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Anaerobic denitrification and biotechnological potentials of filamentous fungi isolated from coastal marine sediment

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Abstract

The anaerobic denitrification, antibacterial and enzyme-producing potentials of filamentous fungi isolated from sediment collected from the coastal region of the Niger Delta in Bonny Island, Nigeria were investigated. The fungi were aerobically isolated using spread plate method on sterile acidified potato dextrose agar. The capability of isolates to survive under anoxic environment was assessed by incubating cultured plates in anaerobic jars. Four isolates were studied for growth and denitrifying capacity in mineral salts supplemented with nitrate, glucose and peptone for 14 days under anaerobic conditions. Agar diffusion assay was employed for antibacterial activity of isolates against fish and shellfish pathogens (*Aeromonas hydrophila* and *Vibrio parahaemolyticus*). The isolates were screened for production of amylase, cellulase, protease and lipase using starch agar, cellulose agar, skim milk agar and tributyrin agar correspondingly. Twelve fungal isolates of the genera *Fusarium*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Acremonium* and *Trichoderma* were isolated at different frequencies with *Aspergillus* predominating. A decrease in nitrate concentration and accumulation of nitrite and ammonia were observed at the end of the denitrification study. Nine fungal isolates (75%) were found active against the pathogens. The percentage composition of amylase, cellulase, protease and lipase producing strains were 83.3, 83.3, 75.0 and 66.7% respectively. The denitrification pattern and organic substrate degradation ability observed showed that fungi can play a role in nitrogen and carbon cycles occurring in marine sediment. The results also revealed that the indigenous multiple enzyme-producing fungi with antibacterial potential isolated in this study can be efficiently used for biotechnological applications.

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Introduction

An alternative respiration in prokaryotes that helps them to survive in the absence of oxygen is anaerobic denitrification. Nevertheless, current studies have shown that fungi can also undergo anaerobic respiration. It has been reported that denitrifying fungi do not complete the denitrification process to nitrogen rather they form nitrous oxide (N_2O) which contributes to an elevated greenhouse gases (Ronaldand and Laughlin, 2002; Shoun *et al*., 1992). However, some authors have reported that during denitrifying process many fungal denitrifiers are able to produce molecules of hybrid N_2O or $N_2$by combining N- atoms from nitrite (NO_2^-) with other nitrogen compounds such as ammonium (NH_4^+) orazide (Hayatsu *et al*., 2008; Shoun *et al*., 1992).

Furthermore, ammonia fermentations (ammonifications) occur, in anaerobic conditions, when $NO_3^-$is reduced toNH_4^+ (Mouton *et al*., 2012). Therefore, fungi is able to perform multiple respiration depending on the oxygen levels in their environment (Catherine and Raghukumar, 2009; Greben *et al*., 2007; Takaya, 2002). Most fungi occurring in marine sediment are facultative with terrestrial origins but their function in breaking down aquatic organic compound and in cycling of nutrients in the sea is scantily known in comparison with their terrestrial counterparts (Clipson, 2006; Fell and Newell, 1998).

Marine microbes at times accumulate structurally distinctive bioactive secondary metabolites that are not found in terrestrial microbes to enable them to acclimatize and live in the marine ecosystem which is characterized by unique conditions that differ from those found in other environment (Bhakuni and Rawat, 2005). Marine microbes are now being used as a source for novel enzymes since enzymes from microorganisms are comparatively more active and stable than similar enzymes gotten from animals and plants (Ghosh *et al*., 2005). Enzymes such as amylase, cellulase, protease and lipase have dominated the world market owing to their commercial applications (Falch, 1991). In the emergent field of marine biotechnology, marine fungi have useful properties such as the production of new products and improvement of industrial processes.

Application and screening of fungi for new metabolites and enzymes are the main goals of modern research to achieve environment friendly technological expansion (Maria *et al*., 2005). Therefore, in the present study, filamentous fungi from coastal marine sediment were investigated for their anaerobic denitrification, antibacterial and extracellular enzyme-producing potentials. This is to understand the role they play in this ecological niche as well as the possibility of using them for biotechnological applications.

Materials and methods

Sample Collection

Marine sediment was collected with a corer from the coastal region of the Niger Delta in Bonny Island. It was extruded into sterile plastic container and taken to the laboratory, immediately, for analyses.

Isolationof filamentous fungi

Twenty five gramme of coastal marine sediment was suspended in 225 ml of sterile normal saline. Ten-fold serial dilutions up to 10^{-6} were prepared. Sub samples of 0.1 ml of the dilutions were cultured on sterile plates of acidified potato dextrose agar (Oxiod) supplemented with 1.0% sodium chloride using spread plate method. The plates were incubated for 3-5 days at 28°C after inoculation. Colonies of fungi were purified by sub culturing aseptically into sterile acidified potato dextrose agar. Pure cultures were maintained as stock culture on acidified potato dextrose agar. Fungi identification was carried out by macroscopic and microscopic examination of the isolates as well as back view of the plate culture (De Hoog *et al*., 2000; Larone, 2011; Samson and De Boer, 1995).

Assessment offungi for growth and nitrate utilization ability under anoxic condition

Four isolates of fungi were screened for growth and denitrification ability under anaerobic condition. The fungi were cultivated in modified mineral salts medium of Mouton *et al*. (2012) containing 5.2 g KH_2PO_4, 1.0 g $MgSO_4.7H_2O$, 1.0 g KCl, 6.4 g K2HPO4, 0.02 g $FeSO_4$, 0.02 g $ZnSO_4.7H_2O$, 0.008 g $CuSO_4.5H_2O$ and 0.85 g $NaNO_3$ in 1 litre of distilled water: The medium was supplemented with 10% glucose and 10% peptone and dispensed in 100 ml amounts into250 ml conical flasks. The flasks were sterilized at 121°C for 15 mins. On cooling each flask was aseptically inoculated in duplicate with 5 mm diameter mycelial plug of a seven day old culture of each fungal isolates. The flasks were incubated in anaerobic jars at 28°C for 14 days. The mycelia were harvested by filtration using cheesecloth and were allowed to dry to a constant weight at 80°C in an oven. Growth was determined as dry weight in 100 ml of culture medium at the end of the experiment. Nitrite and ammonia formed as well as nitrate concentrations were determined by spectrophoto-metric method (APHA, 1998). At zero hour and at the end of the experiment, the dissolved oxygen was determined by spectrophotometric method (Pai *et al*., 1993). The chemicals used were all of analytical grade.

Antimicrobial assay

The antibacterial activity of the fungal isolates against fish and shellfish pathogenic *Aeromonashydrophila* and *Vibrio parahaemolyticus* was assessed using agar diffusion assay.

The fungi strains were grown on acidified potato dextrose agar for 7 days at 28°C. The mycelialdiscs (5 mm diameter) were then excised and placed on plates of nutrient agar freshly seeded with 0.1 ml of 24 hr old broth culture of each tested bacterial strains. Incubation of the plates was for 24 hr at 37°C. Clearancezones were measured and recorded indicating antibacterial activity.

Qualitative enzyme activity

Extracellular enzyme production by fungi was demonstrated by substrate digestion in agar plates inoculated with 5 mm discs of mycelia and subsequent incubation at 28°C for a period of 3-5 days. The assessment of amylase activity was done by cultivating the fungi on starch agar plates. One percent iodine solution was used in flooding the plates after incubation. A clear zone around the colony showed positive result and the presence of blue coloursur rounding the colony indicated absence of amylase. Assay for protease was carried out by cultivating the filamentous fungi on skim milk agar plates. Protease activity was established by clearance of opaque milk proteins around the colony after incubation. Lipase activity was screened by cultivating the fungi on tributyrin agar (Oxoid) plates. Zone of clearance around the colony after incubation indicated positive result. Cellulase activity was performed by cultivating the fungi on cellulose agar plates. After incubation, one percent congo red was used in flooding the plates. The cellulose presence was shown by zone of clearance surrounding the colony.

Statistical Analysis

Standard deviations for each of the experimental results were calculated using Excel Spreadsheets, with Microsoft excel software. Differences between treatments were examined for significance by one-way Analysis of Variance.

Results and discussion

The frequency of occurrence of different filamentous fungal genera from coastal marine sediment is presented in Table 1. The genera *Fusarium*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Acremonium* and *Trichoderma* were isolated at different frequencies with *Aspergillus* predominating.

Table 1. Frequency of isolation of fungal genera in coastal marine sediment.

Genus	Number of isolates
Aspergillus	4 (33.33)
Fusarium	2 (16.67)
Penicillium	2 (16.67)
Cladosporium	2 (16.67)
Trichoderma	1(8.33)
Acremonium	1 (8.33)
Total	12 (100)

Numbers in parentheses represent percentage frequencies.

The greatest species diversity of fungi is found in the marine sediments (Pivkin *et al*., 2006). Diverse substrates on land and in the ocean have been effectively colonized by representatives of the genus *Aspergillus* which are physiologically very versatile (Damare *et al*., 2006b). The dominance of *Aspergillus* taxa in marine sediments has also been reported by Das *et al*. (2009) and Catherine and Raghukumar (2009).The fungi isolated in this study are known terrestrial fungi and have been isolated by other workers from the marine environments (Burgaud *et al*., 2013; Damare *et al*., 2006b; Mathan *et al*., 2013; Pindi, 2012; Raghukumar *et al*., 2004; Singh *et al*., 2011; Vrijmoed, 2000). Simonato *et al*. (2006) reported that the colonization of deep-sea habitats by terrestrial fungi may be owing to their capability to change the composition of their membrane to with stand elevated hydrostatic pressure.

Table 2. Nitrate utilization by fungal isolates under anaerobic culture condition.

Culture	Nitrate concentration (mg/L) ± S.D.	
	Zero hour	Day 14
Aspergillus sp. A2	0.83± 01	0.11± 01
Fusarium sp. F2	0.82± 03	0.1± 04
Penicillium sp. P1	0.81 ± 02	0.09± 05
Acremonium sp.	0.8 ± 01	0.08± 03

All the isolates were able to grow an aerobically. Four isolates screened for denitrification activity under anaerobic condition showed a denitrification pattern where NO_3^-(Table 2) was utilized and NO_2^- (Table 3) and ammonia (Table 4) were produced.

Table 3. Nitrite formation by fungal isolates under anaerobic culture condition.

Culture	Nitrite concentration (mg/L) ± S.D.	
	Zero hour	Day 14
Aspergillus sp. A2	0.02 ± 02	63.0 ± 04
Fusarium sp. F2	0.01 ± 01	62.0 ± 02
Penicillium sp. P1	0.02 ± 01	61.5 ± 03
Acremonium sp.	0.02 ± 02	62.5 ± 02

Table 4. Ammonia formation by fungal isolates under anaerobic culture condition.

Culture	Ammonia concentration (mg/L) ± S.D.	
	Zero hour	Day 14
Aspergillus sp. A2	0.11 ± 03	1.15 ± 01
Fusarium sp. F2	0.12 ± 02	1.16 ± 03
Penicillium sp. P1	0.11 ± 04	1.15 ± 01
Acremonium sp.	0.13 ± 01	1.17 ± 03

This denitrification pattern is known to take place in fungi (Hayatsu *et al*., 2008; Shoun *et al*., 1992; Zhou *et al*., 2002) and have been observed in fungi isolated from marine sediments (Catherine and Raghukumar, 2009; Mouton *et al*., 2012). Increase in fungal biomass (Table 5) was also observed showing that dissimilatory nitrate reduction is an energy yielding reaction (Catherine and Raghukumar, 2009). These data suggest that fungi may have the potential to play a role in the nitrogen cycle of this ecological niche with the prevailing hypoxic and anoxic conditions.

Sewage or animal residues of high nitrogen content can be treated with denitrification process to lower leaching of nitrate to groundwater.

Table 5. Fungal biomass under anaerobic culture condition.

Culture	Dry weight of mycelia (g/100 ml)
*Aspergillus*sp. A2	1.27 ± 03
Fusarium sp. F2	1.22± 02
Penicillium sp. P1	1.2 ± 04
Acremonium sp.	1.15 ± 05

The antibacterial activities of fungal isolates against fish and shellfish pathogens are shown in Table 6. The result showed that fungal strains inhibited the pathogens. The antagonistic fungi belong to the genera *Aspergillus* (4 strains), *Fusarium* (2 strains), *Penicillium* (2 strains) and *Acremonium* (1 strain) (Table 6).

Table 6. Antibacterial activity of fungal isolates against fish and shellfish pathogens.

Fungal isolates	Inhibition zone (mm) ± S.D.	
	Aeromonas hydrophila	*Vibrio parahae-molyticus*
Aspergillus sp. A1	14± 03	13 ± 01
Aspergillus sp. A2	18 ± 02	15 ± 03
Aspergillus sp. A3	14 ± 04	13 ± 01
Aspergillus sp. A4	15 ± 01	13 ± 03
Fusarium sp. F1	14± 02	13± 04
Fusarium sp. F2	13± 05	16± 02
Penicillium sp. P1	13± 06	14± 05
Penicillium sp. P2	14± 03	13± 07
Cladosporium sp. C1	-	-
Cladosporium sp. C2	-	-
Trichoderma sp.	-	-
Acremonium sp.	13± 01	13± 02

- = no inhibitions.

Marine fungi antagonistic to pathogenic bacteria have also been reported by other workers (Ariole *et al*., 2014a; Mathan *et al*., 2011; Samuel *et al*., 2011; Svahn *et al*., 2012; Swathi *et al*., 2013). The main contributors to bioactive metabolites of fungal origin are *Aspergillus* species (Bugni *et al*., 2000; Parvatkar *et al*., 2009).

Dreyfuss and Chapella (1994) reported that about 4,000biologically active secondary metabolites from fungi have been described. Fungisuch as *Fusarium*, *Aspergillus*, *Penicillium* and *Acremonium* are known for production of a number of bioactive metabolites (Dreyfuss and Chapella, 1994). In this study, 75% of fungal isolates exhibited antibacterial activity, showing the potential of marine fungi as producers of novel metabolites. The produced bioactive compounds probably act as a chemical defense that enables fungi compete for nutrients (Fenical and Jensen, 1993; Gallo *et al*., 2004).

The result of substrate degrading ability of fungi from coastal marine sediment is presented in Table 7. The enzyme-producing fungi percentage composition are 83.3, 83.3, 75.0 and 66.7% for amylase producing strains, cellulase producing strains, protease producing strains and lipase producing strains respectively.

Table 7. Composition and substrate degrading ability of fungi isolated from coastal marine sediment.

Fungal isolates	Enzymes			
	Amylase (83.3%)	Cellulase (83.3%)	Protease (75.0%)	Lipase (66.7%)
Aspergillus sp. A1	+	-	+	+
Aspergillus sp. A2	+	+	+	+
Aspergillus sp. A3	+	+	+	-
Aspergillus sp. A4	+	+	+	-
Fusarium sp. F1	+	+	-	+
Fusarium sp. F2	+	+	+	-
Penicillium sp. P1	-	+	+	+
Penicillium sp. P2	+	+	-	+
Cladosporium sp. C1	+	+	-	+
Cladosporium sp. C2	-	+	+	+
Trichoderma sp.	+	+	+	-
Acremonium sp.	+	-	+	+

(+) indicates positive activity; (-) indicates negative activity.

Isolation of these multienzyme producing filamentous fungi implies that fungi might play a key function in decomposition of detritus and can be an invaluable source of enzymes for industrial applications. The ability of microbial isolates to degrade starch, cellulose, proteins and lipids has been demonstrated by other researchers (Ariole *et al*., 2014b; Damare *et al*., 2006a; Maria *et al*., 2005; Smitha *et al*., 2014).

Conclusion

In the present study, filamentous fungi were isolated from coastal marine sediment collected from the Niger Delta region in Bonny Island, Nigeria. Four of the isolates grown under anaerobic condition clearly expressed denitrifying ability. At the end of denitrification experiment, nitrate utilization with biomass increase as well as nitrite and ammonia formation was observed. Seventy five percent of the isolates were antagonistic to fish and shellfish pathogenic *Aeromonas hydrophila* and *Vibrio parahaemolyticus*. All the fungi isolates were found to possess at least three hydrolytic enzymes (amylase, cellulase, protease and lipase) employed. The indigenous multiple enzyme producing filamentous fungi with denitrification potential could participate in organic matter degradation and in nitrogen cycle occurring in marine sediment. The results also showed that marine fungi are invaluable source of enzymes and metabolites for industrial and aquacultural applications.

References

APHA. 1998. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association. Washington DC, USA.

Ariole CN, Ihunwo HO, Ozuru CN. 2014a. Isolation of marine green alga-associated microorganisms and their antimicrobial activity against fish pathogenic *Aeromonas* strain. Journal of Chemical, Biological and Physical Sciences **4(4),** 3279-3286.

Ariole CN, Nwogu HA, Chuku PW. 2014b. Enzymatic Activities of Intestinal Bacteria Isolated from Farmed *Clariasgariepinus*. International Journal of Aquaculture **4(18),** 108-112.

Bhakuni DS, **Rawat DS.** 2005**.** Bioactive Marine Natural Products. Springer: New York and Anamaya Publisher**,** New Delhi, India.

Bugni TS, Abbanat D, Bernan VS, Maiese WM, Greenstein M, Wagoner RMV, Ireland CM. 2000.Yanuthones: novel metabolites from a marine isolate of *Aspergillus niger*, J. Organic Chem **65**, 7195-7200.

Burgaud G, Woehlke S, Redou V, Orsi W, Beaudoin D, Barbier G, Biddle JF, Edgcomb VP. 2013. Deciphering presence and activity of fungal communities in marine sediments using a model estuarine system. Aquatic Microbial Ecol **70**, 45-62.

Catherine SJ, Raghukumar C. 2009. Anaerobic denitrification in fungi from the coastal marine sediments off Goa, India. Mycol Res **113**, 100-9.

Clipson N, Otte M, Landy E. 2006. Biogeochemical roles of fungi in the marine and extreme habitats. In: Gadd GM, Ed. Fungi in biogeochemical cycle Cambridge University Press, New York 436-461.

Damare S, Raghukumar C, Muraleedharan UD, Raghukumar S. 2006a. Deep-sea fungi as a source of alkaline and cold- tolerant proteases, Enzy. And Microb. Technol **39**, 172-181.

Damare S, Raghukumar C. Raghukumar S. 2006b. Fungi in deep-sea sediments of the Central Indian Basin. Deep-Sea Res. I **53**, 14-27.

DasS, Lyla PS, Khan SA. 2009. Filamentous fungal population and species diversity from the continental slope of Bay of Bengal, India. – Acta Oecologica **35**, 269–279.

De Hoog GS, Guarro J, Gené J, Figueras MJ. 2000. Atlas of clinical fungi, 2nd ed. Central bureau voor Schimmel cultures, Utrecht.

Dreyfuss MM, Chapella IH. 1994. Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. In: Gullo VP, ed. The discovery of natural products with therapeutic potential, Butterworth –Heinemann, Boston, 49-80.

Falch EA. 1991. Industrial enzymes - developments in production and application, Biotechnol. Adv **9**, 643-658.

Fell JW, Newell SY. 1998. Biochemical and molecular methods for the study of marine fungi. In: Cooksey KE, ed. Molecular approaches to the study of the ocean. London, UK: Chapman and Hall 259–283.

Fenical W, Jensen PR. 1993. Marine micro-organisms: a new biomedical resource. In: Attaway DH, Zaborsky OR (eds) Marine biotechnol. Plenum Press, New York **1**, 419-457.

Gallo ML, Seldes AM, Cabrera GM. 2004. Antibiotic long chain and α, β-unsaturated aldehydes from the culture of the marine fungus *Cladosporium* sp. Biochem. Syst. Ecol **32**, 545-551.

Ghosh D, Saham I, Sana B, Mukherjee J. 2005. Marine Enzymes. Adv. Biochem. Eng. Biot **96**, 189-218.

Greben HA, Joubert LM, Tjatji MP, Whites HE, Botha A. 2007. Biological nitrate removal from synthetic wastewater using a fungal consortium in one stage bioreactors. Water SA **33**, 285-290.

Hayatsu M, Tago K, Saito M. 2008. Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci. Plant Nutri **54**, 33-45.

Larone DH. 2011. Medically important fungi*:* A guide to identification. 5th ed. American Society for Microbiology (ASM) Press, Washington, DC, 485.

Maria GL, Sridhar KR, Raviraja NS. 2005. Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India, J. Agricultural Technol **I**, 67-79.

Mathan S, Anton SA, Kumaran J, Prakash S. 2011. Anticancer and Antimicrobial Activity of *Aspergillus protuberus* SP1 Isolated from Marine Sediments of South Indian Coast. Chin. J. Nat. Med **94**, 0286-0292.

Mathan S, Subramanian V, Nagamony S, Ganapathy K. 2013. Isolation of endophytic fungi from marine algae and its bioactivity, *Int. J. Res. Pharm. Sci* **4(1),** 45-49.

Mouton M, Postma F, Wilsenach J, Botha A. 2012. Diversity and characterization of culturable fungi from marine sediment collected from St. Helena Bay, South Africa. Microbial Ecology **64**, 311-319.

Muffler K, Sana B, Mukherjee J, Ulber R. 2015. Marine Enzymes- Production & Applications. In: Se- Kwon Kim, Ed. Springer Handbook of Marine Biotechnology. Part B, Springer, Berlin Heidelberg 413-429.

Pai SC, Gong GC, Liu KK. 1993. Determination of dissolved oxygen in seawater by direct spectrophotometry of total iodine. Marine Chemistry **41**, 343-351.

Parvatkar R, Souza C, Tripathi A, Naik CG. 2009. Aspemolides A and B, butenolides from a marine-derived fungus *Aspergillus terreus*, *Phytochem* **70**, 128-132.

Pindi PK. 2012. Diversity of fungi at various depths of marine water. Research in Biotechnology **3**, 62-66.

Pivkin MV, Kuznetsova TA, Sova VV. 2006. Marine fungi and their metabolites: Dalnauka: Vladivostok 133.

Raghukumar C, Raghukumar S, Sheelu G, Gupta SM, Nagender NB, Rao BR. 2004. Buried in time: culturable fungi in a deep-sea sediment core from the Chagos Trench, Indian Ocean. Deep-Sea Res. Part-I **51**, 1759–1768.

Ronald J, Laughlin R. 2002. Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. Soil Science Society of America Journal **66**, 1540-1548.

Samson RA, De Boer E. 1995. Introduction to food-borne fungi, 4th rev. ed. Koninklijke Nederlandse Akademie van Wetenschappen, Central bureauvoor Schimmel cultures, Baarn 322.

Samuel P, Prince L, Prabakaran P. 2011. Antibacterial Activity of Marine derived Fungi Collected from South East Coast of Tamilnadu. India. J. Microbiol. Biotech. Res **1(4),** 86-94.

Shoun H, Kim DH, Uchiyama H, Sugiyama J. 1992. Denitrifcation by fungi. *FEMS Microbiology Letters* **94**, 277-282.

Simonato F, Campanaro S, Lauro FM, Vezzi A, D'Angelo M, Vitulo N, Valle G, Bartlett DH. 2006. Piezophilic adaptation: a genomic point of view. J. of Biotechnol **126(1),** 11-25.

Singh P, Raghukumar C, Verma P, Shouche Y. 2011. Fungal community analysis in the deep-sea sediments of the central Indian basin by culture-independent approach, Microbial Ecology **61**, 507-517.

Smitha SL, Correya NS, Philip R. 2014. Marine fungi as a potential source of enzymes and antibiotics. Int. J. Res. Mar. Sci **3(1),** 5-10.

Svahn KS, Goransson U, El-Seedi H, Bohlin L, Larsson DGJ, Olsen B, Chryssanthou E. 2012. Antimicrobial activity of filamentous fungi isolated from highly antibiotic contaminated river sediment. Ecology and Epidemiology **2**, 11591-11596.

Swathi J, Narendra K, Sowjanya KM, Krishna Satya A. 2013. Marine fungal metabolites as a rich source of bioactive compounds. Glob. J. Environ. Biochem **1(1),** 014-026.

Takaya N. 2002. Dissimilatory nitrate reduction metabolisms and their control in fungi. Journal of Bioscience and Bioengineering **94**, 506-510.

Vrijmoed LLP. 2000. Isolation and culture of higher filamentous fungi, In: Hyde KD, Pointing SM, Eds. Marine mycology – a practical approach, Fungal Diversity Research, Hong Kong, 1-20.

Zhou Z, Takaya N, Nakamura A, Yamaguchi M, Takeo K, Shoun H. 2002. Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi. Journal of Biological Chemistry **277**, 1892-1896.