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Review

Alkalophilic and alkali-tolerant soil fungi from *Celtis tala* and *Scutia buxifolia* forests in eastern Buenos Aires province (Argentina)

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ABSTRACT

Most soil fungi grow in acid environments; however, some of them are able to grow in alkaline conditions. This study is based on the need to establish a link between the ecological studies related to the fungal diversity of the alkaline soils of Argentina, and the development of biotechnological processes. Saprotrophic fungi have an enormous dispersal potential and an efficient enzymatic system that guarantees their biological role. They can degrade a great variety of carbon sources from plants tissues, and their decomposing activity is essential for the redistribution of nutrients in the soil. The alkalophilic and alkali-tolerant fungi that grow in *Celtis tala* and *Scutia buxifolia* forests soils from eastern Buenos Aires province (Argentina) have been here described. Many of the fungal strains tested presented the capacity of producing amylases, cellulases, keratinases and other proteases and chitinases at high pH levels.

KEYWORDS: soil-fungi, diversity, alkaline enzymes, *Celtis tala*, *Scutia buxifolia* forests

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INTRODUCTION

The aim of this work was to establish a relationship between ecological studies on fungal diversity and the potential development of biotechnological processes. The isolation of alkali- tolerant and alkalophilic fungal species from the soil of *Celtis tala* Gill ex Planch and *Scutia buxifolia* Reiss from the coast of the Province Buenos Aires is not only a contribution to understanding of microbial diversity, but also a search for microbial sources of alkali-tolerant and alkaline-active enzymes. These enzymes are of interest for bioconversion, food production and industrial treatment of textile fibers and industrial wastes. These processes are generally performed in alkaline conditions where it is necessary to take aggressive traditional chemical techniques. Therefore, an environmentally- sound alternative is using enzymes from microbial sources. So far, there are no studies integrating microbial diversity from alkaline soils of Argentina and the enzymes of biotechnological interest produced by these fungi.

Fungi perform a crucial role in the descomposition and mineralization of both animal and plant organic matter so they are responsible of soil fertility. Among the microorganisms living in the soil, fungi are the most abundant in terms of

both their biomass and physiological activity, contributing to 75% to 95% of the soil microbial biomass [1]. In addition, together with bacteria, they are responsible for 90% of the total flux of energy from decomposition of organic matter [2, 3]. In the ecosystem, fungi also interact either directly or indirectly with other microorganisms, being they a food source for bacteria, insects nematodes and other fungi [4]. Fungi release a great variety of exudates to the environment, including the enzymes responsible for the degradation of the substrates that they use, and polysaccharides and glycoproteins that form the matrix that protects and lubricates hyphae. These latter compounds have adhesive properties which keep soil particles together [5]. In places where the availability of nutrients is limiting for the development of plants, the presence of certain fungi establishing mycorrhizal association is essential for the evolution of plant communities [6]. These fungi contribute to the stabilization of soils, by means of the effects of aggregation and stabilization of soil particles [7].

Diversity of soil fungi: Effect of depth and pH on frequency and species diversity

In three types of soils from Magdalena, Buenos Aires, Argentina (35° 11´ S, 57° 17´ O) was determined and isolated a total of 84 fungal species [8, 9]. Fifty-nine of these species were found in the Argialboll soil (43 anamorphic Ascomycota; 5 Zygomycota; 8 teleomorphic Ascomycota and 2 mycelia sterilia), 61 species in Natracualf one (48 anamorphic Ascomycota; 6 Zygomycota; 5 teleomorphic Ascomycota; 2 mycelia sterilia) and 65 in Rendoll one (50 anamorphic Ascomycota; 8 Zygomycota; 5 teleomorphic Ascomycota; 2 mycelia sterilia). The species isolated belonged to 43 genera, the most numerous of which were *Acremonium***,** *Aspergillus* and *Penicillium.* Anamorphic forms was the group with the highest number of species identified, varying from 74 to 82% of the total mycobiota identified. The teleomorphs of Ascomycota varied between 4 and 16%, depending of the soil type and depth.

In the Argialboll and Rendoll soils, this group was more abundant in the lower horizons. The Zygomycota showed similar values. In the Argialboll and Rendoll soils they presented a higher number of species in the upper horizons. In contrast, in the Natracualf one, most of the species of Ascomycota were found in the upper horizons, whereas most species of Zygomycota were more abundant in the deeper horizons**.** On the same way, Giri *et al.* [10] found that the species distribution is regulated by the availability of organic matter and by the relationship between the oxygen and carbon dioxide present in the pore spaces of the soil at different depths. Fungal species tolerant to carbon dioxide are distributed in the deeper horizons [11]. In addition, 41 of the isolated species were present in the three types of soil of *Celtis tala* and *Scutia buxifolia* forest, but their distribution patterns were different depending on the soil type, the soil pH, and the isolation method used, showing: i) typical species of alkaline soils like *Clonostachys rosea*, *Paecilomyces lilacinus*, *Verticillium albo-atrum*, and species of *Acremonium* and *Aspergillus*; ii) species related to calcareous soils like *Trichoderma* sp.; and iii) species that were isolated only in the Natracualf soil like *Neosartorya stramenia* and *Neurospora tetrasperma* (Table 1). The components belong to fungal communities identified such as Ascomycota (anamorphs and teleomorphs) and Zygomycota are in agreement with those obtained previously for the same forest soils [12, 13] as well as for agricultural soils of the same region [14]. The species number of teleomorphs of Ascomycota isolated was higher than that obtained in other works carried out in Rendolls of Magdalena, Argentina [12] and in calcic soils from Greece [15].

Technically, the soil system is formed by all the horizons (from the layer formed by dead leaves to the basal rock). Nevertheless, the researchers of soil fungi communities have limited mainly their studies to the horizon in the top 2-10 cm of the profile [16], because most of the fungal descomposition activity occurs in the upper horizons. Therefore, little is known about the structure of fungal communities in deeper horizons. Burges [17] points out that the distribution of fungi in the soil is not homogeneous; there are numerous factors that condition the growth and settlement of different fungal communities. The differences observed in the physico-chemical

Table 1. Fungal strains used for enzymatic analysis and their isolation substrates.

Fungal strain	Soil type and horizon deeper[a]	Isolation pH[b]	Fungal strain	Soil type and horizon deeper	Isolation pH
ASCOMYCOTA					
Acremonium cerealis (928[c], a[d])	R, A (S, 20)	6,8,11	*Penicillium frequentans* (*, a)	R, A, N (S, 20)	6,8,11
Acremonium killiense (958, a)	R, A, N (S, 20)	6,8,11	*Penicillium megasporum* (*, a)	A (20)	8
Acremonium murorum (927, a)	R, A, N (S, 20)	6,8,11	*Penicillum restrictum* (954, a)	R, A, N (S, 20)	6,8,11
Acremonium rutilum (*, a)	N (20)	6,8,11	*Penicillium rubrum* (*, a)	R, A, N (S, 20)	6,8,11
Acremonium sp. 1(*, a)	R, A, N (S, 20)	6,8,11	*Penicillium thomii* (945, a)	R, A, N (S, 20)	6,8,11
Acremonium sp. 2 (*, a)	R, A (20)	6,8,11	*Penicillium sp.* 1(*, a)	R, A, N (S, 20)	6,8,11
Acrostalagmus luteo-albus (427, a)	R, A, N (S, 20)	6,8,11	*Penicillium sp.* 2 (*, a)	R, A, N (S, 20)	6
Alternaria alternata (*, a)	N (20)	11	*Penicillium sp.* 3 (*, a)	A, N (20)	6
Aspergillus niger (845, a)	R, A (S, 20)	6,8,11	*Pestalotiopsis guepinii* (929, a)	R, A, N (S, 20)	6,8,11
Aspergillus sidowii (931, a)	R, A (S, 20)	6,8	*Phialocephala sp.* (*, a)	N (20)	6
Aspergillus terreus (*, a)	R, A, N (S, 20)	6,8,11	*Phialophora fastigiata* (942, a)	R, N (S, 20)	6,8,11
Aspergillus terreus (994, a)	N (S)	6	*Phoma herbarum* (*, a)	A (20)	6
Aspergillus ustus (981, a)	R, A, N (S, 20)	6,8,11	*Scopulariopsis brevicaulis* (947, a)	R (S)	11
Chloridium virescens (*, a)	R, N (S, 20)	11	*Stachybotrys elegans* (997, a)	N (S)	11
Chrysosporium aff xerophillum (*, a)	R, A (S, 20)	6,8,11	*Stachybotrys chartarum* (922, a)	R, A, N (S, 20)	8,11
Chrysosporium sp. (951, a)	R (20)	11	*Talaromyces flavus var flavus* (838, t)	R, A, N (S, 20)	6,8
Cladosporium cladosporioides (953, a)	R, A, N (S, 20)	6,8,11	*Talaromyces stipitatus* (835, t)	R, A, N (S, 20)	6,8
Clonostachys rosea (930, a)	R, A, N (S, 20)	6,8,11	*Talaromyces sp.* (*, t)	R, A, N (S, 20)	6,8
Clonostachys sp. (*, a)	R, N (20)	11	*Thielavia heterotallica* (*, t)	R, A (S, 20)	6
Curvularia lunata (934, a)	N (S)	6,8,11	*Trichoderma hamatum* (*, a)	R, A, N (S, 20)	6,8
Cylindrocarpon didymun (962, a)	R, A, N (S, 20)	6,8,11	*Trichoderma harzianum* (*, a)	R, A, N (S, 20)	6,8
Cylindrocarpon lucidum (956, a)	R, N (S, 20)	6,8	*Trichoderma koningii* (*, a)	R, A, N (S, 20)	6,8
Cylindrocarpon olidum (923, a)	R, A, N (S, 20)	6,8,11	*Trichoderma saturnisporum* (788, a)	R, A, N (S, 20)	6,8

Table 1 continued..

Cylindrocarpon sp. (937, a)	R, A, N (S, 20)	6,8,11	*Verticillium albo-atrum* (941, a)	R (S, 20)	6,8
Chaetomium globosum (950, t)	A (20)	6	*Verticillium nigrescens* (939, a)	R, A (S, 20)	11
Doratomyces microsporus (*, a)	R (S)	6,8	*Volutella ciliata* (946, a)	A, N (S, 20)	8,11
Doratomyces stemonitis (*, a)	R, A, N (S, 20)	6,8,11	*Wardomyces inflatus* (955, a)	R, A (S, 20)	6,8
Epicoccum nigrum (940, a)	R, A, N (S, 20)	6,8,11	*Westerdikella dispersa* (830, t)	A (S, 20)	6,8
Fusarium equisetii (*, a)	N (20)	6	ZYGOMYCOTA		
Fusarium oxysporum (961, a)	R, A, N (S, 20)	6,8,11	*Absidia cylindrospora* (*)	N (20)	8
Fusarium semitectum (935, a)	R, A, N (S, 20)	6,8,11	*Absidia spinosa* (960)	R, A, N (S, 20)	6,8
Fusarium solani (936, a)	R, A, N (S, 20)	6,8,11	*Coemansia pectinata* (*)	R (S)	6
Fusarium sp. (*, a)	R, A, N (S, 20)	6,8,11	*Cunninghamella elegans* (985)	R, A, N (S, 20)	6,8
Humicola fuscoatra (*, a)	R, A, N (S, 20)	6,8	*Gongronella butleri* (991)	R, A, N (S, 20)	6,8
Humicola grisea (933, a)	R, A (S, 20)	6,8,11	*Mortierella sp.* (988)	R, A, N (S, 20)	6,8,11
Metarrhizium anisopliae (993, a)	R (S, 20)	8	*Mucor hiemalis* (982)	R (S, 20)	6,8,11
Microsphaeropsis olivacea (944, a)	N (20)	6	*Mucor mucedo* (*)	R, N (S, 20)	6,8,11
Myrothecium cinctum (*, a)	R, N (S, 20)	6,8,11	*Rhizopus stolonifer* (957)	R (S, 20)	6,8,11
Neosartorya stramenia (833, t)	N (S)	6,8	*Zygorrhynchus moelleri* (990)	A (S)	6
Neosartorya sp. (*, t)	R, A (20)	6,11	MYCELIA STERILIA		
Neurospora tetrasperma (837, t)	N (S)	6	Dematiaceous sterile mycelium1 (989)	R, A, N (S, 20)	6,8,11
Paecilomyces lilacinus (952, a)	R, A, N (S, 20)	6,8,11	Dematiaceous sterile mycelium 2 (*)	R, A, N (S, 20)	8,11
Paecilomyces lilacinus (983, a)	N (S)	6,8,11			
Penicillium chrysogenum (*, a)	R, A, N (S, 20)	6,8,11			

[a]Type of soil from where each fungal strain was isolated (Argialboll, A; Natracualf, N; Rendoll, R) on the superficial horizon (S), the horizon deeper than 20 cm (20) on agar-medium at several. [b]isolation pH values (6, 8, or 11). La Plata Spegazzini Culture Collection= [c]LPSC number. [d]anamorphic state. [e]telemorphic state. [*]without LPSC number.

conditions of the soil as depth increases determine changes in these communities. It has been observed that the horizons richer in organic matter, contained a higher species number and fungal biomass. Therefore the vertical distribution of the fungal communities may be attributed to the decrease in the availability of carbon observed in the deeper layers [15, 18, 19, 20]. Meanwhile Aon and Colaneri [21] showed that the biochemical activities tend to be higher in the most superfical layers of agricultural soil (0-8 cm) according to the concentration of nutrients, in particular, the availability of oxygen. On the other hand, the organic substrates in the different horizons of the soil are present in different degrees of decomposition and humification; therefore, the knowledge of the capacities of decomposition of the fungal species might also contribute to the characterization of their distribution pattern.

The previous results obtained by Elíades *et al*. [8] have shown that *Penicillium* and *Trichoderma* species are the most representative ones in the fungal communities of the upper horizons of calcic soils (Rendoll). On the other hand, species of *Acremonium*, *Aspergillus* and *Cladosporium cladosporioides* showed higher frequencies in the deeper horizons. However, these tendencies were not observed in sodic soils (Natracualf). Grishkan and Nevo [22] pointed out that the fungal diversity in a soil from Israel shows variations as depth increases and that this would be largely related to the conditions of the soil (temperature, humidity, and exposure to light). They concluded that the predominant species in the upper layers, i.e. in those with highest exposure to the light, were dematiaceous fungi and that, as depth increases, the main species were *Aspergillus* and *Penicillium*, since the deeper horizons are protected from diseccation and light. In previous studies carried out in alkaline soils from Argentina, the distribution of species of *Aspergillus* and *Penicillium* and dematiaceous species did not follow a pattern related to the different degree of exposure to light but rather to the conditions of pH [8, 9, 13]. Garret [22] pointed out that many species tend to decrease as depth increases, but that others are habitually found in the lower horizons. Most Zygomycota are usually confined to the upper horizons, whereas species of *Acremonium, Oididendron, Paecilomyces, Penicillium* and, *Sagenomella* are commonly found at higher depths [24].

Most fungi grow in acid soils; however, some of them are able to grow in alkaline conditions. The fungal especies were grouped according Nagai *et al*. [25] definition: i) alkalophobic strains, which could not grow at pH 10; ii) alkali-tolerant ones, which grew at pH 10; and iii) alkalophilic ones, which could grow at pH up to 10, but not at pH 5-6. Because of their complex interaction with other factors, and the local variation, the pH effects are hard to evaluate. Although fungi also have the ability to change the environment pH during their growth, *in vitro* they grow better in a pH ranging from 5 to 7. Some species grow, although more slowly, at extreme pH values. This capacity is related to the maintenance of an adequate internal ionic balance and pH [26]. Extreme environmental conditions such as high or low pH may inhibit fungal growth as well as enzyme activity and stability mainly because of the effect that H+/OH− concentration has on the availability and ionic forms of nutrients, the ionization of the enzymes active sites, and/or protein denaturation [25, 27, 28].

In calcareous soils like Rendolls, which usually pH varies between 7.5 and 8.5, alkalinity is usually originated in the presence of $CaCO_3$, whereas in sodic Natracualf soils, with pH >8, alkalinity is due to the dominant presence of the Na^+ ion in the interchange complex. Alkaline soils, i.e. those without sodium, can lead to a deficiency in certain nutrients such as iron, zinc, manganese and boron in the plants, whereas the presence of sodium can lead to aireation problems due to the deterioration of the physical structure, which, in turn, can induce changes in the availability of nutrients [29]. The decomposing action of soil fungi is essential for the contribution of organic matter to the ecosystems of saline soils [30]. Several authors such as Warcup [31], Stenton [32], Mukerji [33], Pugh and Dickinson [34], Rai *et al*. [35] and Nagai *et al*. [25, 36] have analyzed the fungal composition of alkaline soils. In most of these studies, the range of pH used in the isolation medium varied from acid to neutral. Since these acid media are not appropriate for the

growth of some alkalophilic fungi, it is necessary to take into account that the pH of the isolation medium is key for the evaluation of the total diversity of the fungal species. In this sense other authors such as Vardavakis [15], Cabello and Arambarri [12] have studied the mycobiota of soils with high contents of calcium carbonate.

In previous works on the fungal composition of the soil of *Celtis tala* and *Scutia buxifolia* forests of Magdalena, Province of Buenos Aires, Argentina [8, 12, 37] it was found a specific pattern of alkalophilic and alkali-tolerant fungal species associated with these calcareous soils, independently of the degree of disturbance. It was also observed that the variation in the pH of the isolation medium influenced the frequency of appearance of species and differences were detected in the composition of the communities according to the different pHs used for their isolation (6, 8 or 11). The specific differences found when using different values of pH in the isolation medium, suggests an adaptive response of the mycobiota, which might be also expressed in their natural habitat, with significant consequences for the ecosystem [12]. Besides, the changes in pH in natural conditions can alter the frequency distribution of these species [17]. *Acremonium* spp., *Acrostalagmus luteo- albus*, *Clonostachys rosea, Clonostachys* sp, *Cylindro-carpon didymun*, *C. olidum*, *Doratomyces micro-sporus, D. stemonitis*, *Fusarium* spp., *Humicola fuscoatra, H. grisea* and *Paecilomyces lilacinus* were found to be more frequently isolated at pH 8 and 11. The species of *Penicillium* and *Trichoderma* were isolated at acid pH. This fact is in agreement with Gams [38] who point that the species of *Penicillium* prefer low pH values whereas the species of *Trichoderma* present a positive correlation with the content of calcium in the soil. Among *Ascomycota,* the species of *Talaromyces* were isolated only up to pH 8 in all the soils. However, *Neosartorya stramenia* was isolated at pH 11 in the deeper horizon of the Rendoll [12]. Among the species of *Zygomycota, Absidia spinosa* and *Mortierella* sp. were isolated more frequently at pH 6 and 8. The species of *Aspergillus,* such as *A. niger*, *A. sidowii, A. terreus* and *A. ustus,* showed higher frequencies at pH 11. *Acremonium murorum*, which is an alkalophilic species [25], presented the highest frequencies at higher pH levels and also evidenced an augmentation as depth increased in all types of soil [8, 9]. These findings are of great interest since most fungi are dominant in acid soils because these low-pH environments are not appropriate for the proliferation of bacteria or actinomycetes; thus, fungi monopolize the use of organic substrates and degradation activity [39]. Cabello and Arambarri [12] observed that species of this genus were isolated mainly from acid culture media.

The pH of the isolation medium influences the number of species isolated. In the three types of soil the number of species isolated was higher in the deeper than 20 cm, in all the pHs of the isolation medium. The number of species isolated showed a decrease as the pH of the isolation medium increased.

Fungal abilities for enzyme production

Saprotrophic fungi have a great dispersal potential and an efficient enzymatic system that guarantees their biological role [38]. They can degrade a large variety of carbon sources, from simple sugars, such as hexose or pentose, to molecules more difficult to use, such as disaccharides, starch, pectin and fats, and have an essential role in the decomposition of structural polymers of plants, such as cellulose, hemicellulose and lignin. In soils with pH values between 7.2 and 10, there are species able to grow in highly alkaline environments. This adaptive capacity suggests that they are able to secrete alkaline-tolerant or alkaline-active enzymes required for the degradation of the substrates present in the soil.

There is currently a growing trend to replace some traditional chemical processes by biotechnological processes that involve the use of microorganisms and/or their enzymes, since they not only constitute a less aggressive alternative for the environment, but are also economically more viable. The development of enzymatic products of microbial origin is usually based on an initial screening of a large number of microorganisms adapted to different environmental conditions, with the aim to obtain enzymes with physical and biochemical characteristics for the particular application that wants to be carried out. These enzymes

are currently looked for in microorganisms that inhabit extreme environments [40].

The enzymatic abilities of the species isolated from a soil, varied according to the type of isolate tested, wich could be related with the subculturing time and the culture media used [41, 42]. Amylolytic, cellulolytic and proteolytic activities of the especies isolated from *Celtis tala* and *Scutia buxifolia* forests soils, were determined at two pH levels (6 and 9) (Table 2). Of the species tested 21% showed amylolytic activity in plate at alkaline pH [9]. Between them *Fusarium* species did not produce amylases with the culture conditions used, nevertheless the species of *Aspergillus* tested showed good amylolytic activity at pH 9. Meanwhile Hankin and Anagnostakis [43] observed activity in the species of *Fusarium* and did not detect amylolytic activity in species of *Aspergillus*. The cellulolytic ability of 55 soil fungi species was tested by Elíades [9] who found that 17% showed positive activity. Meenakshi [44] studied in detail the production of different alkaline enzymes produced by fungi and by Actinomycetes isolated from the soil and dead leaves in Thailand. This screening showed a higher percentage of cellulolytic activity in plate at alkaline pHs, 26% of the species tested showed positive activity. However, when this activity was evaluated in culture medium, only two showed activity. Meanwhile Söderström [45] determined that 52% of the species tested were able to degradate cellulose but at neutral pH values.

Of the species tested on their capacity to produce proteases, 54% presented positive activity at both 6 and 9 pH. Meenakshi [44] determined that 52% of the species tested on their capacity to produce proteases *in vitro* at pH 9.4 showed positive activity. Söderström [45] isolated 60 fungal species from a forest soil and separated them by their ability to use different substrates. He found that 85% of the species used proteins at neutral pHs. Hankin and Anagnostakis [43] pointed out that species of *Aspergillus, Fusarium,* and *Mucor* produced proteases *in vitro* assays at pH 6.0. Elíades [9] demostrate that species of Zygomycota showed no enzymatic activity *in vitro* at pH 9, which is consistent with the alcalophobic character of these fungi.

After cellulose, chitin is the second most abundant compound in the biosphere [46]. It is the main compound of the cytoskeleton of insects and an essential structural component of the cell wall of fungi. Based on genomic data, Seidl [47] was able to establish that bacteria produce between two and four chitinases, whereas the fungal genome contains genes that can express between 10 and 25 different chitinases. Elíades [9], determinate the chitinolytic activity in plate of 32 fungal species isolated from alkaline soils and demostrate that 28 % of the strains tested were able to degradate cromogenic substrate Chitin Azure. In all the strains the degradation was evident both 6 and 9 pH. The reason why fungi possess so many chitinases is not well known. However, three potential physiological roles of these enzymes are currently being studied. These include: the degradation of the external chitin present in the remainings of dead hyphae and exoskeletons of arthropods, the use of degradation products as nutrient sources, the remodeling of fungal cells during hyphal growth, branching, fusion and autolysis and, finally, competition and defense against other fungi or arthropods. Seidl [47] also pointed out that there are diverse subgroups of chitinases and certain species present a higher number of enzymes involved in the degradation of chitin than others. Among the species analyzed on their capacity to degrade Chitin Azure, *Metharrhizium anisopliae,* a species which has been reported to be entomopathogenic, showed positive activity [48]. Seidl [47] found that *Stachybotrys elegans* and *M. anisopliae* express specific chitinases in the culture medium.

Elíades *et al*. [41] determined the capacity to produce keratinolytic enzymes from crude extracts obtained from solid and liquid media. In this study *Acremonium murorum*, *Acremonium* sp.1, *Aspergillus niger*, *Aspergillus sidowii*, *Chrysosporium sp.*, *Cladosporium cladosporioides*, *Cylindrocarpon didymun*, *Cylindrocarpon olidum*, *Metarrhizium anisopliae*, *Neurospora tetrasperma*, *Paecilomyces lilacinus*, *Periconia minutissima* and *Westerdikella dispersa* showed keratinolytic activity. These species could thus be of interest for the isolation of alkaline proteases that can efficiently degrade keratine. None of the species tested showed differences depending on the culture medium analyzed. The values of activity found in saprotrophic species isolated from the soil were similar to those obtained for *Trichophyton ajelloi*, a human dermatophyte [49, 50]. The ability

Table 2. Screening for enzymatic activities (amylase; cellulase; chitinase; keratinase; protease). The symbols + and – indicate presence or absence of enzyme production respectively.

Tested strains	amylase	chitinase	cellulase	keratinase	protease	Tested strains	amylase	chitinase	cellulase	keratinase	protease
A. cerealis (928[c], a[d])	+		-	+	+	*P. frequentans* (*, a)	-		-		-
A. killiense (958, a)	+		-	+	+	*P. megasporum* (*, a)					-
A. murorum (927, a)		+	-	+	+	*P. restrictum* (954, a)			-		-
A. rutilum (*, a)	-		-		-	*P. rubrum* (*, a)					-
Acremonium sp. 1(*, a)				+	-	*P. thomii* (945, a)	-		-		+
Acremonium sp. 2 (*, a)	-					*Penicillium sp. 1*(*, a)					
A. luteo-albus (427, a)	-	-	-	+	+	*Penicillium sp. 2* (*, a)					
A. alternata (*, a)	-		-			*Penicillium sp. 3* (*, a)					
A. niger (845, a)	+	+	-	+	+	*P. guepinii* (929, a)	+	-	-	-	+
A. sidowii (931, a)	+	+	-	+	+	*Phialocephala sp* (*, a)	-		+	-	+
A. terreus (*, a)	+	+	-	+	+	*P. fastigiata* (942, a)					+
A. terreus (994, a)	+	+	-	+	+	*Phoma herbarum* (*, a)	-		-		-
A. ustus (981, a)	-		-	+		*S. brevicaulis* (947, a)	+	+	-		+
C. virescens (*, a)						*S. elegans* (997, a)	-		-		+
C. aff xerophillum (*, a)	+		-		+	*S. chartarum* (922, a)	-	+	+	+	+
Chrysosporium sp. (951, a)	+		-	+	+	*T. flavus* var *flavus* (838, t)	-		-		-
C. cladosporioides (953, a)	-	-	-	+	-	*T. stipitatus* (835, t)	-	-	-	+	-
C. rosea (930, a)	-	-	-	+	-	*Talaromyces sp.* (*, t)					
Clonostachys sp. (*, a)	-		-			*T. heterotallica* (*, t)	-		-		-
C. lunata (934, a)						*T. hamatum* (*, a)	-				-
C. didymun (962, a)	-	-	-	+	-	*T. harzianum* (*, a)	-				+

Table 2 continued..

C. lucidum (956, a)	-	-	-		-	*T. koningii* (*, a)	-				-
C. olidum (923, a)	+	-	-	+	+	*T. saturnisporum* (788, a)	-	-			+
Cylindrocarpon sp (937, a)						*V. albo-atrum* (941, a)	-	-	-	+	+
C. globosum (950, t)		-				*V. nigrescens* (939, a)	-	-	-		+
D. microsporus (*, a)						*V. ciliata* (946, a)	-		-		-
D. stemonitis (*, a)				-	-	*W. inflatus* (955, a)		-			-
E. nigrum (940, a)	-		-		+	*W. dispersa* (830, t)	+		+	+	+
F. equisetii (*, a)					-	ZYGOMYCOTA					
F. oxysporum (961, a)	-	-	-	+	+	*A. cylindrospora* (*)					
F. semitectum (935, a)	-	-	-	+	+	*A. spinosa* (960)	-		-		-
F. solani (936, a)	-	-	+	+	+	*C. pectinata* (*)	-		-		-
Fusarium sp. (*, a)						*C. elegans* (985)	-		-		-
H. fuscoatra (*, a)	-		-		-	*G. butleri* (991)		-			
H. grisea (933, a)	-	+	-	-	-	*Mortierella sp*. (988)		-			
M. anisopliae (993, a)	-	+	-	+	+	*M. hiemalis* (982)					
M. olivacea (944, a)	-		-		-	*Mucor mucedo* (*)					
Myrothecium cinctum (*, a)	-	-	-		-	*R. stolonifer* (957)					
N. stramenia (833, t)			-		-	*Z. moelleri* (990)	-		-		-
Neosartorya sp. (*, t)						*MYCELIA STERILIA*					
N. tetrasperma (837, t)	-		+	+	+	D. sterile mycelium1 (989)	-	-	-	-	+
P. lilacinus (952, a)	-	-	-	+	+	D. sterile mycelium *2* (*)	-	-	-	-	+
P. lilacinus (983, a)	-	-	-	+	+						
P. chrysogenum (*, a)	-		-		+						

[c]telemorphic state. [d]anamorphic state. [*]without LPSC number.

to produce proteolytic enzymes that degradde keratine is widely distributed among the members of the fungal community of alkaline environments. The characterization of the enzymatic pool could be of interest in leather technology not only for the removal of hair but also for other humid processes (such as soaking and re-liming), where the non-colagenic proteolytic action is of great importance. In addition, a faster disorganization of the keratine fibers may shorten the time of composting in the degradation process of the hair residue [49, 50].

It should be pointed out that it would be of interest to find out saprotrophic, non-pathogenic organisms able to produce these enzymes in an environment where alkalinity is a natural condition and where they can express their maximal enzymatic capacity.

CONCLUSION

We propose that the fungal species isolated from alkaline soils, such here studied, is a contribution to the knowledge of the fungal diversity with biotechnological potential. In this sense, this finding shows a review about ability to produce alkaline enzymes by autochtonous soil fungi from *Celtis tala* and *Scutia buxifolia* forests and related regions. At present there are no meaning full data available that integrate the knowledge of the mycobiota associated with alkaline soils in Argentina and the enzymatic abilities with biotechnological interest from those fungi.

From these data is evidence that some species have a great potential for producing several enzyme activities like *Acremonium* spp., *Aspergillus* spp., *Stachybotrys chartarum*, *Westerdikella dispersa,* while others only showed few enzyme abilities like Zygomycota.

The detection of alkaline enzymatic activities in most of the strains isolated in alkaline soils, indicates that the fungi here have an enzymatic pool suitable for their growth and development in extreme environments.

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REFERENCES

1. Gaspar, M. L., Cabello, M. N., Pollero, R. J., and Aon, M. A. 2001, Current Microbilogy, 42, 339-334.
2. Kjøller, A. and Struwe, S. 1982, Oikos, 39, 389- 422.
3. Schürer, J., Clarolm, M., and Rosswoll, T. 1985, Soil biology and Biochemistry, 17, 611- 618.
4. Trappe, J. M. and Luoma, D. L. 1992, The fungal community, Carroll, G. and Wicklow, D. T. Marcel Dekker (Eds), New York, USA, pp 17-27.
5. Ritz, K. and Young, I. M. 2004, Mycologist, 18(2), 52-59.
6. Janos, D. P. 1980, Biotropica, 12, 56-64
7. Wright, S. F. and Upadhyaya, A. 1998, Plant and Soil, 198, 97-107.
8. Elíades, L. A., Cabello, M. N., and Voget C. E. 2006, Soil microfungi Diversity in Celtis tala and Scutia buxifolia forests in Eastern Buenos Aires Province (Argentina), 2(2), 229-249.
9. Elíades, L. A. 2009, Tesis doctoral, Fac. De Ciencias Naturales y Museo, UNLP, pp 198.
10. Giri, B., Giang, P. H., Kumari, R., Prasad, R., and Varma, A. 2005, Micro-organisms in soils: Roles in genesis and functions, Buscot, F. and Varma, S. (Eds.), Heidelberg, Germany: Springer-Verlag, 195-212.
11. Burges, A. and Fenton, E. 1953, Transactions of British Mycological Society, 36, 104-110.
12. Cabello, M. N. and Arambarri, A. M. 2002, Diversity in soil fungi from Microbiological Research, 157, 115-125.
13. Elíades, L. A., Bucsinszky, A. M., and Cabello, M. N. 2004, Boletín Micológico, 19, 41-47.
14. Cabello, M. N., Aon, M. A., and Velázquez, M. S. 2003, Boletín de la Sociedad Argentina de Botánica, 38, 225-232.

15. Vardavakis, E. 1990, Mycologia, 82, 715-726.
16. Bills, F. G., Christensen, M., Powell, M., and Thorn, G. 2004, Biodiversity of fungi: Inventory and monitoring methods, Muller, G. M., Bills, F. G., and Foster M. S, (Eds) Elsevier Academic press, San Diego. Cap., 13, 271-302.
17. Burges, A. 1963, Transactions of British Mycological Society, 46, 1-14.
18. Pandey, R. R., Chaturvedi, A. P., and Dwivedi, R. S. 1991, Proc. Nat. Acad. Sci. India Sec. B-Biol. Sci., 61, 97-108.
19. Bogoev, V. M., Kenarova, A. E., Vasilev, V. L., and Gyosheva, M. M. 2002, Folia Microbiologica, 47, 56-60.
20. Fierer, N. Schimel, J. P., and Holden, P. A. 2003, Soil Biology and Biochemistry, 35, 167-176.
21. Aon, M. A. and Colaneri, A. C. 2001, Applied Soil Ecology, 18, 255-270.
22. Grishkan, I. and Nevo, E. 2008, Giornale Botanico Italiano, 142(1), 51-58.
23. Garret, S. D. 1951, New Phytologist, 50, 149-166.
24. Widden, P. and Parkinson, D. 1973, Canadian Journal of Botany, 51, 2275-2290.
25. Nagai, K., Sakai, T., Ratiatmodjo, R. M., Suzuki, K., Gams, W., and Okada, G. 1995, Mycoscience, 36, 247-256.
26. Battley, E. H. and Bartlet, E. J. 1966, Antonie van Leeuwenhoek, 32, 245- 255.
27. Pointing, S. B., Buswell, J. A., Gareth Jones, E. B., and Vrijmoed, L. L. P. 1999, Mycological Research, 103, 696-700.
28. Rosset, J. and Barlocher, F. 1985, Aquatic Transactions of British Mycological Society, 84, 137-145.
29. Marschner, H. 1995, Mineral nutrition of higher plants, 2nd Edition, Academic press, London, p 889.
30. Torzilli, A. P., Sikaroodi, M., Chalkley, D., and Gillevet, P. M. 2006, Mycologia, 98, 690-698.
31. Warcup, J. H. 1951, Transactions of the British Mycological Society, 34, 376-399.
32. Stenton, H. 1953, The soil fungi of Wicken Fen, Transactions of the British Mycological Society, 36, 304-314.
33. Mukerji, K. G. 1965, Mycopathology and Mycology Applied, 29, 339-341.
34. Pugh, G. J. F. and Dickinson, C. H. 1965, Transactions of the British Mycological Society, 48, 279-285.
35. Rai, J. N., Agarwal, S. C. and Tewari, J. P. 1971, Journal of the Indian Botanical Society, 50, 63-74.
36. Nagai, K., Suzuki, K., and Okada, G. 1998, Mycoscience, 39, 293-298.
37. Elíades, L. A., Voget, C., and Cabello, M. N. 2006, Darwiniana, 44(1), 64-73.
38. Gams, W. 1992, Fungi in vegetation science, Winterhoff, W. (Ed.), Kluwer Academic publishers, London, pp 184.
39. Bolton, H. Jr., Fredrikson, J. K., and Elliot, L. E. 1993, Soil microbial ecology Metting, Marcel Dekker, F. B. Jr. (Ed), New York, 27-63.
40. Kladwang, W., Bhumirattana, A., and Hywel-jones, N. 2003, Fungal Diversity, 13, 69-84.
41. Elíades, L. A., Voget, C. E., Saparrat, M. C. N., and Cabello, M. N. 2010, World Journal of Microbiology and Biotechnology, 26(11), 2105.
42. Domsch, H. K. and Gams, W. 1969, Soil Biology and Biochemistry, 1, 29-36.
43. Hankin, L. and Anagnostakis, S. L. 1975, Mycologia, 67, 597-607.
44. Meenakshi, V. R. 2004, Project Completition Report, National Chemical Laboratory, Pune pp 421.
45. Söderström, B. E. 1975, Transactions of the British Mycological Society, 65, 419-425.
46. Tharanathan, R. N. and Kittur, F. S. 2003, Reviews in Food Science and Nutrition, 43, 61-87.
47. Seidl, V. 2008, Fungal Biology Reviews, 22, 36-42.
48. Freimoser, F. H., Hu, G., and S. T. Leger, R. J. 2005, Microbiology, 151, 361-371.
49. Galarza, B. C., Goya, L. M., Cantera, C. S., and Reinoso, H. E. 2002, Biotransformación fúngica del pelo vacuno. Parte I. Tecnologia del Cuero, 47, 19-27.
50. Galarza, B. C., Goya, L. M., Cantera, C. S., Reinoso, H. E. and Lopez, L. M. I. 2004, JSLTC 88 nº 3, 93-98.