

## Composition and characterization of fungal communities from different composted materials

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**Résumé** – L'analyse des communautés de champignons provenant des composts préparés avec différentes matières premières a été menée pour évaluer l'abondance et la fréquence des espèces qui pourraient constituer un risque pour les plantes, les animaux ou la santé humaine. Un total de  $40\,405 \times 10^3$  propagules correspondant à 90 espèces a été dénombré dans 30 échantillons de deux composts de composition différente. Douze de ces espèces sont thermo-tolérantes, trois sont thermophiles et les autres sont des espèces mésophiles. *Acrodontium crateriforme*, est l'espèce la plus abondante, présente dans presque la moitié des échantillons de compost préparé principalement à partir de déchets de poils de l'industrie du cuir. D'autres espèces, *Aspergillus* spp., *Monocillium mucidum*, *Penicillium* spp., *Paecilomyces variotii*, *Candida* sp. et *Humicola grisea* var. *thermoidea* étaient aussi présentes. Le compost composé de déchets de *Ligustrum* et d'écorces de riz mélangés avec des déjections de poulets est caractérisé par la présence de *Aspergillus fumigatus*, espèce présente dans presque tous les échantillons, et par *Penicillium* spp., *Fusarium* spp., *Emericella nidulans*, *Emericella rugulosa* et *Humicola fuscoatra*. Toutes ces espèces ont été mentionnées dans d'autres composts de différentes origines. Plusieurs d'entre elles sont importantes dans la biodégradation et d'autres sont des antagonistes *vis-à-vis* des agents pathogènes. Les deux composts peuvent être utilisés séparément ou ensembles pour améliorer la nutrition du sol et participer à la lutte biologique.

**Abstract** – The analysis of fungal communities of two composts prepared with different raw materials were conducted to evaluate the abundance and frequency of species that could constitute a risk for plant, animal or human health. A total of  $40\,405 \times 10^3$  propagules corresponding to 90 species were found in 30 samples of two composts of different composition. Twelve of the species were thermotolerant, three were thermophilic and the other species were mesophilic. *Acrodontium crateriforme* present in nearly half of the samples of compost prepared mainly with hair waste from leather industry was the most abundant species. Several other species as *Aspergillus* spp., *Monocillium mucidum*, *Penicillium* spp., *Paecilomyces variotii*, *Candida* sp. and *Humicola grisea* var. *thermoidea* were present. The community prepared with *Ligustrum* pruning wastes and rice hull mixed with chicken mess was characterized by *Aspergillus fumigatus*, present in nearly all samples, and by *Penicillium* spp., *Fusarium* spp., *Emericella nidulans*, *Emericella rugulosa* and *Humicola fuscoatra*. All the species are commonly found in composts of different origins. Several are important in biodegradation and in suppressive pathogen abilities. Both composts can be used separately or together to enhance the nutritional and suppressive abilities of soils.

**Basidiomycetes anamorphs / oleaginous fungi / thermophilic / thermotolerant / mesophilic fungi**

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## INTRODUCTION

Composting is the biological conversion of solid organic waste into fertilizer and other products, in a stable substance that can be handled, stored, transported and applied to field without affecting the environment. The high organic matter content and the biological activity make compost also effective in erosion control, biofiltration, bioremediation and production of biogas (Anastasi *et al.*, 2005). The use of composts could constitute a suitable way to avoid some foreign chemical products and also constitute soil conditioner and organic fertilizer (Hoitink & Boehm, 1999). Substantial changes in chemical composition, microbial populations and species abundance during the various temperature stages along the composting process occur. At the end of the process, when compost is ready to be used and material is no longer self heating, temperatures fall and the finished compost has a high microbial diversity (Persson *et al.*, 1995).

Fungi play an important role in composting and compost applications, especially for their ability to use complex carbon sources and to suppress soil-borne and foliar plant pathogens (Hadar & Mandelbaum, 1992). Probably their presence were not incidental as nearly all of them are capable of degrading at least one of the major polymer component of hemicelluloses, cellulose and pectin in litter and other organic resources (Domsch *et al.*, 1980). Proper composting effectively destroys pathogen and weed seeds through the metabolic heat generated by microorganisms during the process (Crawford, 1983), but composts may be re-colonized after the end of the process mainly by soil microorganisms (Chang & Hudson, 1967).

The use of hair save unhairing techniques has conducted to an increasing production of waste hair in the leather manufacturing industry. The composting hair waste of bovine hide for agricultural fertilization is one of its most promising uses due to the high nitrogen content of hair (Barrena *et al.*, 2006). The complementary plant waste, as co-substrate, gives a more stable product at the end of the process. Composting ornamental plant wastes and wastes with high nitrogen content co-substrates is a frequent practice for organic fertilizer production.

The aim of this study was to describe the species composition and their abundance in fungal communities present in commercial mature composts produced with different waste materials and used in agriculture, in order to detect potential plant, animal or human pathogens.

## MATERIALS AND METHODS

### Composts sampling procedure

Two types of commercial composts prepared with different waste products were analyzed. One was prepared using 50% of waste hair from the leather manufacturing industry, 45% of bovine rumen and 5% of *Eucalyptus* sp. sawdust as raw materials (HL). The other was prepared with rice hull mixed with chicken mess 50% and 50% of *Ligustrum* sp. pruning waste (LG).

In the manufactures, composts were stocked in piles of the following dimensions: 0.90 m high  $\times$  1.5 m large  $\times$  150 m long. From each of the 15 piles of both types of composts, five fresh samples of 100 g were taken from the 10 cm under the surface and nearly 500 g of this composed sample was placed in a

polyethylene bag, transported to laboratory and stored at 5°C until processing one day after sampling. Then nearly 100 g of each sample were dried to determine the percentage of compost humidity. A quantity of fresh compost equivalent to 10 g of dry compost was used to fungal analysis of samples.

### **Fungal isolation**

Fungal isolations were performed using the dilution plate method. Colonies obtained by this method are derived from fungi that have exploited any material. Compost dilution was prepared using the equivalent of 10 g of dry compost in 90 ml of sterile distilled water and diluted to obtain 1: 1000. One ml of this final dilution was deposited onto Malt-Agar 2% Chloramphenicol 100 µg/l medium. Ten plates from each compost sample were incubated at 25°C and 37°C. The emerging colonies were successively numbered. Each colony was subcultured for identification by means of conventional mycological methods and the main current taxonomic sources were used in identification procedures. Cultures which failed to sporulate following incubation under black light or those for which conventional mycological methods resulted in an uncertain identification were identified by means of molecular methods. DNA was extracted and purified following the protocol of Lee & Taylor (1990). ITS rDNA was amplified with primers ITS 4 and ITS 5 (White *et al.* 1990), visualized with UV light in agarose gel (1%) and the amplified segments were sequenced by Macrogen Korea.

### **Data analysis**

The relative density of isolation was calculated as the number of propagules forming colonies of a given fungus, by gram of dry compost. To evaluate to which extent the complete fungal community was revealed by sampling, the abundance distribution and species accumulation curves of composts incubated at both temperature were performed. Moreover the relative abundance curves were compared to lognormal theoretical model using the Kolmogorov-Smirnov test (Krebs, 1989). Diversity was measured for each incubation condition of both composts by means of Shannon diversity index with computer package MVSP for Windows (Kovach Computing, Anglesey, UK).

To evaluate differences in fungal composition among composts, a simple correspondence analysis using STATISTICA Data Analysis Software Products was carried out using the species with the mean number of propagules equal or higher to  $10 \times 10^3$  per gram dry compost, at least, in any of the composts at 25°C or 37°C (Howard & Robinson, 1995).

## **RESULTS**

A total of  $40\,405 \times 10^3$  propagules corresponding to 90 species were found in 30 compost samples,  $24\,845 \times 10^3$  propagules belonging to 48 taxa from 15 samples of HL compost and  $15\,560 \times 10^3$  propagules belonging to 62 taxa from 15 samples of LG material. A great variability in the number of propagules (Table 1) and in the species composition per g of dry compost (Table 2) was observed in each of the 15 samples of HL and of the 15 samples of LG at both

incubation temperatures. The number of propagules was not proportional to the number of taxa found in the different samples, *i.e.* in HL at 25°C samples 4 and 15 contained the highest number of propagules (respectively  $1832 \times 10^3$  and  $1830 \times 10^3$ ) with 3 and 6 taxa respectively when the lowest number of propagules (31) was observed in sample 3 with 5 taxa. The number of propagules was irregular for a given taxa found in different samples of the same compost at the same incubation temperature, *i.e.* *Acrodontium crateriforme* (J.F.H. Beyma) de Hoog with  $5\,263 \times 10^3$  propagules/g dry compost in HL4 and  $12 \times 10^3$  or  $34 \times 10^3$  propagules in HL2 and HL1 respectively, at 37°C, *Monocillium mucidum* W. Gams with  $1752 \times 10^3$  propagules in HL10,  $6 \times 10^3$  in HL12 and  $171 \times 10^3$  in HL1 at 37°C, *Paecilomyces variotii* Bainier with  $1070 \times 10^3$  propagules in HL15 and  $209 \times 10^3$  propagules in HL13 at 25°C. To have an overview of the global composition of fungal communities of the two composts, the mean number of propagules per g of dry compost was calculated (Table 2).

The species abundance distribution of both compost at 25°C and 37°C fit with lognormal distribution ( $P > 0.05$ ) (Figure 1). The species accumulation curve evidenced the number of species found in each additional sample. The point of achieving the asymptote was after the 12 samples of both composts incubated at 37°C, but when incubated at 25°C yet after the 15 samples of both composts they did not achieve the asymptote (Figure 2).

Table 1. Fungal propagules and taxa from hair (HL) and waste green (LG) composts.

Samples	Hair composts (HL)				Ligustrum waste composts (LG)			
	25°C		37°C		25°C		37°C	
	N° prop.	N° taxa	N° prop.	N° taxa	N° prop.	N° taxa	N° prop.	N° taxa
sample 1	743	9	594	6	423	14	103	9
sample 2	909	10	630	5	755	7	1 321	5
sample 3	31	4	40	3	200	5	211	5
sample 4	1 832	2	5 271	4	1 687	10	1 330	5
sample 5	43	6	253	5	762	8	815	8
sample 6	938	8	425	6	863	10	666	6
sample 7	759	5	458	8	243	5	291	8
sample 8	1 262	7	496	6	524	8	190	2
sample 9	1 495	8	503	4	198	9	266	4
sample 10	451	5	1 836	3	452	6	267	3
sample 11	716	3	12	2	813	8	584	7
sample 12	262	2	100	7	533	7	360	8
sample 13	638	6	137	3	185	7	190	3
sample 14	120	3	1 710	4	57	10	116	4
sample 15	1 830	6	350	4	678	10	478	5
Total N° of propagules and taxa	12 029	39	12 816	28	8 373	47	7 187	35

N° prop.: Number of fungal propagules  $\times 10^{-3}$  for dry g of each sample

Table 2. Species composition and mean number of fungal propagules  $\times 10^{-3}$ /g dry HL and LG composts incubated at 25°C and 37°C.

<i>Taxa</i>	<i>code</i>	<i>HL</i> 25°C	<i>HL</i> 37°C	<i>LG</i> 25°C	<i>LG</i> 37°C
<i>Absidia coerulea</i> Bainier				1,21	
<i>Absidia corymbifera</i> (Cohn) Saccardo & Trotter	<i>Abc</i>	18,07	18,80		2,11
<i>Absidia cylindrospora</i> Hagem		1,33			
<i>Acrodontium crateriforme</i> (J.F.H. Beyma) de Hoog	<i>Acr</i>	137,43	353,96		
<i>Allescheriella crocea</i> (Mont.) S. Hughes					0,38
<i>Amblyosporium spongiosum</i> (Pers.) S. Hughes			6,80		
<i>Arthrotrichum</i> sp.		3,26	0,24		
<i>Ascochyta pisi</i> Lib.				0,38	
<i>Aspergillus caespitosus</i> Raper & Thom	<i>Aca</i>	1,61		4,20	11,03
<i>Aspergillus flavus</i> Link	<i>Afl</i>	22,33	13,80	2,03	1,07
<i>Aspergillus fumigatus</i> Fresen.	<i>Afu</i>	13,41	180,49	107,34	290,24
<i>Aspergillus niger</i> Tiegh.	<i>Ani</i>	0,81	0,80	10,21	6,96
<i>Aspergillus niveus</i> Blochwitz			8,13		
<i>Aspergillus ochraceus</i> K. Whillh.				0,85	
<i>Aspergillus terreus</i> Thom	<i>Ate</i>	3,13	10,40	12,41	3,73
<i>Aspergillus ustus</i> (Bainier) Thom & Church					0,50
<i>Candida</i> sp.(C1)	<i>Can</i>	39,20			
<i>Cephalophora tropica</i> Thaxt.		0,80			
<i>Chaetomium</i> sp.				0,76	
<i>Chrysosporium pannorum</i> (Link) S. Hughes		1,21	2,18		
<i>Chrysosporium</i> sp.					1,40
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	<i>Cla</i>	3,49		9,87	
<i>Cunninghamella echinulata</i> (Thaxt.) Thaxt.		8,67			
<i>Emericella nidulans</i> (Eidam) Vuill.	<i>Emn</i>	17,47	13,00	28,93	29,37
<i>Emericella rugulosa</i> (Thom & Raper) C.R. Benj.	<i>Emr</i>			15,57	20,63
<i>Eupenicillium brefeldianum</i> (B.O. Dodge) Stolk & D.B. Scott		0,73			
<i>Exophiala jeanselmei</i> (Langeron) McGinnis & A.A. Padhye					0,76
<i>Fusarium acuminatum</i> Ellis & Everh.		0,80			
<i>Fusarium equiseti</i> (Corda) Sacc.	<i>Fue</i>			10,59	
<i>Fusarium oxysporum</i> Schlecht.		0,49		5,85	
<i>Fusarium solani</i> (Mart.) Sacc.	<i>Fus</i>	0,40		15,68	1,53
<i>Gliocladium catenulatum</i> J.C. Gilman & E.V. Abbott				1,27	
<i>Gliocladium nigrovirens</i> J.F.H. Beyma					1,80
<i>Gliocladium roseum</i> Bainier				1,43	
<i>Gliomastix murorum</i> (Corda) S. Hughes					0,38
<i>Graphium putredinis</i> (Corda) S. Hughes		1,53		0,05	
<i>Histoplasma capsulatum</i> Darling	<i>His</i>	1,33	3,27	2,20	9,73

Table 2. Species composition and mean number of fungal propagules  $\times 10^{-3}/\text{g}$  dry HL and LG composts incubated at 25°C and 37°C. (continued)

<i>Taxa</i>	<i>code</i>	<i>HL</i> 25°C	<i>HL</i> 37°C	<i>LG</i> 25°C	<i>LG</i> 37°C
<i>Humicola fuscoatra</i> Traaen	<i>Huf</i>		2,47	9,43	27,71
<i>Humicola grisea</i> Traaen					1,60
<i>Humicola grisea</i> var. <i>thermoidea</i> Cooney & R. Emers.	<i>Hgt</i>		49,87		
<i>Hyalodendron lignicola</i> Diddens				0,87	1,60
<i>Monocillium mucidum</i> W. Gams	<i>Mon</i>	15,70	128,63		
<i>Mortierella hyalina</i> (Harz) W. Gams		7,53			
<i>Mucor circinelloides</i> Tiegh.				0,33	
<i>Mucor hiemalis</i> Wehmer	<i>Muh</i>	18,25		4,52	
<i>Myrothecium</i> sp.				4,78	
<i>Neosartorya fischeri</i> (Wehmer) Malloch & Cain		0,60	0,40		
<i>Paecilomyces fulvus</i> Stolk & Samson				2,80	
<i>Paecilomyces variotii</i> Bainier	<i>Pav</i>	85,27	3,27	4,07	
<i>Paecilomyces carneus</i> (Duché & R. Heim) A.H.S. Br. & G. Sm.					1,60
<i>Penicillium chrysogenum</i> Thom				0,23	
<i>Penicillium citreonigrum</i> Dierckx	<i>Pci</i>	98,47	34,20		
<i>Penicillium citrinum</i> Thom		0,81			
<i>Penicillium decumbens</i> Thom	<i>Pde</i>	118,73			
<i>Penicillium funiculosum</i> Thom					1,80
<i>Penicillium islandicum</i> Sopp			0,13		
<i>Penicillium minioluteum</i> Dierckx	<i>Pmi</i>	42,33		103,20	3,20
<i>Penicillium mirabile</i> Beliakova & Milko				5,20	
<i>Penicillium piceum</i> Raper & Fennell		1,67	4,33		5,07
<i>Penicillium purpurogenum</i> Stoll	<i>Ppu</i>	77,40	11,68	29,22	4,07
<i>Penicillium</i> sp.	<i>Pen</i>	38,00	1,13	70,49	4,96
<i>Penicillium variabile</i> Sopp			1,87		
<i>Penicillium verrucosum</i> Dierckx	<i>Pve</i>			44,93	8,00
<i>Phialemonium obovatum</i> W. Gams & McGinnis					0,38
<i>Phoma eupyrena</i> Sacc.				0,93	
<i>Phoma nebulosa</i> (Pers.) Berk.				6,09	
<i>Rhizopus oryzae</i> Went & Prins. Geerl.			0,67		
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.		1,33			
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier		6,90	1,53		
<i>Scytalidium thermophilum</i> (Cooney & R. Emers.) Austwick.					6,53
<i>Sporothrix fungorum</i> de Hoog & G.A. de Vries				0,76	
<i>Sporothrix inflata</i> de Hoog		0,40			
<i>Sporothrix</i> sp.	<i>Sps</i>			8,33	11,13
<i>Staphylotrichum coccosporum</i> J.A. Mey. & Nicot			0,13		
<i>Syncephalastrum racemosum</i> Cohn			0,67		

Table 2. Species composition and mean number of fungal propagules  $\times 10^{-3}/g$  dry HL and LG composts incubated at 25°C and 37°C. (continued)

Taxa	code	HL 25°C	HL 37°C	LG 25°C	LG 37°C
<i>Talaromyces flavus</i> (Klöcker) Stolk & Samson		0,87			
<i>Thermomyces lanuginosus</i> Tsikl.	The			3,20	12,80
<i>Trichoderma harzianum</i> Rifai	Trh			11,79	0,07
<i>Trichoderma koningii</i> Oudem.				2,27	
<i>Trichoderma longibrachiatum</i> Rifai				0,05	
<i>Trichoderma</i> spp.				0,88	
<i>Lecanicillium psalliotae</i> (Treschew) Zare & W. Gams		1,00		0,87	
<i>Verticillium</i> sp.		9,11	1,53		
White sterile mycelia	Ste			1,53	0,87
Sterile mycelia 15	Ste			1,60	
Sterile mycelia 18	Ste				3,27
Grey sterile mycelia	Ste			0,67	
Grey sterile mycelia (23)	Ste			6,53	1,27
Dark sterile mycelia	Ste			1,73	
Dark sterile mycelia 20	Ste				1,60
<b>Total number of taxa 91</b>		<b>48</b>		<b>62</b>	
<b>Total mean number of isolates</b>		<b>801,87</b>	<b>854,37</b>	<b>558,17</b>	<b>479,15</b>

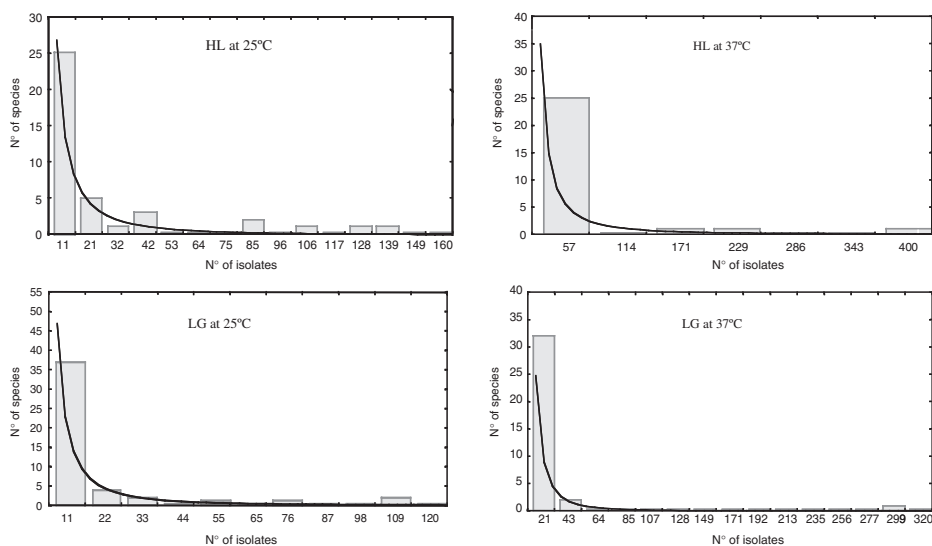


Fig. 1. Lognormal of species abundance distribution from each compost incubated at 25°C and 37°C. Few species were isolated with high frequency and several were rare. The lognormal distribution expected (line) did not differ significantly ( $P > 0.05$ ) from the observed data (Kolmogorov-Smirnov). HL: hair from leather manufacturing industry; LG: *Ligustrum* pruning waste.

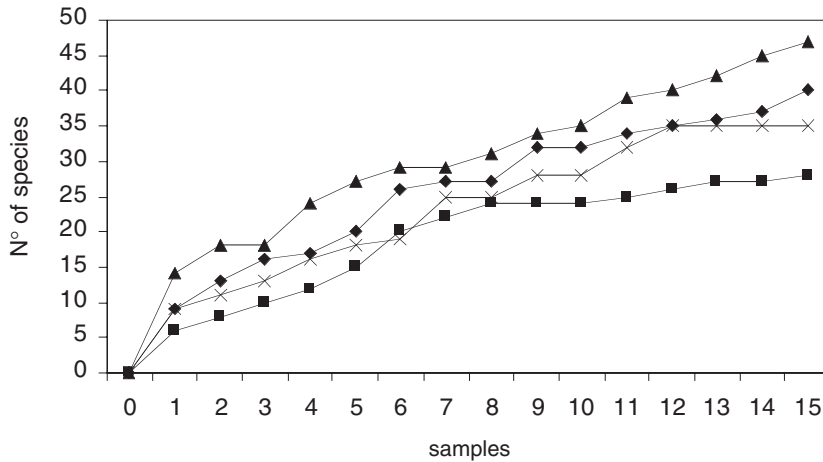


Fig. 2. Species accumulation curves showing the number of species found in each additional compost sample. Symbols indicate: ▲: LG at 25°C; X: LG at 37°C; ◆: HL at 25°C; ■: HL at 37°C. HL: hair from leather manufacturing industry; LG: *Ligustrum* pruning waste.

Table 3. Measures of Index of Diversity for compost fungal communities.

Diversity of composts	Shannon diversity Index ( $H'$ )	Evenness ( $J$ )	Total number of species in each compost ( $S$ )
HL compost			
25°C	2.691	0.730	40
37°C	1.856	0.557	28
LG compost			
25°C	2.770	0.720	47
37°C	1.809	0.509	35

HL: hair from leather manufacturing industry; LG: *Ligustrum* pruning waste.

The Index of diversity of fungal species was higher at 25°C than at 37°C in both composts (Table 3), and several species were exclusively found at 25°C (the most abundant being *Candida* sp., *Cunninghamella echinulata* (Thaxt.) Thaxt., *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Fusarium equiseti* (Corda) Sacc. *Mortierella hyalina* (Harz.) W. Gams, *Mucor* spp., *Penicillium mirabile* Beliakova & Milko, *Phoma* spp.) or at 37°C (the most abundant being *Amblyosporium spongiosum* (Pers.) S. Hughes, *Aspergillus niveus* Blochwitz, *Humicola grisea* var. *thermoidea* Cooney & R. Emers., *Scytalidium thermophilum* (Cooney & R. Emers.) Austwick. Eight species were common to the two composts incubated at either 25°C and 37°C (*Aspergillus flavus* Link, *A. fumigatus* Fresen., *A. niger* Tiegh., *A. terreus* Thom, *Emericella nidulans* (Eidam) Vuill., *Histoplasma capsulatum* Darling, *Penicillium purpurogenum* Stoll and *Penicillium* sp.). Numerous species were found in only one sample (17/40 taxa in HL 25°C, 13/28 in HL 37°C, 21/47 in LG 25°C and 20/35 in LG 37°C).



### Fungal community of hair waste compost (HL)

A total of  $12\,029 \times 10^3$  isolates corresponding to 40 taxa were present in 15 HL samples (corresponding to 15 g dry compost) incubated at 25°C and  $12\,816 \times 10^3$  isolates belonging to 28 taxa from the same number of samples incubated at 37°C were found. From all isolated taxa 11 were exclusively found when compost was incubated at 25°C and 8 only at 37°C. The number of propagules per g dry compost ranged from  $31 \times 10^3$  to  $1832 \times 10^3$  depending on the sample analyzed when incubated at 25°C and from  $12 \times 10^3$  to  $5271 \times 10^3$  when incubated at 37°C, being in average  $802 \times 10^3$  and  $854 \times 10^3$  propagules per g of dry compost when incubated at 25°C and 37°C respectively (Tables 1 and 2).

When referring to the frequency to which the taxa was recovered in the different samples for one compost, *Acrodontium crateriforme* (6/15), *Penicillium purpurogenum* and *Mucor hiemalis* Wehmer (5/15), *Aspergillus flavus* and *Absidia corymbifera* (Cohn) Saccardo & Trotter (4/15). At 37°C, *Aspergillus fumigatus* was the most frequent taxa (13/15), followed by *Absidia corymbifera* (6/15) and *Aspergillus flavus*, *A. terreus* and *Penicillium purpurogenum* (4/15).

When referring to the mean number of propagules per g of dry compost, the highest number of propagules belonged to *Acrodontium crateriforme*, being the most abundant species, recovered at both incubation temperature, with a higher number at 37°C. *Aspergillus fumigatus* and *Monocillium mucidum* were abundant at 37°C with respectively  $180 \times 10^3$  and  $128 \times 10^3$  propagules per g of dry compost. *Penicillium decumbens* Thom, *Penicillium citreonigrum* Dierckx, *Paecilomyces variotii* and *Penicillium purpurogenum*, were abundant at 25°C with  $120 \times 10^3$  to  $78 \times 10^3$  propagules per g of dry compost (Table 2).

### Fungal community of Ligustrum waste compost (LG)

From 15 LG samples incubated at 25°C a total of  $8\,373 \times 10^3$  isolates corresponding to 47 taxa were found and from the same samples, incubated at 37°C,  $7\,187 \times 10^3$  isolates belonging to 35 taxa were found. From all isolated taxa 21 were exclusively found when compost was incubated at 25°C and 13 only at 37°C. The number of propagules ranged from  $57 \times 10^3$  to  $1\,687 \times 10^3$  per g of dry samples incubated at 25°C and from  $103 \times 10^3$  to  $1\,330 \times 10^3$  when incubated at 37°C, being in average  $558 \times 10^3$  and  $479 \times 10^3$  propagules at 25°C and 37°C respectively (Table 1).

*Aspergillus fumigatus* was the most frequent species of LG compost, found in all samples at 37°C and in 11/15 samples at 25°C, followed, at 25°C by *Cladosporium cladosporioides* and *Aspergillus niger* (9/15), *Penicillium* sp. (8/15), *Trichoderma harzianum* Rifai and *Fusarium solani* (Mart.) Sacc (6/15), *Emericella nidulans* (5/15), *F. equiseti* (Corda) Sacc. and *F. oxysporum* Schlecht. (4/15) and at 37°C by *Emericella nidulans* (7/15) and *Aspergillus niger* (6/15), *Absidia corymbifera*, *Aspergillus caespitosus* Raper & Thom, *A. terreus*, *Emericella rugulosa* (Thom & raper) C.R. Benj. and *Penicillium* sp. (4/15).

The highest number of propagules belongs to *Aspergillus fumigatus*. ( $107 \times 10^3$  and  $290 \times 10^3$  propagules per g of compost at 25°C and 37°C respectively). Several other species with densities similar or higher than  $15 \times 10^3$  propagules per g dry compost were found such as, *Penicillium* spp. (at 25°C), *Emericella nidulans* and *E. rugulosa* (at both temperatures), *Humicola fuscoatra* Traaen (at 37°C) and *Fusarium solani* (at 25°C) (Table 2).

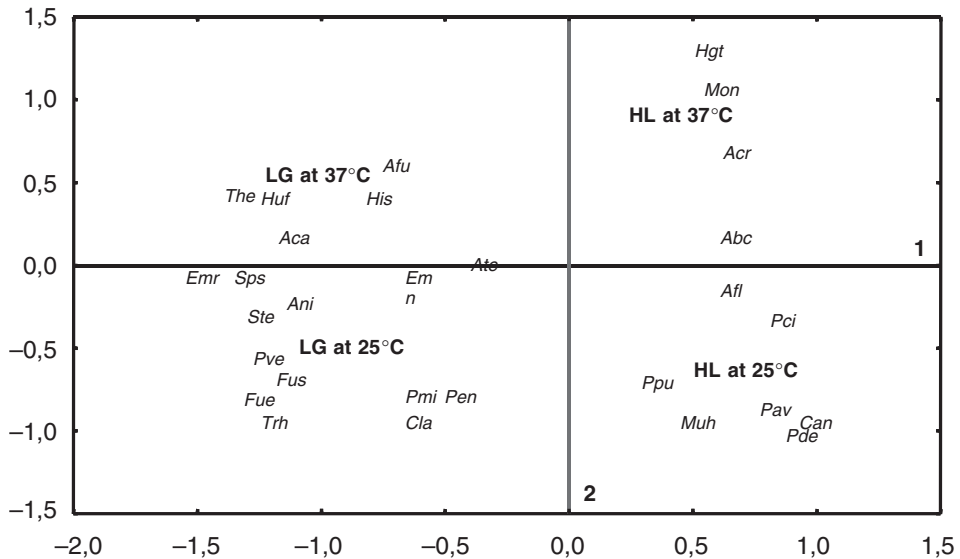


Fig. 3. Simple Correspondence Analysis. Ordination of composts (HL: hair from leather manufacturing industry; LG: *Ligustrum* pruning waste) at 25°C and 37°C according to the fungal composition on the two first axes. 83% of the total inertia was explained by the two first coordinates axes. Variables are the relative density of isolation of species with density equal or higher than  $10 \times 10^3$  propagules per g/dry compost. Codes for the fungal species are indicated in Table 2.

### Comparison of the species composition and species frequency of the fungal communities from both composts

The number of microfungi propagules able to grow on dilution plates was higher in HL dry compost, compared to LG but the mean number of species /100 isolates ranged from 8.42 at 25°C to 7.30 at 37°C at LG compost and from 4.86 at 25°C to 2.28 at 37°C in HL compost.

The simple correspondence analysis carried out on 29 fungal species present with a density equal or higher to  $10 \times 10^3$  propagules / g dry compost showed that the two first coordinate axes explained 83% of the total inertia, indicating a good fit of the data to the model (Figure 3). The axis 1 accounted for 46.46% of the total inertia and separated fungal assemblages of HL from that of LG composts and the second axis accounted for 36.54% and allowed to characterize composts incubated at 37°C and at 25°C, mainly in composts HL.

A set of species including *Acrodontium crateriforme*, *Monocillium mucidum*, *Absidia coerulea* Bainier and *Humicola grisea* var. *thermoidea* characterized HL at 37°C and contributed with 27% to the total inertia of the second axis. *Penicillium citreonigrum*, *Penicillium decumbens*, *Penicillium pupurogenum*, *Aspergillus flavus*, *Candida* sp. and *Paecilomyces variotii*, mainly associated to HL compost, at 25°C contribute with 29.14% to the inertia of this axis.

On the other hand *Aspergillus fumigatus*, *Thermomyces lanuginosus* Tsikl. and *Humicola fuscoatra* contributed with 24.15% to the inertia of this axis, and characterized LG compost incubated at 37°C. Correspondingly, *Penicillium*

*verrucosum* Dierckx, *Penicillium minioluteum* Dierckx, *Penicillium* sp., *Aspergillus niger*, *Fusarium solani*, *Trichoderma harzianum* and sterile mycelia that accounted for 25.06% to this axis characterized the LG compost at 25°C. Some species placed in an intermediate position in relation to the second axis (Figure 3) evidenced that they well developed under the two temperature conditions, as *Aspergillus terreus*, *Emericella nidulans*, *E. rugulosa* and *Sporothrix* sp. in the LG compost or *Absidia corymbifera* and *Aspergillus flavus* in the HL compost.

## DISCUSSION

The two composts analyzed were highly colonized by fungi but were very heterogeneous with regards to the diversity and number of propagules recovered in each sample incubated at either 25°C or 37°C. They appeared as mosaics of micro-habitats, where fungi evolved spatially and temporally. The higher species diversity found at 25°C than at 37°C could reflect that both composts were in the latest stage of evolution being thermophilic species replaced by mesophilic ones (Griensven, 1994). Since at 25°C the number of species did not achieve the asymptote, propagules of new species from the surrounding environment, not recorded before, may colonize the composts.

The composition and abundance of fungal propagules present in both composts were similar to those found in composts prepared with other raw materials (Chang & Hudson, 1967; Straatsma et al. 1994; Van Heerden et al., 2002, Klamer et al., 2001, Tiscornia et al., 2005). The total number of propagules from two Uruguayan grazing land soils that ranged from  $47 \times 10^3$  to  $60 \times 10^3$  per g of dry soil was lower to that of both composts analyzed in the present study. Conversely, the species richness in composts was lower than those in both soils (Bettucci et al., 1993).

Twelve of the species were thermotolerant, having a temperature range for growth from below 20°C to ca 55°C, four species were thermophilic (25-65°C) and the remainders were mesophilic according to Maheshwari et al. (2000). The most important thermotolerant species apart of *Aspergillus* spp were *Absidia corymbifera* and *Acrodontium crateriforme*. The thermophilic fungal species found here, *Humicola grisea* var. *thermoidea*, *Rhizopus oryzae* Went & Prins. Geerl. and *Scytalidium thermophilum* and *Thermomyces lanuginosus* were the same as found in citrus waste (Van Heerden et al., 2002), in compost prepared for mushroom cultures (Straatsma et al., 1994) and from straw litter in chickens nests (Ellis, 1989).

*Fusarium oxysporum*, *Fusarium acuminatum* Ellis & Everh., *Fusarium equiseti* and *Fusarium solani* found in some samples of the composts analyzed here are all plant pathogens. These mesophilic species could re-colonize the semi-pasteurized compost from the outer low temperature layers into the center of the pile (Hoitink & Fahy, 1986). However, species that have chlamydospores, such as *F. oxysporum*, could survive to composts heating (Bollen, 1985).

Species of *Aspergillus* are common soil fungi (Domsch et al., 1980) and frequent colonizers of composts produced with different raw materials (Chang & Hudson, 1967; Hoitink & Boehm, 1999). *A. fumigatus* and *A. niger* present in composts constitute an immunological and infectious risk in several countries. Hence, it is usually recommended to minimize the direct contact to avoid the spores

inhalation (Weber & Kullman, 1993). *Aspergillus flavus* is an important mycotoxigenic species (Samson *et al.*, 1995), although, it was present in low densities.

The absence of sclerotigenous fungi reflected that materials used to prepare the composts were free of them or they could not resist the high temperature of the composting process.

*Thermomyces lanuginosus* is a well known frequent thermotolerant species as *Paecilomyces variotii*. Conversely, *Allescheriella crocea* (Mont) S. Hughes (Basidiomycetous hyphomycetes) is an infrequent endangered species (Degawa *et al.*, 2006) known to grow on rotten bark. *Amblysporium spongiosum*, an other Basidiomycetous hyphomycetes may be associated to rich nitrogen resources. It grows well on ammonium, some amino acids as glycine and asparagine and urea. This fact can explain its presence only in hair leather compost (Yamanaka, 1999).

Several isolated species (of the genera *Chaetomium*, *Emericella*, *Myrothecium*) are antagonist *vis à vis* the nematode *Tilenchus semipenetrans* Cobb present in soil from citric plantations (Viera *et al.*, 2006) being an important condition of this compost. Moreover these species have important antibiotic and degrading abilities. *E. rugulosa* that is able to produce a broad spectrum of antimicrobial compounds (Onifad, 2006) was recovered from some soil where rainfall accumulated water, other water reservoirs or submerged materials (Nasser, 2005) indicating that compost samples where it was present had high water content. This is an interesting phosphate solubilizing microorganism that produces acid and alkaline phosphatases and phytase which mobilize phosphore and enhances the production of pearl millet (Yadaf & Tarafdar, 2007). *Chaetomium* and *Myrothecium* are able to increase cellulose degrading lignocellulosic wastes. Moreover, *Mortierella hyalina* (Harz.) W. Gams, as many *Mortierella* species is able to convert diverse carbon sources into lipids and is thus an oleaginous fungus, being important in transforming insoluble to soluble components when composts are used as soil amendment (Weber & Tribe, 2003).

*Trichoderma* spp. commonly present in composts prepared from high content lignocellulosic resources, as LG, are very important due to their biocontrol ability *vis à vis* several phytopathogens (Lupo *et al.*, 2001; Hoitink & Boehm, 1999; Correa *et al.*, 1995; Kuter *et al.*, 1983). In addition, composts containing *Trichoderma* that have cellulolytic activity could accelerate decomposition of litter debris of deciduous plants improving the input of organic matter into soil (Béguin & Aubert, 1994).

In conclusion, these results evidenced that fungal communities of composts prepared with different materials have, at least, some differences in composition, distribution and frequency of several species. But both composts contain not only common species to other composts that are well known as decomposers of different resources but they are also important by their suppression abilities.

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