorded along with the percent sequence identity (Table 1). The DNA sequences were aligned and compared to each other using the Molecular Evolutionary Genetics Analysis (MEGA) software version 3.3.14.

Morphological Characterization

Initial characterization of isolates growing on nutrient or potato dextrose agar was performed using a Stereo Discovery V8 Stereomicroscope (Carl Zeiss, Germany). Colony morphology was recorded after 6 days, 2 weeks, and 3 weeks of growth. Some samples confirmed as Actinobacteria through 16S rDNA sequence analysis and preliminary morphological observation were transferred to agar containing γ-irradiated carnation leaves to facilitate development of spore structures and these were analyzed by scanning electron microscopy. Individual agar plugs containing microbe-colonized carnation leaves were fixed in 2% glutaraldehyde, 0.1 M sodium cacodylate buffer (pH 7.2–7.4) and Triton-X 100, aspirated for 5 min and fixed overnight. The samples were then processed as described in [9]. The samples were critical-point dried and gold-sputter-coated, and images were recorded with a JEOL 6100 scanning electron microscope.

Bioassays

Each actinomycete isolate was grown on either 1:10 PDA or NA for 14 days at room temperature. The test organisms were: *Rhizoctonia solani*, *Fusarium solani*, *Geotrichum candidum*, *Sclerotinia sclerotiorum*, *Cerospora sp.*, *Phytophthora cinnamomi*, *Colletotrichum lagenarium*, *Verticillium dahliae*, *Trichoderma viride*, and *Pythium ultimum* provided by G.A.S; *Candida albicans* and *Staphylococcus epidermidis*, obtained from the American Type Culture Collection; *Escherichia coli* obtained from the Coli Genetic Stock Center at Yale University; and *Bacillus subtilis*, provided by Dr. A.L. Sonenshein, Tufts University School of Medicine.

For the fungal growth-inhibition assays (except *C. albicans*), the isolates were plated in a “cross” pattern that divided the plate into quarters. After 14 days, 3×3×3-mm plugs of agar containing freshly grown cultures of the fungal test organisms were plated at a location in the central portion of the quadrant. In order to ensure consistency, a paper template was made that provided uniform placement of the fungal test organisms and the position of the bacterial crosses.

To assay inhibition of growth of *C. albicans*, *E. coli*, *B. subtilis*, and *S. epidermidis*, the actinomycete was plated in a line down the center of the plate. After 14 days, each test organism was streaked perpendicular to the growth of the endophyte, starting near the endophyte and extending toward the edge of the plate. A paper template aided in obtaining consistent placement of microbes on all plates. All bioassays were performed in triplicate.

Growth of the test organisms was evaluated after 24, 48, and 72 h. For the fungi, the hyphal growth of the test organism was measured in millimeters and compared to growth of test organism plated in the absence of endophytic isolate. The values shown in Table 2 are the averages from three experiments. Growth of the yeast *C. albicans* and of the bacterial test organisms was inspected after 24, 48, and 72 h and scored as no growth, growth, or partial growth.

Results

Endophytic Actinomycetes Isolated from Amazon Rainforest Sampling Sites

A total of ~300 apparently disease-free plants were sampled and subjected to surface treatment processes to eliminate surface associated microorganisms. Endophytes were isolated from internal plant stem tissues. Cultures that exhibited growth morphology indicative of actinomycetes were selected for further study. All exhibited filamentous growth and produced an earthy odor. Additional morphological observations combined with rDNA analysis confirmed that at least 14 of these isolates were actinomycetes. Of these, one is a *Micromonospora* sp., one is an *Amycolatopsis* sp., and the remaining 12 belong to the genus *Streptomyces*. Of the actinomycetes isolated, only two isolates had the same rRNA sequence (P506A and P513A). All others exhibited differences in rRNA sequence, morphology, and growth inhibition patterns. In addition, 16S partial ribosomal sequences did not show 100% identity with any organisms existing in GenBank on June 4, 2008 (Table 1). There were 14 actinomycete isolates obtained from 12 different plant species representing ten plant families, suggesting that endophytic actinomycetes are likely widespread throughout plant phylogeny (Fig. 1).

The source plant and identifying features of each actinomycete is summarized in Table 1. The morphologies indicated in Table 1 refer to those seen when the organisms were grown for 2 weeks on PDA with one exception; the clear liquid observed on the colony surface of P1403H was observed when grown on nutrient agar. The majority of the microbes show more robust growth on NA than PDA, with the exception of P1303B and P1801B which prefer PDA. All endophytes for which spores were

observed, except P503A, produce barrel-shaped spores about 1 μM in length. The spores of P503A were less blunt-ended and rounder than all other spore structures observed (Fig. 2).

The combined morphological and rDNA sequences suggest that all 14 organisms are unique. Although P506A and P513A have the same rDNA sequence over the 1,200 nucleotides sequenced, these two organisms have distinguishable growth morphologies and different growth inhibition profiles. When grown on PDA, P506A produces yellow- to mocha-colored colonies that produce narrow, vertical projections visible with the naked eye. When grown on 1× PDA, P513A, like P506A, produces brownish colonies, but unlike P506A, it secretes metabolites into the medium. P513A colonies are rounded and raised yet appear to collapse in the center over time, leading to a wrinkled appearance by the naked eye without the dramatic vertical projections seen in P506A. The colonies appear wet and not at all chalky and are surrounded by a halo not seen in P506A colonies.

Two other organisms sharing similarities are P1400C and P1400D. While both cultures were isolated from the same plant and exhibit a similar growth inhibition profile, the growth morphology and rDNA sequence data differ. On both PDA and NA, P1400C colonies appear raised, produce darker mycelia than P1400D and secrete an orange-brown metabolite not observed with P1400D growth. The reverse side of the P1400D colonies is reddish brown as opposed to the grey of P1400C. Although P1400C and P1400D share *S. hygroscopicus* as the closest relative in GenBank, out of approximately 1,200 nucleotides sequenced, there are differences at 16 locations.

Phylogenetic Analyses

Standard methodologies were used to create a phylogenetic tree. The 16S rDNA sequences were placed in Megalign from DNAStar at default settings comparing the relatedness of our organisms to each other, to their closest named relatives in GenBank, and to other known *Streptomyces* species (Fig. 1). None of our organisms matched known species. Interestingly, four endophytes (P1801B, P1303B, P513A, and P506A) show higher identity in 16S rDNA sequence to each other than to any other named relative in GenBank. As expected, P801A and P1318F, the two non-*Streptomyces* species, fall into different clades.

All Isolated Organisms Produce Growth-Inhibitory Substances

Novel organisms are likely to harbor metabolic pathways that may lead to the production of novel secondary metabolites. Based on the fact that these Amazonian actinomycetes appeared different from previously described organisms, it may be the case that they produce novel natural products including, but not limited to, antimicrobial compounds although further structural studies will be necessary to determine if this is the case. In order to test for the production of antibiotics, bioassay tests were performed in which a panel of test fungi, oomycetes, and bacteria were challenged with 14-day-old endophyte cultures. Our panel of test microbes included fungal plant pathogens from the Ascomycota and Basidiomycota, an opportunistic

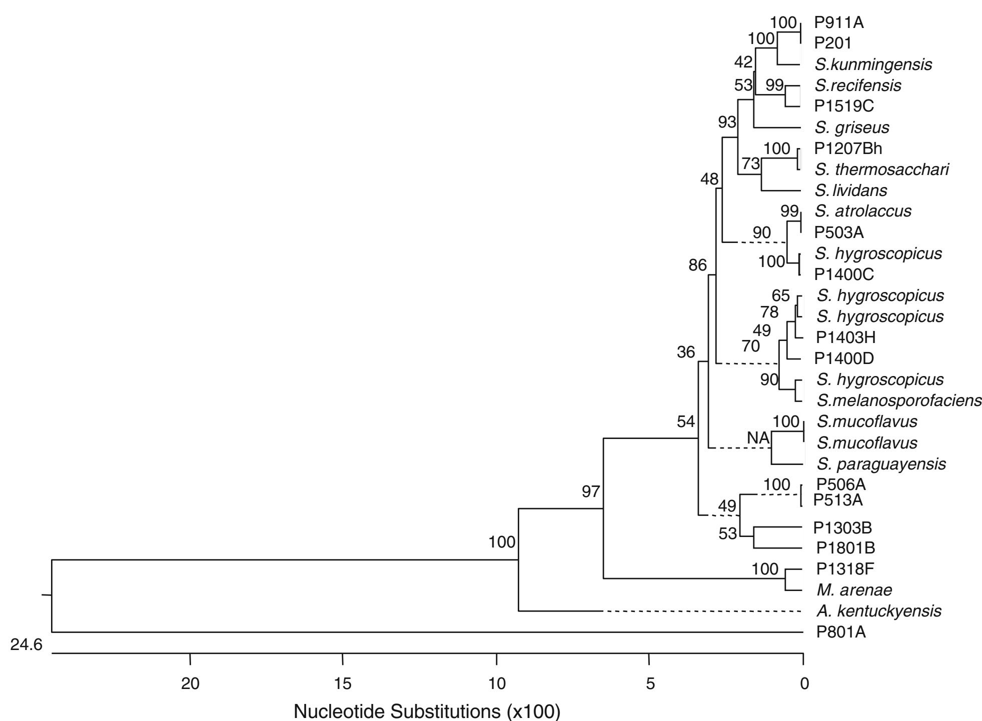

Figure 1 Phylogram based on 16S rDNA sequence alignments. Organisms represented are the endophytes in this study, their closest named GenBank matches, and two other known *Actinomycetes* (*S. lividans* and *S. griseus*). Bootstrap trials = 1,000; seed = 111

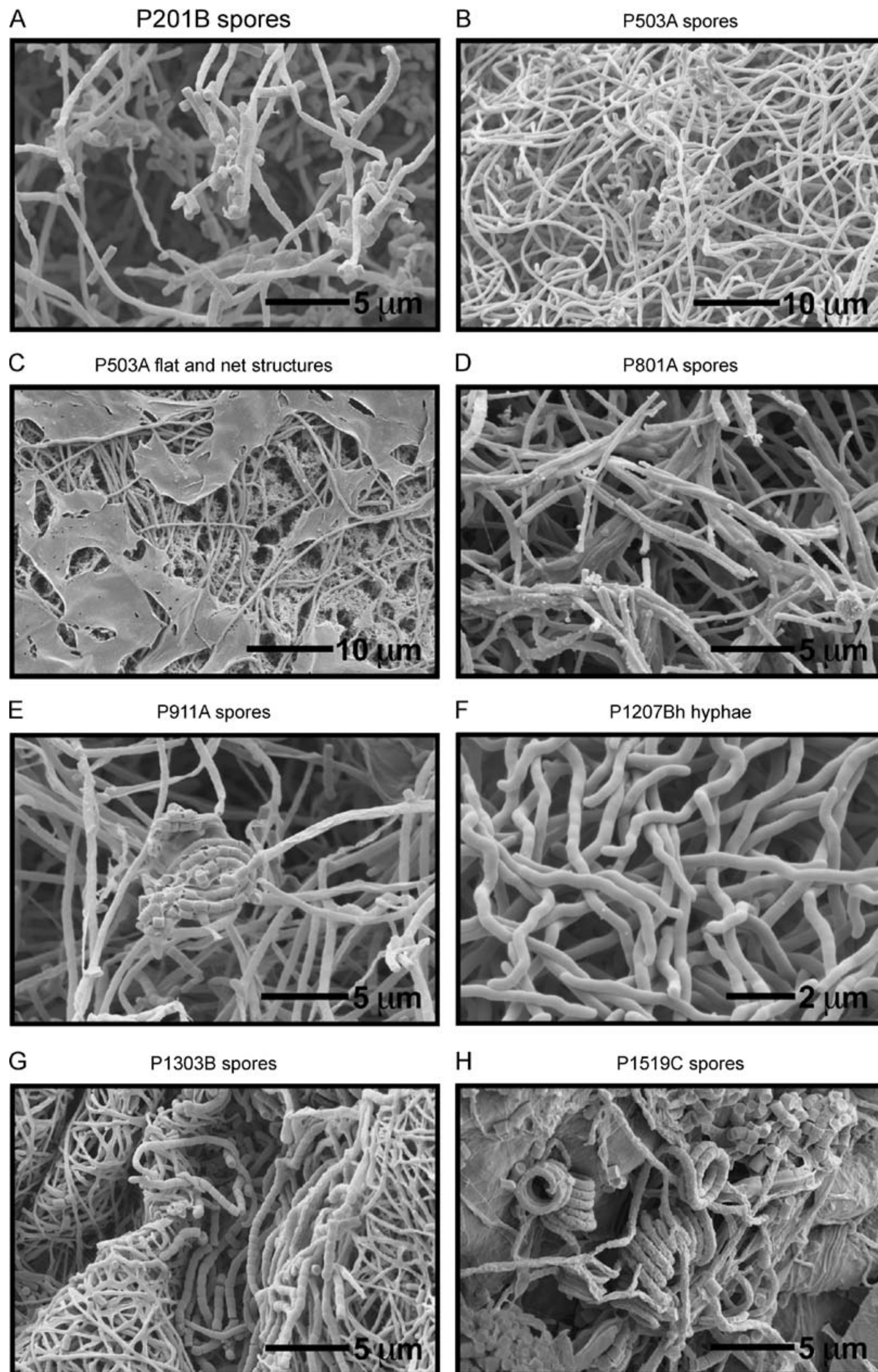

Figure 2 A–**H** Scanning electron micrographs of selected Amazonian *Actinomycetes*. Seven selected actinomycetes are shown. All formed spores under the conditions used except P1207Bh (**F**). Flat and net-like structures were observed in addition to spores and hyphae in the preparation of P503A (**C**)

Table 2 Biological activity of the Peruvian endophytes

Endophyte	*Rhizoctonia*		*Fusarium*		*Geotrichum*		*Sclerotinia*		*Cerospora*		*Colletotrichum*		*Verticilium*		*Trichoderma*		*Pythium*		*Phytophthora*		*Candida*		*S. epidermidis*		*E. coli*		*B. subtilis*	
	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA	PDA	NA
P201B	79	100	26	69	70	74	95	100	67	100	82	100	88	100	87	100	93	100	90	100	0	1	1	1	1	0	2	1
P503A	71	100	83	96	67	100	75	100	33	100	82	100	56	100	74	100	84	100	81	100	1	1	1	1	0	1	2	1
P506A	71	59	45	28	62	24	94	94	22	88	18	79	53	83	43	68	76	60	54	100	1	2	0	2	1	2	1	2
P513A	82	56	60	24	60	6	100	94	33	76	82	68	94	56	48	70	92	17	100	94	1	2	1	2	1	2	0	2
P801A	70	100	52	90	47	100	96	100	33	100	58	100	48	81	67	100	92	100	100	100	1	2	2	2	0	2	2	2
P911A	100	100	75	91	100	100	100	100	86	100	100	100	73	100	91	100	76	100	100	100	1	1	1	1	1	1	1	1
P1207BH	89	100	82	73	91	69	87	100	29	100	36	100	95	100	84	100	100	100	92	100	0	1	0	0	0	0	1	0
P1303B	100	25	90	22	100	-16	93	58	50	38	100	70	71	-33	100	-6	98	19	100	9	0	0	0	0	0	0	1	0
P1318F	28	100	80	88	36	36	46	100	-25	100	40	100	43	100	88	100	20	99	81	100	0	1	0	0	0	0	0	1
P1400C	66	100	68	95	70	82	89	100	80	100	100	100	12	100	-18	100	100	100	100	100	0	1	0	1	1	1	1	1
P1400D	87	98	91	100	100	100	100	100	100	100	100	100	100	100	79	100	99	100	100	100	0	0	NA	2	0	0	0	0
P1403H	91	95	61	94	90	82	95	100	67	100	100	100	85	100	94	100	90	100	81	100	1	1	1	1	0	1	2	1
P1519C	54	100	40	67	100	22	53	100	-14	100	38	100	11	100	57	100	67	100	66	100	0	0	0	0	0	0	0	0
P1801B	100	58	100	38	100	21	100	84	100	71	100	79	100	67	100	58	99	43	100	83	1	0	2	0	1	0	1	0

Test organism growth inhibition due to presence of actinomycete endophytes. Numbers shown indicate percent growth inhibition of each test organism. Endophytes were grown on individual plates for 14 days, after which up to four test organisms were plated onto an endophyte-containing plate. Hyphal growth of each test organism was measured and compared to growth of a test organism grown in the absence of endophyte. No growth of the test organism is considered 100% growth inhibition. Organisms that did not exhibit hyphal growth (all bacteria and *Candida albicans*) were scored on a scale of 0–2. Test organisms with no observable growth (the most robust growth inhibition) are scored as a 2, whereas test organisms showing growth comparable to those grown in the absence of endophyte received a score of 0.

fungal human pathogen (*C. albicans*), two plant pathogenic oomycetes, and representative gram positive and gram negative bacteria.

Inhibition Summary

All endophytes tested demonstrated robust antimicrobial activity (≥90% growth inhibition) against a minimum of six microbial test organisms. A large difference in inhibitory activities and target organism specificity was associated with each endophyte. In addition, choice of growth media affected the spectrum of growth inhibitory activity of some endophytes (Table 2; Fig. 3). For example, P1519C shows 100% inhibition of *Geotrichum* when grown on 1:10 PDA plates but only 22% inhibition on NA plates. Conversely, this same organism exhibited 100% inhibition of *Cerospora* and *Verticillium* when grown on NA plates without showing significant inhibition of these same organisms on 1:10 PDA. Organisms P503A, P801A, P911A, P1400D, 1403H, and P1801B showed a broad spectrum of activity against the largest panel of test organisms. P506A inhibited the lowest number of fungal test organisms; however, it was one of only three endophytes that inhibited all three bacterial test organisms, suggesting a prokaryotic inhibitory preference.

Fungal Inhibition

Each endophyte was able to confer robust growth inhibition of at least three fungal or oomycete test organisms. We quantified the inhibitory activity directed against the fungal test organisms that exhibited mycelial growth (Table 2). On the media used in our bioassays, the dimorphic fungus *C. albicans* grows as a unicellular yeast rather than exhibiting the mycelial morphology typical of the other test fungi. For this reason, we assayed inhibition of *C. albicans* growth using the same criteria as for the bacterial test organisms (see

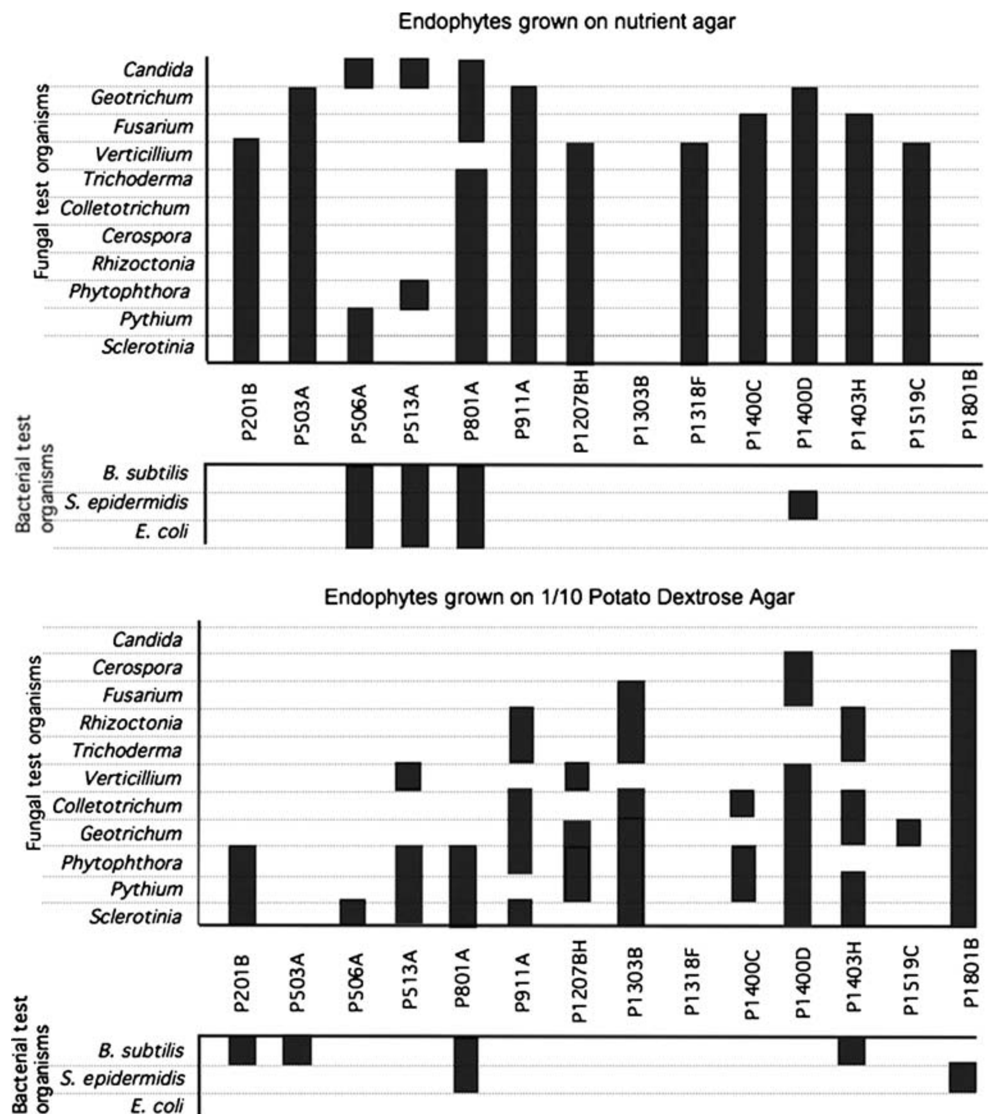

Figure 3 Antibiotic activity of endophytes grown on nutrient agar and 1/10 PDA. Each endophytic actinomycete was grown for 14 days prior to plating of test organisms. A *black bar* indicates ability of the endophyte to inhibit growth of the test organism as measured after 3 days of challenge. Fungal inhibition is defined as growth equal to or less than 10% of that seen by a control in the absence of challenge by endophytic actinomycete. Bacterial and *C. albicans* inhibition is defined as no visible growth of the test organism after 3 days in the presence of the endophyte. Numerical values are shown in Table 2

below). Four endophytic isolates were able to inhibit growth of all ten fungal (non-*Candida*) test organisms when inhibition was defined as growth limited to 10% or less than that observed in the absence of the endopyte (≥90% inhibition; Fig. 3). Interestingly, two of these (P503A and P1801B) could not inhibit any of these test organisms when plated on alternate media, suggesting the importance of media in inducing production of specific secondary metabolites.

Bacterial and Candida Inhibition

A total of eight endophytes exhibited what appeared to be robust inhibition of at least one of the three bacterial test organisms (Fig. 3). Three endophytes exhibited robust inhibition of the yeast *C. albicans*. Robust inhibition was defined as no visible growth of the test organism after 3 days in the presence of the endophyte. Endophytes P506A, P513A, and P801A showed complete inhibition of all three bacterial test organisms as well as *C. albicans*. For P506A and P513A, this inhibition was observed only when the endophytes were grown on NA. P801A lost the ability to fully inhibit *C. albicans* and *E. coli* when grown on 1:10 PDA but retained the ability to inhibit *B. subtilis* and *S. epidermidis* on both media types.

Discussion

A total of 14 actinomycetes were isolated from the inner tissue of healthy plants found in the rainforests of southeastern Peru and the Heath river area of Bolivia. Molecular biology (16S rDNA) combined with morphological data indicate that these organisms are all distinct from each other and from any other sequences deposited in GenBank (as accessed on June 4, 2008). While it is not uncommon for rDNA analysis of environmental samples to indicate the presence of unique microbes, it is significant to note that all of our sequences represent organisms that were cultured and deposited into a permanent microbial culture collection. To our knowledge, only one other endophytic actinomycete has been isolated from the upper Amazon region of Peru [14]. Interestingly, it was isolated from *Monstera*, the same genus from which we isolated two different *Streptomyces* isolates.

While many of the endophytes show a relatively broad spectrum of biological activity, it is important to emphasize that a broad spectrum of activity may be due to multiple compounds secreted by the endophyte, rather than a single inhibitory compound. Furthermore, organisms that demonstrate selective inhibition of only a few organisms may prove valuable in circumstances where selectivity is required. The bioactivity associated with these organisms indicates the potential commercial value of these endophytes and of rainforest plants in general. The prevalence of antimicrobial compounds produced by the endophytes may be explained as a contribution to the host plant in exchange for the nutrients and protection afforded the endophyte by the plant. While this study did not directly demonstrate the role of the actinomycete to the biology of the host plant, it represents a necessary step in understanding the role of these microbes in the forest ecosystem. It is reasonable to hypothesize that because these organisms are present as endophytes, they must be involved in one or more ways in the intricate complex life of the forest.

Finally, it is noteworthy that a group of enthusiastic, though inexperienced, undergraduates could isolate such a large number of potentially novel actinomycetes over a 4-month period. It has been proposed that a successful undergraduate research program should allow investigations where multiple students participate in discovery-based projects. In such projects, each student should perform similar, but nonidentical, tasks in parallel during the initial stages of the project [17, 30]. This study fulfills these criteria and at the same time has provided a wealth of opportunities for continued undergraduate research. Future studies will include purification and characterization of the antimicrobial products as well as further characterization of the endophytic isolates.

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