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Ex situ conservation and exploitation of fungi in Italy

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Abstract

The kingdom Fungi comprises one of the most diverse groups of living organisms. They are numerous, ubiquitous and undertake many roles both independently and in association with other organisms. Fungi display a wide diversity of forms also mirrored by functional diversity and play such a dominant role in human society that they are arguably biotechnologically the most important group of organisms. *Ex situ* conservation of fungi, not only guarantees correct management and conservation of biodiversity, but also its exploitation in different fields. This article describes the major biological resource centres dealing with fungi in Italy and gives several examples of fungal exploitation in different fields of applications.

Keywords: bioremediation, ex situ conservation, exploitation, fungi, yeasts,

Introduction

The sudden acceleration of the processes of alteration or even destruction of natural habitats and the resulting extinction of most of the species sharing these ecosystems has made essential the improvement of approaches for the correct management and conservation of biodiversity. They must be understood not only as conservation *per se*, but also as resources potentially exploitable for biotechnology purposes. Reference collections of biological material are the pillars of biotechnology research because they provide expertise and specialized facilities for acquisition, preservation, identification, characterization and distribution of organisms. Given the rapid development of biomedical research and biotechnology, collections must not only guarantee perfect preservation of strains from a genetic, physiological and morphological point of view, but also need to expand their activities using new technologies to better characterize their preserved organisms (Smith et al. [2008 Smith, D, Ryan, M J and Stackebrandt, E](#). 2008. “The *ex situ* conservation of microorganisms: aiming at a certified quality management”. In *Biotechnology in encyclopedia of life support systems (EOLSS)*. Developed under the auspices of the UNESCO, Edited by: [Delle, H W](#) and [DaSilva, E J](#). 1–30. Oxford, , UK: Eolss Publishers.).

Growing awareness of the role of fungi in ecosystems, biotechnology and disease makes their conservation more important than ever to maintain and assess their valuable biodiversity. Among organisms used in biotechnology, fungi (yeasts and filamentous forms) occupy a prominent place in many industrial and medical fields: they are used in agriculture and forestry, food- and biotech-oriented industry, and in biological control of pests, and they find numerous uses in environmental degradation of both urban and industrial wastes and in remediation of degraded and polluted areas (Zhiqiang [2005 Zhiqiang, A](#). 2005. *Mycology Vol. 22. Handbook of industrial mycology*, 763 New York, NY: Marcel Dekker. ; Anastasi et al. [2008 Anastasi, A, Varese, G C, Bosco, F, Chimirri, F and Filippello Marchisio, V](#). 2008. Bioremediation potential of basidiomycetes isolated from compost. *Bioresource Technol*, 99: 6626–6630. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). There are also, however, negative aspects associated with fungi. They are responsible for important plant diseases and for deterioration of food, raw materials and artefacts. More directly, for humans, they can cause mycoses, mycotoxicosis and fungal and allergic diseases the importance of which has increased exponentially in recent years (Picco et al. [2011 Picco, A M, Angelini, P, Ciccaglione, C, Franceschini, A, Ragazzi, A Rodolfi, M](#). 2011. Biodiversity of emerging pathogenic and invasive fungi in plants, animals and humans in Italy. *Plant Biosyst*, 145 991–999 [[Web of Science ®](#)]).

Based on the definition of biological resource centre (BRC) provided by the organization for economic cooperation and development (OECD), there are currently only three BRCs in Italy: (i) the Industrial Yeasts Collection DBVPG of the Department of Applied Biology of the University of Perugia, for *ex situ* conservation of yeasts and yeast-like organisms; (ii) the *Mycotheca Universitatis Taurinensis* (MUT) of the Department of Plant Biology of the University of Turin and (iii) the International Culture Collection of Toxigenic Fungi and their Secondary Metabolites (ITEM) at the Institute of Sciences of Food Production of the National Council of Research (CNR) of Bari for filamentous fungi. All these Institutions are historical collections affiliated for many years with the European Culture Collection Organization (ECCO) and the World Federation of Culture Collections (WFCC).

At present, the DBVPG maintains about 5000 yeast and yeast-like cultures representing a large percentage of the known species associated either with the food and beverage industry or isolated in tropical and sub-tropical environments of Africa and South America, and the cold and glacial environments of Antarctica, South America and Europe. Since 1997 the DBVPG collection has been accredited by the Italian Ministry of Commerce as an International Depositary Authority for the deposit of patented yeast and yeast-like fungi under the Budapest Treaty. The MUT preserves

about 4500 strains belonging to almost all classes of filamentous fungi isolated from very different habitats and substrata in arctic, temperate, tropical and subtropical zones. It also includes many potentially harmful fungi isolated from human and animal pathological specimens. The collection has a particular interest from an ecological and applicative point of view since many fungi preserved have been characterized for their ecological and physiological properties. It also includes: mycorrhizal strains, biocontrol and bioindicator agents, and antibiotic and enzyme producers to be used in industrial and bioremediation applications. The ITEM collection consists of around 5000 cultures of toxigenic fungi of phytopathological and agro-food interest whose ability to form mycotoxins is the cause of justified apprehension for food safety. For each strain the metabolites produced are indicated, each with its own biological activity. Like other internationally recognized BRCs, the three Italian Collections perform three basic functions: acquisition, conservation (in conditions that guarantee their survival and preservation of their genetic integrity) and distribution of cultures and related information. In addition, they provide a service of identification by means of conventional and molecular procedures. These BRCs thus guarantee as their main institutional remit, the safeguard of genetic resources and diversity of fungi as well as properties of biotechnological interest inherent in those fungi. In addition to the three collections mentioned above, in Italy there are many University Departments with specific fungal collections, often highly specialized, and of crucial importance for the conservation and study of Italian fungal biodiversity.

The next sections will illustrate examples of fungal exploitation devised by Italian scientists.

Industrial exploitation of yeasts in Italy

Research on yeast biotechnology in Italy encompasses an enormous variety of studies involving the activity of yeast cells and metabolites. The impact of such investigations at an industrial scale is so far limited, because most implementation up to now has been only at laboratory level. *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, the most extensively exploited species in production of traditional fermented food and beverages, was reviewed by Romano et al. (2006 Romano, P, Capece, A and Jespersen, L. 2006. "Taxonomic and ecological diversity of food and beverage yeasts". In *Yeasts in food and beverage*, Edited by: Querol, A and Fleet, G. 13–53. Germany: Springer-Verlag. [CrossRef]). Research on the metabolic diversity of so-called "non-conventional" yeasts (NCY) has revealed innumerable and groundbreaking biotechnological properties: a few NCY species have been tested since the 1980s for potential industrial exploitations (Buzzini & Vaughan 2006). A lot of industrial mycological research has been directed at in-depth definition of the overall biological diversity of yeasts conserved in DBVPG, and has been realized through the planning of well-targeted large-scale screening surveys aimed at selecting strains expressing metabolic characteristics of biotechnological interest. In particular, successful use of yeast whole cells and of derived enzymes for producing enantiopure compounds of chemical and pharmacological interest has been recently achieved (Forzato et al. 2008 Forzato, C, Furlan, G, Nitti, P, Pitocco, G, Valentin, E, Zangrando, E and et, al. 2008. Chemo-enzymatic and yeast-catalyzed synthesis of diastereoisomeric gamma-phenil and gamma-n-pyridyl paraconates. *Tetrahedron-Asymmetry*, 19: 2026–2036. [CrossRef], [Web of Science @]; Goretti et al. 2011 Goretti, M, Ponzoni, C, Caselli, E, Marchegiani, E, Cramarossa, M R, Turchetti, B and et, al. 2011. Bioreduction of α , β -unsaturated ketones and aldehydes by non-conventional yeast (NCY) whole-cells. *Biores Technol*, 102: 3993–3998. [CrossRef], [PubMed], [Web of Science @]).

On an industrial scale, several interesting applications have been recently proposed. Regarding biofuels, the Italian chemical group Mossi and Ghisolfi (M&G) recently announced a second-generation 200,000-tons plant for bioethanol production, the first of its kind in Italy. The new plant will initially use 600,000 tons of maize as feedstock and M&G has already lined up local

farmers to deliver grain, as it aims to cover 60% of its feedstock needs through local suppliers (www.gruppomg.com).

Based on the WIPO database (www.wipo.int), several patents involving yeasts (or yeast-related antibodies) have been deposited in the last 10 years by the Italian academy and Italian industry:

1. use of a native strain of *S. cerevisiae* in production of Prosecco wine (WO 2011/004254);
2. use of the methylotrophic yeast *Pichia pastoris* (Guillierm.) Phaff as: (i) a host for the overexpression of toxic chimaeric proteins endowed with ribosome-inactivating activity based on the plant toxin saporin from *Saponaria officinalis* L.(WO 2011/012254) and (ii) a truncated form of α' chain ($\epsilon\alpha'$), the soybean 7S globulin, active in controlling the cholesterol and triglyceride homeostasis in *in vitro* and *in vivo* models (WO 2010/145820);
3. use of a strain of *Metschnikowia pulcherrima* Pitt & M.W. Mill. in combination with lower concentrations of ferric salts or alone, as a highly effective antagonist in biological control of pathogenic fungi responsible for the rotting of post-harvest fruit, the strain retaining its effectiveness under conditions of low temperature and low-oxygen atmosphere (WO 2009/040862);
4. use of anti-idiotypic antibodies which recognize the idiotope of an antibody specific for a yeast killer toxin possessing microbicidal activity (WO 2003/095493).

Industrial exploitation of filamentous fungi in Italy

Fungi as active ingredients of biopesticides

In the last years a new class of pesticides appeared on the market, having living organisms as active ingredients. The rationale of biopesticides is the exploitation against pathogens and parasites of the mechanisms usually used by their competitors in the struggle for life in natural substrata. Nine species of fungi (*Ampelomyces quisqualis* Ces., *Beauveria bassiana* (Bals.-Criv.) Vuill., *Coniothyrium minitans* W.A. Campb., *Gliocladium catenulatum* J.C. Gilman & E.V. Abbott, *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm., *P. lilacinus* (Thom) Samson, *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg, *T. gamsii* Samuels & Druzhin. and *T. harzianum* Rifai) have been included in the list of approved active ingredients (EU Directive 91/414) and are commercialized in Italy. Two, *T. asperellum* and *T. gamsii*, are the active ingredients of commercial biopesticides (Radix and Remedier, Isagro). These strains were in the past misidentified and on the commercial product labels appear as *T. harzianum* and *T. viride*, respectively. They have been isolated from soils in central Italy and Sardinia, respectively but no information is available about the isolation and selection procedures involved.

As a consequence of work done at the University of Naples, an isolate of *T. harzianum* is now available on the market as the active ingredient of a plant biostimulant (BioPlantguard, Saipan). The isolate derived from a suppressive soil near Portici (Naples) has good antagonistic activity, induces resistance and stimulates growth in treated plants. Its genome has been partially sequenced and the secondary metabolites profile has been defined (Finale et al. 2009 [Finale, F.](#), [Ghisalberti, E L.](#), [Sivasithamparam, K.](#), [Marra, R.](#), [Scala, F.](#), [Ruocco, M](#) and [et, al.](#) 2009. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. *Lett Appl Microbiol*, 48: 705–711. [[PubMed](#)], [[Web of Science ®](#)]; Ruocco et al. 2009 [Ruocco, M.](#), [Lanzuise, S.](#), [Vinale, F.](#), [Marra, R.](#), [Turrà, D.](#), [Woo, S L](#) and [et, al.](#) 2009. Identification of a new biocontrol gene in *Trichoderma atroviride*: The role of an ABC transporter membrane pump in the interaction with different plant pathogenic fungi. *Mol Plant-Microbe Inter*, 22: 291–301. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]).

The two other products, Biofox C (ICC Caffaro) and Fusapi[ugrave] (Agrifutur), have as active ingredients saprotrophic *Fusarium oxysporum* Schltdl. isolates and have been on the market as plant biostimulants. They are based on a large amount of research carried out at the University of Turin on soils suppressive for *Fusarium* wilt of carnation (Garibaldi et al. [1992 Garibaldi, A, Aloj, C, Parodi, C and Gullino, M L](#). 1992. “Biological control of Fusarium wilt of carnation”. In *Biological control of plant diseases*, Edited by: [Tjamos, E S, Papavizas, C and Cook, R J](#). 105–108. New York, NY: Plenum Press.). Such research includes strain improvement by mutagenesis, protoplast fusion and transformation, and risk analysis in respect of the deliberate release of antagonists into the environment (Gullino et al. [1995 Gullino, M, Migheli, Q and Mezzalana, M](#). 1995. Risk analysis in the release of biological control agents. *Antagonistic Fusarium oxysporum as a case study. Plant Dis*, 79: 1193–1201.).

Other fungi identified by research at the University of Pisa as suitable active ingredients of biopesticides and very close to commercialization include *Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx isolate I10 and *T. asperellum* isolate I252. The former strain, isolated from a natural woodland soil, was selected during screening for biological control of seed- and soil-borne fungal pathogens by seed application of antagonists (Vannacci & Pecchia [1986 Vannacci, G and Pecchia, S](#). 1986. Evaluation of biological seed treatment for controlling seed-borne inoculum of *Drechslera sorokiniana* on barley. *Med Fac Landbouww Rijksuniv Gent*, 51/2b: 741–750.). It gave interesting results in sterilized soil when applied to barley seeds infected by *Bipolaris sorokiniana* (Sacc.) Shoemaker or to healthy radish seeds sown in *Rhizoctonia solani* J.G. Kühn infested soil. The second strain was selected during studies targeted to find microfungi for use against *Sclerotinia minor* Jagger and other sclerotium-forming pathogens (Vannacci et al. [1989 Vannacci, G, Pecchia, S and Resta, E](#). 1989. Presence of *Sclerotinia minor* Jagger antagonists in soils from different mediterranean areas. *II. Sclerotia decaying fungi. Acta Hort (ISHS)*, 255: 293–302.). Soils from Italy and other Mediterranean countries with a previous history of sclerotinia diseases were baited by sclerotia of *S. minor*. Fungi recovered from sclerotia were tested *in vitro* for viability of decaying sclerotia of different pathogens and were evaluated for their competitive saprobic ability in natural soil. The two selected strains were further investigated in field and greenhouse experiments in collaboration with a private company, SIAPA. The studies indicated that these strains have sufficient potential as biocontrol agents against different pathogens to make them worth marketing. Strains were developed by SIAPA, but have not yet reached the market because of financial difficulties not connected with the research. In the meantime *T. virens* I10 has been transformed by a GFP construct to follow the route of colonization of sclerotia of *S. sclerotiorum* (Lib.) de Bary (Sarrocco et al. [2006 Sarrocco, S, Mikkelsen, L, Vergara, M, Jensen, D F, Lubeck, M and Vannacci, G](#). 2006. Histopathological studies of sclerotia of phytopathogenic fungi parasitized by a GFP transformed *Trichoderma virens* antagonistic strain. *Mycol Res*, 110: 179–187. [[CrossRef](#)], [[PubMed](#)]).

Fungi in soil bioremediation

Soil may be subject to severe degradation from improper agricultural practices, pollution, economic activities, climate change and changes in land use itself. To protect soil as a resource, the European Union has proposed, in the 6th Environmental Action Programme, the Directive on Soil Protection (European Communities Commission (ECC) 2006), that recognizes the environmental importance of soil and its strong relationship with other aspects of the environment. In Western Europe, more than 300,000 potentially contaminated sites have been identified. In Italy 57 sites of national interest have been identified. Bioremediation, involving bioaugmentation and/or biostimulation has emerged as the most promising soil and water clean-up technique for contaminated sites. Fungi possess the biochemical and ecological capacity to degrade organic pollutants and to decrease risk, either by chemical modification or by influencing chemical bioavailability (Harms et al., [2011](#)

[Harms, H](#), [Schlosser, D](#) and [Wick, L Y](#). 2011. Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals. *Nat Rev Microbiol*, 9: 177–192. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). In Italy many studies have shown the efficiency of fungi belonging to different ecophysiological groups in degradation or biosorption of a variety of recalcitrant compounds.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants consisting of two or more fused benzene rings. The persistence of these compounds in the environment is mainly due to their low solubility in water and stable polycondensed aromatic structure. The potential of different *Basidiomycetes* to degrade PAHs in both artificially-spiked and industrially-contaminated soils has been widely demonstrated (Covino et al. [2010](#) [Covino, S](#), [Svobodová, K](#), [Křesinová, Z](#), [Petrucchioli, M](#), [Federici, F](#), [D'Annibale, A](#) and [et, al](#). 2010. In vivo and in vitro polycyclic aromatic hydrocarbons degradation by *Lentinus (Panus) tigrinus* CBS 577.79. *Bioresource Technol*, 101: 3004–3012. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]; Federici et al. [2011](#) [Federici, E](#), [Giubilei, M A](#), [Cajthaml, T](#), [Petrucchioli, M](#) and [D'Annibale, A](#). 2011. *Lentinus (Panus) tigrinus* augmentation of a hystorically contaminated soil: Matrix decontamination and structure and function of the resident bacterial community. *J Hazard Mater*, 186: 1263–1270. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]) and in several cases potential involvement of ligninolytic enzymes (Anastasi et al. [2009](#) [Anastasi, A](#), [Coppola, T](#), [Prigione, V](#) and [Varese, G C](#). 2009. Pyrene degradation and detoxification in soil by a consortium of basidiomycetes isolated from compost: Role of laccases and peroxidases. *J Hazard Mater*, 165: 1229–1233. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)], 2010) and the effects of mobilizing agents has been evaluated (Giubilei et al. [2009](#) [Giubilei, M A](#), [Leonardi, V](#), [Federici, E](#), [Covino, S](#), [Šašek, V](#), [Novotny, C](#) and [et, al](#). 2009. Effect of mobilizing agents on mycoremediation and impact on the indigenous microbiota. *J Chem Technol Biot*, 84: 836–844. [[CrossRef](#)], [[Web of Science ®](#)]). Many anamorphic fungi (*Aspergillus*, *Penicillium* and *Stachybotrys* spp.) have been also reported as being able to degrade PAHs (Capotorti et al. [2004](#) [Capotorti, G](#), [Digianvincenzo, P](#), [Cesti, P](#), [Bernardi, A](#) and [Guglielmetti, G](#). 2004. Pyrene and benzo(a)pyrene metabolism by an *Aspergillus terreus* strain isolated from a polycyclic aromatic hydrocarbons polluted soil. *Biodegradation*, 15: 79–85. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]), but the mechanisms involved in biodegradation are still unidentified.

Polychlorinated biphenyls (PCBs) are xenobiotic compounds extremely resistant to chemical and biological degradation, and they persist for years in the environment. The potential of mitosporic fungi for bioremediation of PCBs has been studied by Tigini et al. ([2009](#) [Tigini V](#), [Prigione V](#), [Di Toro S](#), [Fava F](#), [Varese GC](#). 2009. Isolation and characterisation of polychlorinated biphenyl (PCB) degrading fungi from a historically contaminated soil. *Microb Cell Fact* 8: Available: <http://www.microbialcellfactories.com/content/8/1/5>), who characterized the native fungi of soil from an old dump site contaminated by about 0.9 g/kg of Aroclor 1260 PCBs, and monitored changes after aerobic biotreatment with a complex of bacteria and fungi. The original contaminated soil contained low levels of only a few fungal species. Six of the 21 strains isolated from the treated soil (*Fusarium solani* (Mart.) Sacc., *Penicillium chrysogenum* Thom, *P. digitatum* (Pers.) Sacc. and *Scedosporium apiospermum* (Sacc.) Sacc. ex Castell. & Chalm) were found to degrade PCBs apparently without the involvement of ligninolytic enzymes.

Girlanda et al. ([2009](#) [Girlanda, M](#), [Favero-Longo, S E](#), [Lazzari, A](#), [Segreto, R](#), [Perotto, S](#) and [Siniscalco, C](#). 2009. Indigenous microfungi and plants reduce soil nonylphenol contamination and stimulate resident microfungal communities. *Appl Microbiol Biot*, 82: 359–370. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]) investigated the potential of indigenous fungi (*Aspergillus flavus* Link, *Paecilomyces lilacinus* (Thom) Samson, *Penicillium canescens* Sopp, *P. pinophilum* Hedgc. and *P. restrictum* J.C. Gilman & E.V. Abbott) to remove nonylphenol, the most abundant environmental pollutant with endocrine disrupting activity. The potential of indigenous fungi and

plants to remove nonylphenol was assessed in mesocosm experiments. Introduction of indigenous fungi consistently reduced nonylphenol levels in all substrata, up to *ca.* 70% depletion, whereas introduction of plants proved effective only when initial pollutant levels were high.

Zanaroli et al. (2010 Zanaroli G, Di Toro S, Todaro D, Varese GC, Bertolotto A, Fava F. 2010. Characterization of two diesel fuel degrading microbial consortia enriched from a non acclimated, complex source of microorganisms. *Microb Cell Factories* 9: Available: <http://www.microbialcellfactories.com/content/9/1/10>) studied two mixtures of bacteria and the fungus *Trametes gibbosa* (Pers.) Fr. enriched from a commercial source on diesel and HiQ diesel. Both mixtures exhibited a remarkable capability for biodegradation. In particular, the fungus was outstanding in its ability to degrade diesel hydrocarbons. Presence of both components of the mixture, bacteria and fungi, appeared to be essential for functionality, suggesting that mineralization of hydrocarbons in nature may require the combined efforts of both fungi and bacteria.

Dry olive mill residue (DOR) is very rich in organic matter and nutritionally relevant cations. Sampedro et al. (2009 Sampedro, I, Cajthaml, T, Marinari, S, Petruccioli, M, Grego, S and D'Annibale, A. 2009. Organic matter transformation and detoxification in dry olive mill residue by the saprophytic fungus *Paecilomyces farinosus*.. *Process Biochem*, 44: 216–225. [CrossRef], [Web of Science ®]) investigated the impact of *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G.Sm. on both organic matter modification and detoxification of this waste. Water-soluble phenols were reduced by 45% in 20-week-old *P. farinosus* cultures and phytotoxicity was totally suppressed.

Investigation of a soil fungal community of contaminated agricultural soils in the Valle Latina (organochlorines, heavy metals) and test of the growth response of selected microfungi to the toxic metal vanadium has been carried out (Maggi et al. unpublished data). Six species (*Aspergillus terreus* Thom, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams, *Paecilomyces lilacinus*, *Penicillium citrinum* Thom and *Rhizopus arrhizus* A. Fisch.) were inoculated on malt extract agar with ammonium vanadate added at concentrations of 1, 2, 3 and 6mM. Metal tolerance was shown by all species.

Fungi in bioremediation of wastewaters

Water quality is currently one of the main topics for discussion and regulation in the European Community, because of growing awareness that water will become increasingly valuable. Wastewater treatment is particularly relevant because of its environmental, social and financial implications. Industrial effluents are generally extremely heterogeneous and complex and contain a wide variety of pollutants, such as dyes, heavy metals, surfactants, chlorides and sulphates, which can interact giving rise to complexation (Hai et al. 2007 Hai, F I, Yamamoto, K and Fukushi, K. 2007. Hybrid treatment system for dye wastewater. *Crit Rev Environ Sci Technol*, 37: 315–377. [Taylor & Francis Online], [Web of Science ®]). Physical–chemical treatments traditionally used for removing pollutants do not always give good results and are expensive and energy consuming. They also may require use of further toxic chemicals which can themselves be harmful to both humans and the environment.

Over the last few decades, attention has focused on bioremediation as an attractive, low cost and low environmental impact process that uses living organisms or their enzymes to remove contaminants from wastewater. It can be implemented through two mechanisms: (1) biodegradation which exploits the enzyme pool of the organisms to degrade organic contaminants into less toxic compounds, leading to their mineralization; (2) biosorption which is the binding of solutes to the biomass by processes which do not involve metabolic energy or transport. The role of fungi in the

treatment of wastewater has been extensively researched, and fungi have proved to be suitable organisms for treatment of industrial effluent through both these bioremediation strategies (Kaushik & Malik [2009 Kaushik, P](#) and [Malik, A. 2009](#). Fungal dye decolourization: Recent advances and future potential. *Environ Int*, 35: 127–141. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]).

In biodegradation, fungal mycelium has an additional advantage over single cell organisms when solubilizing insoluble substrata by producing extra-cellular enzymes: because of the increased cell-to-surface ratio, fungi have a greater physical and enzymatic contact with their environment. The extra-cellular nature of fungal enzymes is also advantageous in tolerating high concentrations of the toxicants (Kaushik & Malik [2009 Kaushik, P](#) and [Malik, A. 2009](#). Fungal dye decolourization: Recent advances and future potential. *Environ Int*, 35: 127–141. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). Most of the work related to biodegradation of wastewater pollutants by fungi concentrates on use of wood-rot fungi which produce lignin degrading enzymes (i.e. laccases and peroxidases) which, thanks to their high redox potential, are effective against many recalcitrant pollutants including a wide range of industrial dyes (Anastasi et al. [2010 Anastasi, A, Spina, F, Prigione, V, Tigini, V, Giansanti, P](#) and [Varese, G C. 2010](#). Scale-up of a bioprocess for textile wastewater treatment using *Bjerkandera adusta*.. *Bioresource Technol*, 101: 3067–3075. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). While the literature is full of reports demonstrating the excellent capacity of fungi to degrade pollutants in wastewater, their potential has not yet found a real application, mainly because of the difficulty in selecting organisms capable of growing and degrading in the very variable and restrictive conditions of textile industry wastewaters (Vanhulle et al. [2008 Vanhulle, S, Trovaslet, M, Enaud, E, Lucas, M, Taghavi, S, van der Lelie, D](#) and [et, al. 2008](#). Decolorization, cytotoxicity and genotoxicity reduction during a combined ozonation/fungal treatment of dye-contaminated wastewater. *Environ Sci Technol*, 42: 584–589. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). In addition, most of the screenings have been performed in conditions where the actual degradation efficiency in real effluents has been difficult to measure. Up to now only very few papers deal with continuous treatment, under non-sterile conditions, of real industrial wastewaters in bioreactors (Blanquez et al. [2008 Blanquez, P, Sarrà, A](#) and [Vicent, T. 2008](#). Development of a continuous process to adapt the textile wastewater treatment by fungi to industrial conditions. *Process Biochem*, 43: 1–7. [[CrossRef](#)], [[Web of Science ®](#)]).

Very recently, a bioreactor which operated under industrial conditions using real wastewaters was developed by Anastasi et al. ([2010 Anastasi, A, Spina, F, Prigione, V, Tigini, V, Giansanti, P](#) and [Varese, G C. 2010](#). Scale-up of a bioprocess for textile wastewater treatment using *Bjerkandera adusta*.. *Bioresource Technol*, 101: 3067–3075. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). One strain of *Bjerkandera adusta* (Willd.) P. Karst. was selected from 12 basidiomycetes for its ability to decolorize and detoxify three simulated wastewaters, showing as significant physiological versatility which is very useful for application purposes. To evaluate its true bioremediation potential, this strain was packed in a fixed-bed bioreactor, for treatment of large volumes of a real wastewater. The fungus was effective over 10 cycles of decolourization, remaining active for a very long period, in non-sterile conditions.

Fungi present several advantages over other organisms in biosorption, too. Their cell wall displays a multitude of physical and chemical properties which could be exploited in the binding of pollutants by biosorption. Moreover, separation of mycelial biomass from liquid is simpler than separation of single cell organisms, and the supply of biomass as a biosorbent is guaranteed by their easy and productive growth on different media with low nutritional requirements. Besides, many of the fungi useful for this work are already extensively used in large scale industrial fermentation processes, representing a potential source of cheap adsorbent materials (Gadd [2009 Gadd, M G. 2009](#). Biosorption: Critical review of scientific rationale, environmental importance and significance for pollution treatment. *J Chem Technol Biot*, 84: 13–28. [[CrossRef](#)], [[Web of Science ®](#)]).

Fungi have been employed for pollutant biosorption in either living or dead form, and even when dead they seem to be more effective and environmentally safe (Kaushik & Malik [2009](#) [Kaushik, P](#) and [Malik, A](#). 2009. Fungal dye decolourization: Recent advances and future potential. *Environ Int*, 35: 127–141. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). Studies related to biosorption have been mostly conducted on anamorphic fungi and *Zygomycetes*. *Zygomycetes*, in particular, seem to be suitable for pollutants biosorption because of the particular richness of their cell walls in acid polysaccharides such as chitin and chitosan, which represent about the 50% of the cell wall components (Tigini et al. [2011](#) [Tigini, V](#), [Prigione, V](#), [Donelli, I](#), [Anastasi, A](#), [Freddi, G](#), [Giansanti, P](#) and [et, al](#). 2011. *Cunninghamella elegans* biomass optimisation for textile wastewater biosorption treatment: an analytical and ecotoxicological approach. *App Microbiol Biot*, 90: 343–352. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). These macromolecules are characterized by amino and hydroxyl groups, which are involved in biosorption of dyes, heavy metals and phenolic compounds (Prigione [2009](#) [Prigione, V](#), [Zerlottin, M](#), [Refosco, D](#), [Tigini, V](#), [Anastasi, A](#) and [Varese, G C](#). 2009. Chromium removal from a real tanning effluent by autochthonous and allochthonous fungi. *Bioresource Technol*, 100: 2770–2776. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]).

Recent literature has focused attention on biomass modification by selection of pre-culture medium, biomass pretreatments, configuration of bioreactors and physico-chemical conditions to enhance biosorption (Gadd [2009](#) [Gadd, M G](#). 2009. Biosorption: Critical review of scientific rationale, environmental importance and significance for pollution treatment. *J Chem Technol Biot*, 84: 13–28. [[CrossRef](#)], [[Web of Science ®](#)]; Tigini et al. [2010](#) [Tigini, V](#), [Prigione, V](#), [Giansanti, P](#), [Mangiavillano, A](#), [Pannocchia, A](#) and [Varese, G C](#). 2010. Fungal biosorption, an innovative treatment for the decolourisation and detoxification of textile effluents. *Water*, 2: 550–565. [[CrossRef](#)]). Scaled up pilot trials of biosorption to treat pollutants using *Cunninghamella elegans* Lendn. have shown that this fungus is particularly effective in simultaneously removing dyes, surfactants, salts and heavy metals and toxicity from wastewaters (Tigini et al. [2010](#) [Tigini, V](#), [Prigione, V](#), [Giansanti, P](#), [Mangiavillano, A](#), [Pannocchia, A](#) and [Varese, G C](#). 2010. Fungal biosorption, an innovative treatment for the decolourisation and detoxification of textile effluents. *Water*, 2: 550–565. [[CrossRef](#)]).

Cultivation of fungi of gastronomic value

Ex situ conservation, especially for saprotrophic species growing in culture, might be of great importance for species on the verge of extinction or those strictly confined to very limited or threatened habitats (Courtecuisse [2001](#) [Courtecuisse, R](#). 2001. “Current trends and perspectives for the global conservation of fungi”. In *Fungal conservation. Issue and solutions*, Edited by: [Moore, D](#), [Nauta, M M](#), [Evans, S E](#) and [Rotheroe, M](#). 7–18. Cambridge, , UK: Cambridge University Press. [[CrossRef](#)]). Breeding has contributed much to the spread of the cultivation of *Pleurotus* species for which world commercial production approaches annual rates of 1 Mt, making it the third most abundantly cultivated mushroom (Chang [1996](#) [Chang, S T](#). 1996. “Mushroom research and development – equality and mutual benefit. ”. In *In: Royse DJ, editor. Proceedings of the 2nd International Conference on Mushroom Biology and Mushroom Products. University Park, Pennsylvania State University, 1–10*). In the Mediterranean region, *Pleurotus* cultivation is relatively widespread and accounts for about 10–20% of the area's total mushroom production (Zervakis & Venturella 2002). *Pleurotus nebrodensis* (Inzenga) Quél. has started to be cultivated in the hope of reducing collecting pressures on the species in the wild. The mushroom is grown in a special tunnel and the estimated yield is *ca.* 1–1.5 kg per plastic bag, which works out as *ca.* 1000 kg in a tunnel of 60 square metres. The cultivated *P. nebrodensis* mushrooms retain the same characteristic aroma and flavour as found in the wild basidiomata which is not the case with other *Pleurotus* species (i.e. oyster mushroom). *Ex situ* cultivation of *P. nebrodensis* also provides an

additional income for local farmers, who can offer a cheaper product than that collected from the wild, and this in turn reduces pressure on the wild population.

Another important example of *ex situ* exploitation in Italy is truffle cultivation. From the end of the last century, natural production of white truffles (*Tuber magnatum* Pico) and black truffles (*T. melanosporum* Vittad.) has shown a decline due to environmental changes and different biological factors (Hall et al. [2007 Hall, I R, Brown, G T and Zambonelli, A. 2007. Taming the truffle](#), 304Portland, Oregon, USA: Timber Press.). This has resulted in a strong increase in commercial prices with consequent sophistications and food adulteration. Perhaps even more importantly numbers of truffle hunters have increased, with concomitant problems for a *in situ* conservation of these hypogeous fungi. In the past attention was given to the natural environment where the truffles grow. The auto-ecology of these ascomycetes has been described in different Italian Regions to enhance not only the environmental management but also conservation (Venturella et al. [2006 Venturella, G, Pecorella, E, Saitta, A, Zambonelli, A and Morara, M. 2006. Ecology and distribution of hypogeous fungi from Sicily \(southern Italy\). Cryptogamie Mycol, 27: 201–217. \[Web of Science ®\]](#); Salerni et al. [2010 Salerni, E, Baglioni, F, Mazzei, T, Gardin, L, Ciabatti, F, Leopardi, P and et, al. 2010. Efectos de las diversas técnicas culturales sobre la producción de Tuber aestivum Vitt. y de Tuber melanosporum Vitt. en dos plantaciones experimentales en Italia central. ZIZAK, 7: 47–62. \). The ectomycorrhizal \(ECM\) symbiosis of truffles is a biotechnology quite hard to reproduce in artificial conditions. Positive results have been obtained in the laboratory and greenhouse \(Donnini et al. 2010\). However, the plantation of artificial truffle areas needs respect of both the natural habitat and the presence and ecology of native species if genetic pollution is to be avoided. Hence, many studies have been made to distinguish and identify ascomata and ectomycorrhizae by morphological and molecular analyses \(Mello et al. \[2006 Mello, A, Murat, C and Bonfante, P. 2006. Truffles: Much more than aprized and local fungal delicacy. FEMS Microbiol Lett, 260: 1–8. \\[CrossRef\\], \\[PubMed\\], \\[Web of Science ®\\]\]\(#\); Pacioni et al. \[2010 Pacioni, G, Leonardi, M, Taglienti, A, Cozzolino, S, Ritota, M, Sequi, P and et, al. 2010. Internal structure and quality assessment of fresh truffle Tuber melanosporum by means of magnetic resonance imaging spectroscopy. Plant Biosyst, 144: 826–832. \\[Taylor & Francis Online\\], \\[Web of Science ®\\]\]\(#\)\).](#)

Host plants are only a small component among a large number of other variables all important for successful plantation: choice of soil, environment, altitude, exposure and site slope inclination are just some of the others. Adequate planning to establish and maintain the plantation are also fundamental for rapid and high quality truffle production (Donnini et al. 2010). Unfortunately, the truffle lifecycle is toolong for short-term results and there are many variables that interact, such as allelochemicals (Splivallo et al. [2010 Splivallo, R, Ottonello, S, Mello, A and Karlovsky, P. 2010. Truffle volatiles: From chemical ecology to aroma biosynthesis. NewPhytol, 189: 688–699. \[CrossRef\], \[Web of Science ®\]](#)), and other saprobic or ectomycorrhizal fungi and bacteria (Barbieri et al. [2005 Barbieri, E, Gioacchini, A M, Zambonelli, A, Bertini, L and Stocchi, V. 2005. Determination of microbial VOCs from Staphylococcus pasteurii against Tuber borchii using SPME and gas chromatography/ion trap mass spectrometry. Rapid Commun Mass Sp, 19: 3411–3415. \[CrossRef\], \[PubMed\], \[Web of Science ®\]](#); Mello et al. [2010 Mello, A, Miozzi, L, Vizzini, A, Napoli, C, Kowalchuk, G and Bonfante, P. 2010. Bacterial and fungal communities associated to Tuber magnatum-productive niches. Plant Biosyst, 144: 323–332. \[Taylor & Francis Online\], \[Web of Science ®\]](#)). Truffle orchards can be assessed on the basis of fruitbody production or by ECM analyses of root samples to study the competition with other ECM fungi (Donnini et al. [2008 Donnini, D, Baciarelli Falini, L, Di Massimo, G, Benucci, G and Bencivenga, M. 2008. Competizione o sinergia di funghi ectomicorrizici in tartufoie coltivate. Micol Ital, 37: 46–51. ; Napoli et al. \[2010 Napoli, C, Mello, A, Borra, A, Vizzini, A, Sourzat, P and Bonfante, P. 2010. Tuber melanosporum, when dominant, affects fungal dynamics in truffle grounds. New Phytol, 185:\]\(#\)](#)

237–247. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). There is often a very significant diversity of ECM fungi, and in some case-studies results suggest an ECM equilibrium of a fungal community rather than real competition between species (Donnini et al. [2008](#) [Donnini, D.](#), [Baciarelli Falini, L.](#), [Di Massimo, G.](#), [Benucci, G.](#) and [Bencivenga, M.](#) 2008. Competizione o sinergia di funghi ectomicorrizici in tartufaie coltivate. *Micol Ital*, 37: 46–51. ; Iotti et al. [2010](#) [Iotti, M.](#), [Lancellotti, E.](#), [Hall, I](#) and [Zambonelli, A.](#) 2010. The ectomycorrhizal community in natural *Tuber borchii* grounds. *FEMS Microbiol Ecol*, 72: 250–260. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). Cultivation of truffles with great gastronomic and commercial value is becoming an important agricultural activity, because of a new emphasis on organic foods, improved biodiversity conservation and increased income from local products. There are already truffle orchards with a high production of black truffles: from 50 to 100 kg/ha, or even more, for *T. melanosporum* and *T. aestivum* Vittad. (Donnini et al. 2010). Good results have also obtained with *T.borchii* Vittad. in Italy and other countries (Hall et al. [2007](#) [Hall, I R.](#), [Brown, G T](#) and [Zambonelli, A.](#) 2007. *Taming the truffle*, 304Portland, Oregon, USA: Timber Press.). In contrast cultivation of *T. magnatum* has remained difficult and at best only partially successful. Although many aspects of the biology of *T. magnatum* remain unknown; there are some plantations producing this species (Donnini et al. 2010). Truffle cultivation is an alternative activity which can help to solve rural economic problems, but it is not simple, because each situation requires different management. The results of local experimentation cannot always, therefore, be applied to plantations further afield. Data about the genome sequence of *T. melanosporum* now becoming available (Martin et al. [2010](#) [Martin, F.](#), [Kohler, A.](#), [Murat, C.](#), [Balestrini, R.](#), [Coutinho, P M.](#), [Jaillon, O](#) and [et, al.](#) 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature*, 464: 1033–1038. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]) will surely open a window on the complexities of the life-cycle and culture of this truffle.

Medicinal mushrooms

Many species of macrofungi have been used as medicines for 5000 years or more. However, the use of medicinal mushrooms in Europe pales when compared with their use in the Far East: even now about 10 species are regularly used in traditional Chinese medicine. In the last decades, many scientific studies on medicinal mushrooms have been carried out: the traditional applications of many fungi have been confirmed and new wider uses found. Research has focused on fungi with anti-cancer and immune system enhancing activities and many bioactive substances with immunomodulating effects have been isolated (Bochers et al. [2008](#) [Bochers, A T.](#), [Krishnamurthy, A.](#), [Keen, C L.](#), [Meyers, F J](#) and [Gershwin, M E.](#) 2008. The immunobiology of mushrooms. *Exp Biol Med*, 233: 259–276. [[CrossRef](#)], [[Web of Science ®](#)]). These are mainly (-glucan protein with different lateral chains peculiar to each species, amino acids such as lysine and tryptophan, as well as nicotinic acid, riboflavin (vitamin B₂), pantothenic acid and vitamins B, C and K. In addition, medicinal mushrooms contain terpenes and steroids, some of which have demonstrated antibacterial and antiviral activity. Interest in research on the medicinal value of mushrooms has increased in line with demand for mushrooms products used by many, not only in the Orient, as sources of functional and medical food. Even if fungi do not contribute much as basic food, they can nevertheless significantly diversify the human diet, providing important chemical compounds for the “nutraceutical” industry (Chang & Miles [2004](#) [Chang, S T](#) and [Miles, P G.](#) 2004. *Mushrooms cultivation, nutritional value, medicinal effect, and environmental impact.* , 2nd ed, 451Boca Raton, FL: CRC Press. [[CrossRef](#)]; Saltarelli et al. [2009](#) [Saltarelli, R.](#), [Ceccaroli, P.](#), [Iotti, M.](#), [Zambonelli, A.](#), [Buffalini, M.](#), [Casadei, L.](#) and [et, al.](#) 2009. Biochemical characterisation and antioxidant activity of mycelium of *Ganoderma lucidum* from Central Italy. *Food Chem*, 116: 143–151. [[CrossRef](#)], [[Web of Science ®](#)]).

The consequent need for large quantities of mycelium and basidiomata of medicinal mushrooms for scientific research and human consumption has made the cultivation of different species crucial. Several medicinal mushrooms are rare in nature or have a limited distribution. Although it is possible to extract medicinal substances from mycelium of cultivated species, the quality and quantity of molecules contained in basidiomata are different (Reshetnikov et al. 2001). Basidioma production needs specific environmental conditions and is the most difficult step in mushroom cultivation. To stimulate the medicinal fungi market in Italy, high quality products, obtained from well-known strains and substrata, will be critically important. That in turn requires synergy between University researchers and growers. The University of Pavia keeps a collection of lignicolous fungal strains isolated from basidiomata collected in several Italian regions (Altobelli et al. unpublished data). All strains were collected in unpolluted areas and accurately identified to guarantee species discrimination which is considered one of the critical points (Paterson Russell 2006 [Paterson Russell, R M.](#) 2006. Ganoderma – A therapeutic fungal biofactory. *Phytochemistry*, 67: 1985–2001. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)]). *Ganoderma lucidum* (Curtis) P. Karst. and *Trametes versicolor* (L.) Lloyd have been cultivated using different techniques to obtain basidiomata, and chemical characterization of those fruitbodies is in progress. The exotic *Lentinula edodes* (Berk.) Pegler has been also cultivated using strains obtained from mushroom farms. There have also been studies in Italy on the *Pleurotus eryngii* (DC.) species-complex. It comprises edible fungi, growing as saprobes on root residues of plants of the *Apiaceae*, with a large commercial and biotechnological potential. Studies based on enzyme and RAPD-PCR analysis (Zervakis et al. 2001 [Zervakis, G I](#), [Venturella, G](#) and [Papadopoulou, K.](#) 2001. Genetic polymorphism and taxonomic infrastructure of the *Pleurotus eryngii* species-complex as determined by RAPD analysis, isozyme profiles and ecomorphological characters. *Microbiology*, 147: 3183–3194. [[CrossRef](#)], [[PubMed](#)], [[Web of Science ®](#)], [[CSA](#)]) clearly separated *P. nebrodensis* from varieties of *P. eryngii*. In the Mediterranean area these *taxa* are very popular as edible mushrooms. The chemical composition and nutritional value (LaGuardia et al. 2005) of *Pleurotus* mushrooms makes them suitable in every kind of diet, including the hypocaloric. They are also considered a good source of vitamins and mineral salts. Investigations on the *P. eryngii* species-complex as a source of anti-tumour and immunomodulating polysaccharides are continuing at the University of Palermo. The ability of organic extracts from *Pleurotus* species to induce anti-proliferative, anti-invasive and pro-apoptotic effects on human colon carcinoma HT-29 and HCT-116 cells are currently being investigated. In particular, anti-cancer activities of phenolic antioxidants and polysaccharides are being tested singly and in combination, including *in vivo* experiments using immunodeficient nude mice as experimental metastasis models.

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