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Alkalithermophilic actinomycetes in a subtropical area of Jujuy, Argentina

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ABSTRACT

The objective of this study was to examine the alkalithermophilic actinomycete communities in the subtropical environment of Jujuy, Argentina, characterized by sugarcane crops. *Laceyella putida, Laceyella sacchari, Thermoactinomyces intermedius, Thermoactinomyces vulgaris* and *Thermoflavimicrobium dichotomicum* were isolated on the media with novobiocin, from sugar cane plants and renewal rhizospheres, and grass and wood soils. Soil pH was almost neutral or lightly alkaline, except for grass soil acidified by lactic liquor. A smaller number of actinomycetes was found on the living plants and bagasse (recently obtained or stored according to the Ritter method) with respect to decomposed leaves on the soil. Thermophilic species of *Laceyella*, *Thermoactinomyces*, *Thermoflavimicrobium, Saccharomonospora, Streptomyces* and *Thermononospora* were isolated on the media without novobiocin, from composted sugar cane residues. Air captured near composted bagasse piles, contained alkalithermophilic actinomycete spores.

Keywords: actinomycetes, subtropical soils, sugar cane, bagasse

RESUMEN

Actinomicetos termoalcalófilos del área subtropical de Jujuy, Argentina. El objetivo de este trabajo fue examinar los actinomicetos termoalcalófilos presentes en el área subtropical de Jujuy, Argentina, caracterizada por el cultivo de la caña de azúcar. Se aislaron en medio con novobiocina las especies *Laceyella putida, Laceyella sacchari, Thermoactinomyces intermedius, Thermoactinomyces vulgaris* y *Thermoflavimicrobium dichotomicum* a partir de la rizósfera de plantas y de renuevos de caña de azúcar, así como de suelos de pastura y de monte natural. El pH de los suelos era casi neutro a ligeramente alcalino, excepto en un solo caso en que el suelo estaba acidificado por licor láctico. El número de actinomicetos encontrados sobre los tejidos vivos y en el bagazo recién obtenido o almacenado según el método de Ritter fue pequeño en comparación con el observado sobre las hojas en descomposición. *L. sacchari* predominó respecto de *T. vulgaris*. Se aislaron especies termoalcalófilas de *Laceyella*, *Thermoactinomyces*, *Thermoflavimicrobium, Saccharomonospora, Streptomyces* y *Thermononospora* de los residuos compostados de caña de azúcar utilizando medio sin novobiocina. El aire capturado cerca de pilas de bagazo en compostaje contenía esporos de estos organismos.

Palabras clave: actinomicetos, suelos subtropicales, caña de azúcar, bagazo

INTRODUCTION

Many different kinds of alkaliphilic microorganisms, including bacteria belonging to the genera *Bacillus*, *Micrococcus*, *Pseudomonas*, *Streptomyces,* and *Thermoactinomyces,* and eukaryotes, such as yeasts and filamentous fungi, have been isolated from a variety of environments. Alkaliphilic microorganisms coexist with neutrophilic microorganisms. The frequency of alkaliphilic microorganisms in neutral ordinary soil samples is 10^2 to 10^5/g of soil, which corresponds to 1/10 to 1/100 of the population of the neutrophilic microorganisms (5). Alkaliphilic microorganisms grow very well at pH values of 8.5 and above, and cannot grow at pH value of 6.5 (12).

Thermoactinomyces and other alkalithermophilic actinomycete genera with very resistant endospores, grow in compost, rotten hay, manure, and soil (10). Alkalithermophilic actinomycetes, exceptionally well adapted for dispersal, are the most common etiological agents of hypersensitivity pneumonitis (9).

We hypothetized that thermophilic actinomycetes are better suited for colonizing soils, and overheated stores of residual materials than living vegetal surfaces. The objective of this study was to examine the alkalithermophilic actinomycete communities in the subtropical environment of Jujuy (Argentina) characterized by sugarcane crops.

MATERIALS AND METHODS

Soil and living plant samples were collected from the subtropical zone at 463 m above sea level (23º48'S, 64º47'W), having a mean annual temperature of 23.7 ºC, and annual precipitation of 827 mm (2). From each 10 by 10 m area, five soil cores were obtained from 0 to 10 - 20 cm depth by using a tubular sampler. Cores were placed in sealed ziplock bags. Ten samples of sugar cane leaves and stems, were collected from a sugar cane plantation. Ten fresh bagasse samples were collected from a sugar cane mill. Twenty samples of bagasse stored according to the Ritter method, were taken from one year old and recent piles. Five samples of residual foliage were taken after harvest. All samples were placed in sealed bags. Soil cores were extruded and homogenized to create a single large composite sample. Vegetal materials were cut and homogenized to make a single large sample. The samples were split into portions for analysis. Twenty-five g sub-samples were mixed with 225 ml of sterile neutral water with 0.05% Tween 80, on a mixer for 2 min, and they were used for analysis. Actinomycetes were isolated by the serial-dilution-spread plate technique. Dilutions (10^{-1} to 10^{-3}) were plated onto a pH 7.5 half-strength tryptone soy agar containing 50 mg of cycloheximide and 25 mg of novobiocin per ml. Plates were incubated at 50 °C for 4 to 10 days to allow the actinomycetes to sporulate, and then colonies were counted (7).

Sugar cane residues were collected from another site of the subtropical zone 575 m above sea level (24°13'S, 64°52'W), having a mean annual temperature of 21.6 °C, and annual precipitation of 645 mm (2). Twelve samples were taken from overheated sugar cane residue piles and were placed in sealed bags. Three samples of dust generated during the processing of compost piles, were collected from air using a Porton impinger with sterile phosphate-buffered saline (137 mM NaCl, 2.5 mM KH_2PO_4, 6.9 mM K_2HPO_4, pH 7.3), and connected to a portable pump. Composted residues were cut and homogenized. The air dust suspensions and serial dilutions of composted residues were plated onto the medium without novobiocin.

The colonies were picked and transferred to tryptone soy agar slants, incubated at 50 °C until sporulated and stored at 4 °C until used for identification. Isolates were classified on the basis of phenotypic characteristics: colour of aerial mycelium; sporophore morphology; pigments; degradation of arbutin, gelatin, starch, xanthine; utilization of L-arabinose, mannitol, sucrose, D-xylose; growth at 30 °C and on pH 10 medium (7, 13).

Kruskal and Wallis comparison test was used to test for significant differences between sample data. Significance was determined as a α value of < 0.05 (3).

RESULTS

The percentage of alkalithermophilic actinomycetes strains in soils collected from five sites in Ledesma Department (Jujuy) with a haplic phaeozem ground is shown in figure 1. Soil pH was almost neutral or lightly alkaline, except for soil acidified by Ritter liquor. The total number of alkalithermophilic actinomycetes of sugar cane rhizosphere differed significantly from that observed in the soil where sugar cane had been recently planted and the soil acidified. The soils with old established vegetal cover did not show any significant difference among them. The difference in the number of isolated *T. vulgaris* coming from soils with natural cover (grass and wood) was significant compared to the other samples.

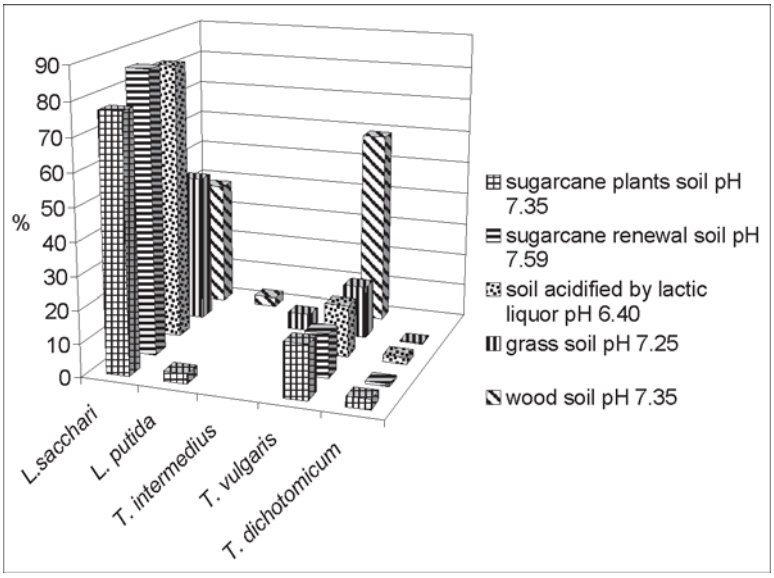

Figure 1. Alkalithermophilic actinomycetes (*Laceyella sacchari, Laceyella putida, Thermoactinomyces vulgaris, Thermoactinomyces intermedius*, *Thermoflavimicrobium dichotomicum*) from five sites in Ledesma Department (Jujuy) with a haplic phaeozem ground. The average total counts were 11800, 8300, 9033, 7262, and 7175 CFU/g (dry weight) in sugarcane plant soil[a], sugarcane renewal soil[b], soil acidified by lactic liquor[b], grass soil[c], and wood soil[c], respectively (different letters show significant difference in actinomycetes total number).

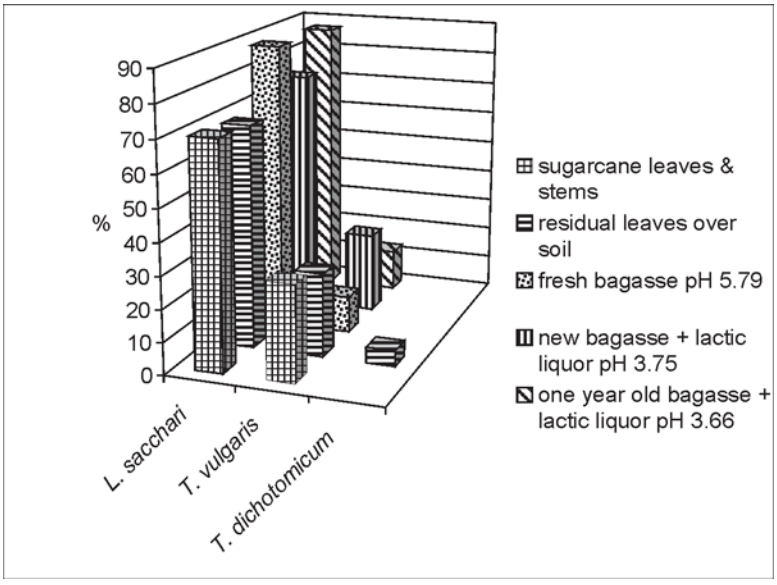

Figure 2. Alkalithermophilic actinomycetes (*Laceyella sacchari, Thermoactinomyces vulgaris, Thermoflavimicrobium dichotomicum*) from samples of living sugar cane plants, residual leaves, fresh and stored bagasse, taken in Ledesma Department, Jujuy. The average total counts were 150, 1700, 115, 105, and 155 CFU/g (dry weight) in sugar cane leaves and stems[a], residual foliage lying on soil[b], fresh bagasse[a], and one year old[a] and recent piles[a] of bagasse stored according to the Ritter method, respectively (different letters show significant difference in actinomycetes total number).

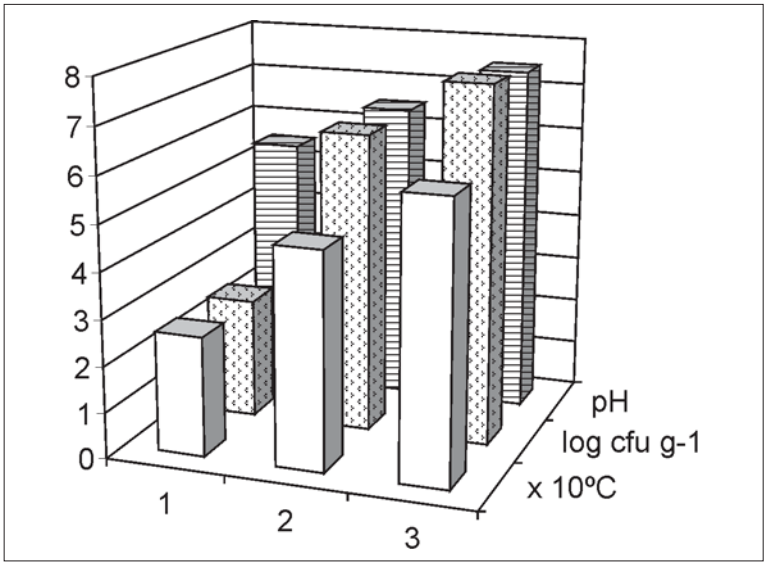

Figure 3. Alkalithermophilic actinomycetes numbers on media without novobiocin, pH and temperatures at three times during sugar cane residue composting. The average total counts were 4 x 10^2, 3 x 10^6, and 5 x 10^7 CFU/g (dry weight) in compost at 26 ºC, 47 ºC, and 60 ºC, and pH 5.5, 6.5 and 7.5, respectively.

Some thermoactinomycete strains isolated from soil samples showed antagonistic activity to gram-positive bacteria growing as culture contaminants.

The incidence of alkalithermophilic actinomycete species on sugar cane leaves and stems, residual leaves after harvest, fresh bagasse, and one year old and re-

cent piles of bagasse acidified by lactic liquor according to the Ritter method (Ledesma Department, Jujuy) is shown in Figure 2. The difference among the number of actinomycetes on the living plant and bagasse was significant with regard to the decomposed leaves on the soil. The difference among the total number of thermophilic actinomycetes in the sugar cane and the fresh or acidified bagasse was not significant, but *L. sacchari* predominated over *T. vulgaris*.

The incidence of alkalithermophilic actinomycete species in composted sugar cane residues from San Pedro Department (Jujuy), at different stages, is shown in Figure 3. Temperature increased to a maximum of 60 °C and later it decreased. The pH, tested in field with indicator paper, increased from 5.5 to 7.5. Thermophilic species of *Laceyella*, *Thermoactinomyces*, *Thermoflavimicrobium, Saccharomonospora, Streptomyces* and *Thermononospora* grew on the isolation media without novobiocin. Air captured near composted bagasse piles, contained 700 alkalithermophilic actinomycetes spores/m^3.

DISCUSSION

Although alkalithermophiles require alkaline conditions and elevated temperatures for optimal growth, these factors do not necessarily restrict the distribution of alkalithermophiles to very distinct niches where both conditions are provided. Many alkalithermophiles have also been isolated from mesobiotic, slightly acidic to neutrophilic habitats (12). Some actinomycete genera (e.g. *Thermoactinomyces, Laceyella, Saccharomonospora*) are strictly thermophilic, while other genera (e.g. *Streptomyces*, *Thermomonospora*) contain some thermophilic species (10). *Thermoactinomyces* and *Laceyella* species are common in natural high- temperature habitats, such as leaf and compost heaps, and in overheated stores of plant materials such as hay, bagasse and grain. Their resistant spores are disseminated in a variety of soils (8).

Sugar cane bagasse leaves the mill containing about 50% water and 3-6% sugar, providing ideal conditions for microbial growth. The Ritter method is a safe way of bagasse storage, which retards microbial decay and reduces airborne organic dust. The bagasse is moistened with a liquor containing lactic bacteria before storage and the pH drops to about 4 (1).

When untreated sugar cane residues are stored, maximum temperatures greater than 50 °C are common and sometimes remain above 40 °C for long periods. After initial fungal growth, actinomycetes predominate during the composting process, and they must degrade solid organic substrates using hydrolytic extracellular enzymes and/or membrane-bound enzymes (cellulase, proteases, amylase) (8). Some commercial fertilizers include thermoactinomycetes which are effective for farm product growth and for prevention of plant diseases (11).

Actinomycete spores are suited for aerial dispersal; they have dry, hydrophobic surfaces and are easily detached by disturbance of the substrate or air movement. Their numbers in outdoor air are soon diluted, but very large numbers can occur indoors when deteriorated materials are handled (6).

Bagassosis is due to the synergistic action of bagasse fibers and actinomycete spores with an immunological component. *L. sacchari* spores are an important cause of this disease, because this species is usually more abundant in bagasse than *T. vulgaris* (6). Sera from patients show cross-reactivity against antigens from different actinomycete species because they are sensitized to multiple species of thermophilic actinomycetes present in the environment. We have tested sera from patients against bagasse actinomycete antigens by immunoprecipitation (4).

The *Laceyella* and *Thermoactinomyces* genera were found in soils, on living plants, and bagasse samples, but *Thermoflavimicrobium* was isolated from some soils. *Laceyella* strains constituted 72% of the total counts. This genus appears to be the most important in ecological function. Alkalithermophilic actinomycetes do not multiply on the living plants, the few isolated strains proceeded from soil dust. The allergenic *L. sacchari* and *T. vulgaris* were detected in all samples.

Six genera of thermophilic and thermotolerant actinomycetes, including *Laceyella* and *Thermoactinomyces,* were detected in compost samples. Also, allergenic species were isolated from air captured near composted bagasse piles.

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