ORIGINAL ARTICLE

Spatial distribution of fungal microflora in the sediment of a brackish lake (Lake Alimini Grande, Italy) used for fish production and bathing

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Key words

Sediments • Brackish waters • Fungi

Summary

A study of the distribution of fungal microflora was conducted on the sediment of Lake Alimini Grande in order to contribute to the evaluation of the ecosystem characteristics that can effect the process of decomposition. The isolation and identification of fungal species and ergosterol analysis were performed on sediment samples taken from 33 monitoring stations in autumn and winter. Altogether, 24 strains belonging to 8 genera were isolated. Trichoderma spp (41.6%) and Aspergillus spp (20.8%) were the dominant genera: in particular, Trichoderma was present near the Traugnano marsh, whereas Aspergillus was isolated in the area of connection to the sea.

Introduction

In the last few years there was an increasing interest in the dynamics of fungal flora in the environment in terms of clinical aspects and ecology.
In fact, opportunistic fungi have emerged during the past decade as important causes of morbidity and mortality in immunocompromised patients [1].
In the environment, the decomposition of plant detritus, greatly mediated by living organisms (fungi, bacteria and aquatic invertebrates) is a process that affects nutrient dynamics in freshwater bodies [2]. The initial phases of the decomposition process of dead plant material present in the aquatic system, especially cellulose, lignin and pectin, are mainly due to the action of micronfungi [3, 4]. Microfungi also have an important role on the decaying material palatable to invertebrates. In fact, only after the detritus has been colonized by fungi can shredder macroinvertebrates [5] convert the coarse organic matter (Coarse Particulate Organic Matter, CPOM) into smaller particles (Fine Particulate Organic Matter, FPOM), on which collectors feed.
In lake Alimini Grande, a study of the distribution of fungal microflora was carried out in the sediment, which is the main site of mineralisation of organic matter in shallow coastal environments. The lake is a basin a few metres deep and is situated north of Otranto (Lecce-Italy). It is connected to the sea via a channel and receives amounts of freshwater from Lake Alimini Piccolo, also called Fontanelle, to which it's connected via a channel named "Strittu" and via the "Zuddeo" channel that receives runoff waters from adjoining land. North of the lake there is a marshy area, the "Traugnano marsh", whereas the vast sandy shore adjacent to the mouth is popular among the bathers in summer. The waters of the lake are also used for the extensive farming of valuable fish species.
The progressive water quality deterioration in Lake Alimini Grande, most probably due to the anthropisation of the adjacent areas [6, 7] can negatively affect not only the health of the lake ecosystem but also human health as a consequence of the productive (fishfarming) and recreational (bathing) uses of these waters. This has led to a multidisciplinary study for the evaluation of ecosystem characteristics that can affect the decomposition process, that is the renewal of primary energy sources [8] and consequently the turnover of the main elements in order to predict the biological load that the lake can tolerate. The study also was conducted to give information about infective risk associated to utilization of basin for productive and recreational use.

Materials and methods

Isolation of fungal microflora: 33 sampling stations were selected situated throughout the lake area (Fig. 1). From each station, samples of sediment were taken with a bucket, transferred into sterile containers and then taken to the laboratory in refrigerated boxes and stored until analysis.
Sampling was conducted in autumn (October) and in winter (February).
The values of temperature, pH, dissolved oxygen and salinity were measured in situ (Tabs. II and III).
The isolation of fungi from the sediment was conducted using two methods: direct inoculation of each sample on Malt extract agar (Difco) and Sabouraud dextrose agar (Difco) supplemented with 0.5 g/l chloramphenicol (Difco): 1:2 dilution of each sample in sterile physiological, after 60 min shaking, were poured onto Malt extract

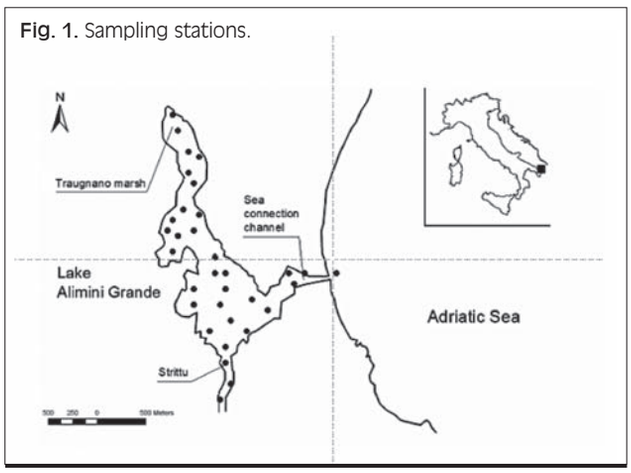

Fig. 1. Sampling stations.

agar and onto Sabouraud dextrose agar supplemented with 0.5 g/l chloramphenicol. Tests were conducted in triple incubation at 25°C for 15-20 days.
The isolation strains were identified based on the micro- and macroscopic morphological characters.
Ergosterol analysis: ergosterol levels in the fungal biomass contained in the sediment of Lake Alimini Grande was evaluated with the method described by Newell et al. [9].
Ergosterol is a typical component of the cell membrane of eumycota. Its presence can serve as an indicator of fungal biomass in the sediment.
The analysis was conducted on samples taken from two stations with different chemical and physical characteristics: one was situated in the centre of the lake and the other one was located near the Traugnano marsh. The sediment was drawn with a bucket and then taken to the laboratory in refrigerated boxes. Samples were supplemented with absolute ethanol (25 g of sediment in 5 ml of ethanol) and stored at 8°C until analysis. The trial was conducted in triple.

Results

Altogether, 24 strains belonging to 8 genera were isolated (Tab. I). Trichoderma and Aspergillus were the dominant genera: Trichoderma (41.6%) was mostly present near the Traugnano marsh; Aspergillus (20.8%) was isolated in the area of connection to the sea where there is a larger amount of dissolved oxygen (> 8 mg/l).
The central area of the lake was the less colonized by fungi. These data were confirmed by ergosterol analysis. Ergosterol, as indicator of fungal biomass [10, 11], was almost absent in the samples of sediment taken from the centre of the Lake (0.0123 μg/g sediment). Higher values (0.837 μg/g sediment) were recorded along the shoreline. Similar data were observed in winter.

Discussion

The results of this study are the first data available on the fungal species involved in the decomposition of allochthonous plant material (mainly *Phragmites australis* in lake Alimini Grande). The variety of fungi isolated is probably underestimated given to the relatively short period in which the research was carried out. It emerges, however, that decomposition in lake Alimini Grande is also due to the abundance of filamentous fungi not typically aquatic, in line with other data in literature [3, 4, 12, 13] that find in the lake the optimal conditions to complete their life cycles.
The species belonging to genus *Trichoderma* have in fact better colonised the lake. These are saprophytic fungi with intense cellulolityc and chitinolytic activity to which belong ubiquitous species mainly found in the soil but that are capable of colonising aquatic environments in the presence of favourable nutritional conditions [14, 15, 16].
T. harzianum and *T. viridae* species, isolated from the sediment of Lake Alimini Grande, have an important role as decomposers [17, 18]. Moreover, genus *Trichoderma* is reported to be an indicator of pollution in lakes, rivers and other streams as it is susceptible to certain environmental pollutants. In particular, *T. harzianum* has an oxidative system capable of degrading organic chlorine compounds [14, 19]. It is advisable to investigate if there is a relation between the presence of fungi belonging to this genus and the possible presence of contaminants in Lake Alimini Grande.
Additionally, attention should be paid to the hygienic and sanitary implications deriving from the recreational

Tab. I. Frequency and spatial distribution of fungal genera on sediments of Lake Alimini Grande.

Genera	Frequency (%)	Spatial distribution
Trichoderma	41.6	Traugnano marsh. Strittu mouth. West and est coastal zone
Aspergillus	20.8	Strittu mouth
Acremonium	8.3	Strittu mouth
Talaromyces	8.3	Strittu
Penicillium	8.3	Central zone west coastal zone
Mariannea	4.1	Mouth
Mucor	4.1	Strittu
Cephalosporium	4.1	Traugnano marsh

Tab. II. Physico-chemical parameters in the waters of Lake Alimini Grande (October).

	Temperature (°C)	pH	Salinity (‰)	Dissolved oxygen (ml/l)
Traugnano marsh	21.55	8.26	34.51	7.57
Strittu	19.50	8.18	29.66	7.85
Sea Connection channel	21.55	8.29	34.55	8.11

Tab. III. Physico-chemical parameters in the waters of Lake Alimini Grande (February).

	Temperature (°C)	pH	Salinity (‰)	Dissolved oxygen (ml/l)
Traugnano marsh	10.00	8.18	24.42	10.46
Strittu	9.80	8.22	8.43	9.40
Sea Connection channel	7.20	8.15	34.98	10.12

and productive use of the waters of Lake Alimini Grande and of the coast strip into which it flows. Several data [20] have been published about the clinical importance of the filamentous fungal genus *Trichoderma*, indicating that *Trichoderma* strains, besides their agricultural and biotechnological importance, may be potential opportunistic pathogens in immunocompromised hosts. In fact, previously considered a saprophytic organism with low pathogenicity, several centers have recently reported infections caused principally by *Trichoderma longibrachiatum* [21, 22]. Although the genus *Trichoderma* is composed of numerous species, recent molecular studies indicate that virtually all human infections are caused by a single taxonomic "section" composed of *T. longibrachiatum* [23]. *Trichoderma spp.* have been reported to cause pulmonary, cerebral, soft tissue and disseminated infections in immunocompromised patients, including those with bone marrow or solid organ transplantation [21, 22, 24].

The identification of zones with a different colonisation of fungal microflora within the lake can be related to various chemical and physical factors such as salinity and oxygen concentration, to different anthropic load and to the different distribution of vegetation (*Phragmites australis*) along the shores of the lake [25]. In the area of connection to the sea, for example, where *Phragmites australis* is totally absent, fungi of the genus *Aspergillus* are prevalent.

References

[1] Walsh TJ, Groll AH. *Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first centuty*. Transpl Infect Dis 1999;1:247-61.

[2] Mille-Lindblom C, Tranvik LJ. *Antagonism between bacteria and fungi an decomposing aquatic plant litter*. Microb Ecol 2003;45:173-82.

[3] Barlocher F, Kendrick B. *Dynamics of fungal population on leaves in a stream*. J Ecol 1974;62:761-91.

[4] Rossi L, Fano EA, Basset A, Fanelli C, Fabbri AA. *An experimental study af a microfungal comunity on palnt detritus in a mediterranean woodland stream*. Mycologya 1983;75:887-96.

[5] Rossi L, Fano EF. *Role of fungi in the trophic niche of the congeneric detrivorous Asellus aquaticus and A. coxalis (Isopode)*. Oikos 1979;32:380-5.

[6] Montagna MT, Bagordo F, De Donno A, Carrozini F. *Indagini microbiologiche nelle acque dei Laghi Alimini*. Ann Ig 1998;10:75-83.

[7] Montagna MT, De Donno A, Gabutti G, Aralla C, Bagordo F, Erroi R, et al. *"Master Plan": un progetto per la salute dei Laghi Alimini*. OER Puglia 1999;2:12-5.

[8] Basset A, Sangiorgio F, Polimeno P, Coccioli D, Fiocca A, Vignes F, et al. *Fonti di variazione della composizione di Phragmites australis (cav) Trin nel lago Alimini Grande*. IX Congresso Nazionale Società Italiana di Ecologia 1999.

[9] Newell SY, Arsuffi TL, Fallon RD. *Fondamental procedures for determing ergosterol content of decaying plant material by liquid chromatography*. Appl Environ Microbiol 1988;54:1876-988.

[10] Pasanen A, Yli-Pietila K, Pasanen P, Kalliokoski P, Tarahanen. *Ergosterol content in various fungal species and biocontaminated building materials*. Appl Environ Microbiol 1999;65:138-42.

[11] Michael JP, Meyer JL. *Fungal biomass of leaf litter species during decay in an Appalachian stream*. J N Am Benthol Soc 1996;15:421-32.

[12] Lamore BJ, Goos RD. *Wood-inhabiting fungi of freshwater stream in Rhode Island*. Mycologia 1978;70:1025-34.

[13] Graca MAS, Ferreira RCF. *The ability of selected acquatic hyphomycetes and terrestrial fungi to decompose leaves in freshwater*. Sydowia 1995;47:167-79.

[14] Samuels GJ. *Trichoderma: a review of biology and systematics of genus*. Mycol Res 1996;100:923-35.

[15] Kredics L, Manczinger L, Antal Z, Penzes A, Szekeres A, Kevei F, et al. *In vitro water activity and pH dependence of mycelial growth and extracellular enzyme activities of Trichiderma strains with biocontrol potential*. J Appl Microbiol 2004;96:491-8.

[16] Kredics L, Antal Z, Manczinger L. *Influence of water potential on growth, enyme secretion and in vitro enzyme activities of Trichoderma harzianum at different temperatures*. Curr Microbiol 2000;40:310-4.

[17] Markovich NA, Kononova GL. *Lytic enzymes of Trichoderma and their role in protecting plants from fungal diseasea*. Prikl Biokim Mikrobiol 2003;39:389-400.

[18] Sandhya C, Adapa LK, Nampoothiri KM, Binod P, Szakacs G, Pandey A. *Extracellular chitinase production by Trichoderma harzianum in submerged fermentation*. J Basic Microbiol 2004;44:49-58.

[19] Bulgheroni A, Guglielmetti M. *Analisi qualitativa della flora fungina presente in un impianto di depurazione a fanghi attivi per scarichi industriali*. Mic Ital 1998;2:45-52.

[20] Kredics L, Antal Z, Doczi I, Manczinger L, Kevei F, Nagy E. *Clinical importance of the genus Trichoderma. A review*. Acta Microbial Immunol Hung 2003;50:105-17.

[21] Gautheret A, Dromer F, Bourhis JH, Andremont A. *Trichoderma pseirdokoningii as a cause of fatal infection in a bone marrow transplant recipient*. Clin Infect Dis 1995;20:1063-4.

[22] Richter S, Cormican MG, Pfaller MA, et al. *Fatal disseminated Trichoderma longibrachiatirm infection in an adult bone marrow transplant patient: species identification and review of the literature*. J Clin Microbiol 1999;37:1154-60.

[23] Kuhls K, Lieckfeldt E, Borner T, Gueho E. *Molecular identification of human pathogenic Trichoderma isolates of Trichoderma longibrachiatirm and Trichoderma citroinuiride*. Men Mycol 1999;37:25-33.

[24] Groll AH, Walsh TJ. *Uncommon opportunistic fungi: new nosocomial threats*. Clin Microbiol Infect 2001;7:8-24.

[25] Burke DJ, Hamerlynck EP, Hahn D. *Interactions among plant species and microorganisms in salt marsh sediments*. Appl Environ Microbiol 2002;68:1157-64.

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