Malaysian Journal of Microbiology, Vol 16(3) 2020, pp. 153-158
DOI: http://dx.doi.org/10.21161/mjm.180234

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Isolation and characterisation of nitrilase producing *Aspergillus* species recovered from solid waste leachates at two dump sites in Lagos, Nigeria

Adewale Kayode Ogunyemi[1]*, Bukola Caroline Ogunyemi[2], Abiola Lateefat Ategun[2], Bamidele Abiodun Iwalokun[3], Joseph Ikosi Ike[2], Matthew Olusoji Ilori[1] and Olukayode Oladipo Amund[1]

[1]Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria.
[2]Department of Chemical Science, Yaba College of Technology, Yaba, Lagos, Nigeria.
[3]Department of Microbiology, Molecular Biology Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.
Email: waleogunyemi2002@yahoo.com

Received 12 September 2018; Received in revised form 8 October 2019; Accepted 14 December 2019

ABSTRACT

Aims: Nitrile compounds are cyanogenic intermediates, products, byproducts and waste products of agriculture, chemical and pharmaceutical industries and fossil fuels degradation. The enzymatic hydrolysis of nitriles to non-toxic carboxylic acids or amides plays an increasingly important role in environment remediation. This study aimed at exploring the potential of *Aspergilli* in the detoxification nitrile compounds at two selected dump sites in Lagos, Nigeria.
Methodology and results: Decomposing solid waste leachate samples (SWL) were randomly collected at two selected dump sites namely Olusosun (Ojota) and Isolo (Oke-Afa). Samples per site were pooled, processed by selective enrichment and screened for the presence of Aspergilli by culture technique and intergenic spacer sequencing (ITS). Biomass generation and pH changes in the culture fluids were monitored at 4-days interval by dry weight measurements. Nitrilase production was determined spectrophotometrically. Two nitrilase producing *Aspergillus* strains: *Aspergillus fumigatus* strain WO2 with accession number MF78882 and *Aspergillus niger* strain WO7 with accession number MH542673 were identified. Growth investigation revealed biomass generations of 17.8 g and 23.8 g dry weight per one liter media for *A. fumigatus* strain WO2 and *A. niger* strain WO7 respectively. Progressive pH monitoring showed decline from 7.2 to 4.5 and 7.2 to 6.2 was obtained for strains WO2 and WO7 respectively, during nitrilase production at different yields of 0.0150 and 0.0161 mg/mL/min respectively.
Conclusion, significance and impact of study: This study supports the studied dump sites as important sources of nitrilase-producing *A. fumigatus* and *A niger* strains with potentials as cost-effective environmental bioremediation agents in Nigeria.

Keywords: Nitriles, Nitrilase, solid waste leachate, *Aspergillus* strains, bioremediation

INTRODUCTION

The distributions of nitrilase activities in nitrilase producing microorganisms especially filamentous fungi are difficult to assess (Gong *et al.*, 2012b). Microbial nitrilases have been identified in the fungal genera include *Fusarium*, *Gibberella*, *Aspergillus* and *Penicillium* (Banerjee *et al.*, 2002; Gong *et al.*, 2012a). However, information available on fungal nitrilase is scanty right from decades. Several fungal strains have been able to hydrolyze indole-3-acetonitrile (IAN) into indole-3-acetic acid and they are from the genera of *Fusarium*, *Gibberella*, *Aspergillus*, and *Penicillium* (Thimann and Mahadevan, 1964). Nitrilases in fungi were described in many strains such as *Fusarium oxysporum* ssp. melonis, *F. solani* O1, *Aspergillus niger* K10 or *Penicillium multicolor* CCF 2244 (Goldlust and Bohak, 1989; Vejvoda *et al.*, 2008; Šnajdrová *et al.*, 2004; Kaplan *et al.*, 2006a). As regards to activity and gene screening results, filamentous fungi are a found to be rich source of nitrilases as compared to their widely examined bacterial counterparts. All known fungal nitrilases are inducible like those from bacteria. 2-cyanopyridine seems to be a good inducer of nitrilases in filamentous fungi; however, its expression in suitable hosts is required for biotechnological applications due to the low specific activity of nitrilases from native producers (Martínková *et al.*, 2009). The facts are not been farfetched why more studies are on the bacterial nitrilases than fungal nitrilases (Gong *et al.*, 2012b). Nitrilase activity in *Streptomyces* (Khandelwal *et al.*, 2007) was induced by benzonitrile as in the fungus *F. solani* (Harper, 1977). Some aliphatic

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(3) 2020, pp. 153-158
DOI: http://dx.doi.org/10.21161/mjm.180234

nitriles were also used for nitrilase activity induction, this includes valeronitrile in some filamentous fungi. In Czech Republic, a research group isolated and identified nitrilases from *A. niger* K10 and *F. solani* O1 and have proved to be promising biocatalyst tools in nitrile transformation (Winkler *et al.*, 2009). Both these new fungal nitrilases were purified, immobilized, and used to synthesize carboxylic acids (Kaplan *et al.*, 2006b; Vejvoda *et al.*, 2008; Kaplan *et al.*, 2011). Moreover, the aforementioned *F. solani* IMI 196840 nitrilase was further investigated (Vejvoda *et al.*, 2010). Kaplan *et al*. (2006b) were of opinion that aromatic nitrilases were efficiently induced in several species of filamentous fungi. While knowledge of the structure and function of bacterial nitrilases has substantially improved in the last two decades (Banerjee *et al.*, 2002; O'Reilly and Turner, 2003; Singh *et al.*, 2006), almost no further work has been devoted to nitrilases from filamentous fungi until recently. This study aimed at characterizing fungal species that are suitable candidates for application on sites polluted with both aliphatic and aromatic nitriles as well as cassava processing wastes which contain cyanogenic glycosides.

MATERIALS AND METHODS

Sample collection for isolation of fungi

For the fungal isolation, solid waste leachates (SWL) were collected from the landfill sites at two locations, Olusosun, Ojota (Coordinates: N 6°29'21.8''; E 3°23'29.3'') and Oke-Afa, Isolo (N 6°27'11.0002''; E 3°23'44.9999'') in sterile sample bottles, properly labeled and stored at 4 °C, and processed within 24 h. Figure 1 shows the satellite view of dump sites and sampling points.

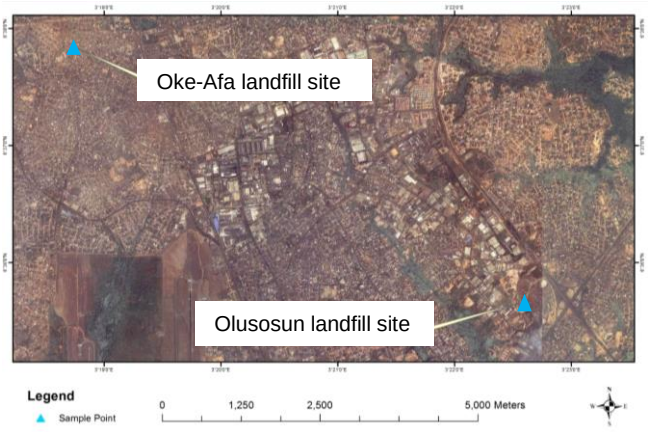

Figure 1: Satellite image of Olusosun and Oke-Afa landfill sites in Lagos State, Nigeria.

Media and culture conditions

The fungal strains capable of utilizing glutaronitrile as the sole carbon and nitrogen sources were isolated from SWL by selective enrichment culture technique (Santoshkumar *et al.* 2011) About 1.0 g of the SWL was suspended in 50 mL of mineral salts medium (MSM) (containing in g/L of K_2HPO_4, 2.5; KH_2PO_4, 2.0; $MgSO_4{\cdot}7H_2O$, 0.5; $MnSO_4{\cdot}4H_2O$, 0.1; $CaCl_2{\cdot}2H_2O$, 0.06; $FeSO_4{\cdot}7H_2O$, 0.1; $Na_2MoO_7{\cdot}2H_2O$, 0.006) supplemented with glutaronitrile (0.2% v/v) in a 250 mL Erlenmeyer flask. The flasks were incubated in an orbital shaker (180 rpm) at 32 °C for 7 days. For further enrichment, 5% inoculum was transferred to fresh mineral salts medium (MSM) containing the same concentration of glutaronitrile. After several repeated subcultures, the culture was streaked on mineral salts agar (MSA) plates containing glutaronitrile. The colonies that grew on agar plates containing glutaronitrile but not on control plates (without glutaronitrile) were selected for further identification. The purity of the culture was checked periodically by plating on agar plates. Nitrilase assay was performed as previously described by Almatawah *et al.* (1999) and Gupta *et al.* (2010).

Identification of nitrilase producing organisms

The isolates were then identified using appropriate microscopic and macroscopic characteristics and ITS gene sequences and stored as agar slants at 4 °C until needed.

Cultural and morphological characteristics

The fresh culture samples were used for the microscopy. The samples were subcultured on a fresh potato dextrose agar plate and further processed by Lactophenol staining as described by Ogunyemi *et al.* (2010). They were examined for spore formation and other characteristics by using a compound microscope (Hitachi S-3500N model, ThermoNaran, Hitachi technologies, Schaumburg, Illinois, USA). The pure cultures of the fungal isolates were identified according to the identification criteria of mycology (Barnett and Hunter, 1972; de Hoog *et al.*, 2000).

Purification of cultures through single spore isolation

The test organisms were purified through single spore isolation technique (Samapundo *et al.*, 2007). The single conidial isolates were maintained on low nutrient medium for further studies.

Molecular identification

Genomic extraction and its gene detection

The genomic DNA of the strains was extracted and purified following a standard protocol for fungal genomic DNA preparations using Jena Bioscience DNA preparation kits (Germany). The ITS gene was amplified by Polymerase Chain Reaction (PCR) (94 °C for 5 min, 30 cycles consisting of 94 °C for 30 sec, 50 °C for 30 sec, 72 °C for 90 sec followed by a terminal incubation at 72

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(3) 2020, pp. 153-158
DOI: http://dx.doi.org/10.21161/mjm.180234

°C for 10 min) using universal ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-2 (5'-TCCTCCGCTTATTGATATGC-3') primers. The PCR product purification of isolates was carried by adding 2.5 µL of Exo/SAP master mix to 10.0 µL of the PCR product, mixed well and incubated at room temperature for 30 min. The reaction was stopped by heating the mixture at 95 °C for 5 min. The PCR amplified product was purified and the nucleotide sequence was determined with an automated sequencing apparatus (ABI3500XL, Thermo Scientific). The ITS sequences of the strains were searched for homology with the sequences in public databases using the BLAST search program (http://www.ncbi.nlm.nih.gov/) to find closely related fungal ITS gene sequences. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura *et al.*, 2007). The neighbor-Joining evolutionary model was used to construct the phylogenetic tree.

Agarose gel electrophoresis of DNA fragments

The PCR amplified DNA segments were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide, using 100 bp DNA marker (Promega, USA) as DNA standard, Millipore water (blank) was used as negative control. The gel was run for 80 min at 100 V, and the amplified products were observed and imaged by Kodak fluorescent imaging equipment, model IS 4000R (Kodak image station, care stream molecular imaging health Inc. Rochester, NY, USA.).

Growth and substrate utilization studies

About 1.0 mL of 48 h grown pure isolate was inoculated in a 250 mL conical flask containing mineral salts medium (100 mL, pH 7.2 and glutaronitrile (0.2 % v/v) as sole carbon source) and incubated at 30 °C and 150 rpm. The aliphatic, as well as aromatic nitrile compounds were tested for their abilities to support the growth of the nitrilase-producing fungi. The culture broths were sampled at an interval of 48 h and tested for nitrilase activities over a period of 12 days. For each substrate, two sets of controls (the uninoculated MSM with the substrate and the inoculated MSM without any substrate) were put in place to monitor the growth rate of each isolate and to rule out contamination. Growth of the test strains were performed under aerobic batch (submerged) fermentation. Five milliliters of MS was inoculated with the test organisms punched with a cork-borer on solid medium and the 5 mL of each organism was grown in separate Erlenmeyer flasks (100 mL) containing mineral salts medium (50 mL, pH 7.2) which was supplemented with 0.1% (v/v) substrate (Glutaronitrile) as sole carbon source. A flask containing substrate and medium but without organism serves as the control. The flasks were then incubated at 30 °C on a rotary shaker (150 rpm) for 16 days. Growth was evaluated at intervals (4 days) by the dried weight (g/L) in mineral salts medium, while the nitrilase activities were determined by the method described by Almatawah *et al.* (1999) and Gupta *et al.* (2010), by monitoring the production of ammonia using a UV-visible spectrophotometer at 630 nm.

Nitrilase assay

Culture filtrates collected at 48 h intervals from each of the culture flasks were centrifuged (10,000 × *g*, 4 °C, 10 min). The supernatants were used as the source of enzymes. The reaction mixture (3.0 mL) comprised of culture supernatant (1.0 mL), glutaronitrile (1.0 mL) and 1.0 mL phosphate buffer (0.2 M, pH 7.2). Nitrilase activity was measured as described earlier (Almatawah *et al.* 1999; Gupta *et al.* 2010) by monitoring the production of ammonia using a UV-visible spectrophotometer at 630 nm for 10 min. One unit of enzyme activity was defined as 1.0 mM of glutaronitrile oxidized per minute. The uninoculated medium was used as the control in all the experiments. All the experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Filamentous fungi are a rich source of nitrilases distinct in evolution from their widely examined bacterial counterparts (Kaplan *et al.*, 2011). However, fungal nitrilases have been given little attention compared with the bacterial counterpart. In the present study, two nitrile-utilizing fungal species were isolated from solid waste leachates by selective enrichment technique, which were identified as *A. fumigatus* strain WO2 (MG78622) and *A. niger* strain WO7 (MH542673). Macroscopical and microscopical characters *of A. fumigatus* strain WO2 and *A. niger* strain WO7 are presented in Table 1. For *A. fumigatus*, the grayish conidial coloured on PDA, white mycelium, inconspicuous to florescence; exudates were absent; uncoloured to yellowish on the reverse, red brown or green, soluble pigments were absent; sclerotia were absent in all media (Table 2). While *A. niger* on the same medium coloured conidia, white mycelium, uncoloured to dull yellow or grey on the reverse, soluble pigments were absent. Many *Aspergillus* strains are very close in their morphological characters and there is likelihood of identifying them wrongly. Kim *et al.* (2009) and Diba *et al.* (2007) studied the morphological characters for identification of *Aspergillus* sp. isolates from clinical origin.

Evidences from ITS gene sequence analyses as summarized (Table 2) showed that the ITS genes of the strains WO2 and WO7 had 100% similarity to *A. fumigatus* TB1 (MN178806) and *A. niger* (EU386133) respectively. The phylogenetic tree showed two distinct clusters; strains WO2 and WO7 are closely related by being in the same cluster and having genetic distance of about 1% (Figure 2). However, the two nitrile utilizers recovered in this study were further stratified into two different subgroups within their common cluster. The sequence analysis of ITS gene and unrooted

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(3) 2020, pp. 153-158
DOI: http://dx.doi.org/10.21161/mjm.180234

Table 1: Macroscopic and microscopic characters of *Aspergillus* species on potato dextrose agar at 37 °C.

Characters	Test Organisms	
	WO2	WO7
Macroscopic characters		
Colony diameter (µm)	29 ± 1.2	42 ± 2.0
Colony colour	Grayish	Black
Conidia colour	Dark Green	Black
Mycelium	White	Dull White
Exudates	Nil	Nil
Sclerotia	Nil	Nil
Microscopic characters		
Conidiophore length (µm)	122-163	381-462
Conidiophore breadth (µm)	5.1-9.2	9.8-13.4
Conidiophore colour	Uncoloured to Greyish	Hyaline to yellow or slightly brown
Conidiophore size	1.8-2.5	2.2-2.9
Conidiophore surface texture	Smooth to finely roughened	Rough walled
Vesicle diameter (µm)	20.4-22.6	23.2-26.1
Phialides	Uniseriate	Biseriate
Length (µm)	5.5-7.2	4.9-6.2
Breadth (µm)	1.9-2.2	2.3-3.0

Table 2: Genotypic identities of nitrile-degrading bacterial isolates from amplified sequences of ITS fragment of genomic DNA.

Fungal strain	Tentative Identity	GenBank	Closest strain	% identity	GenBank
WO2	*A. fumigatus*	MG786522	*A. fumigatus* TB1	100	MN178806
WO7	*A. niger*	MH542673	*A. niger*	100	EU386133

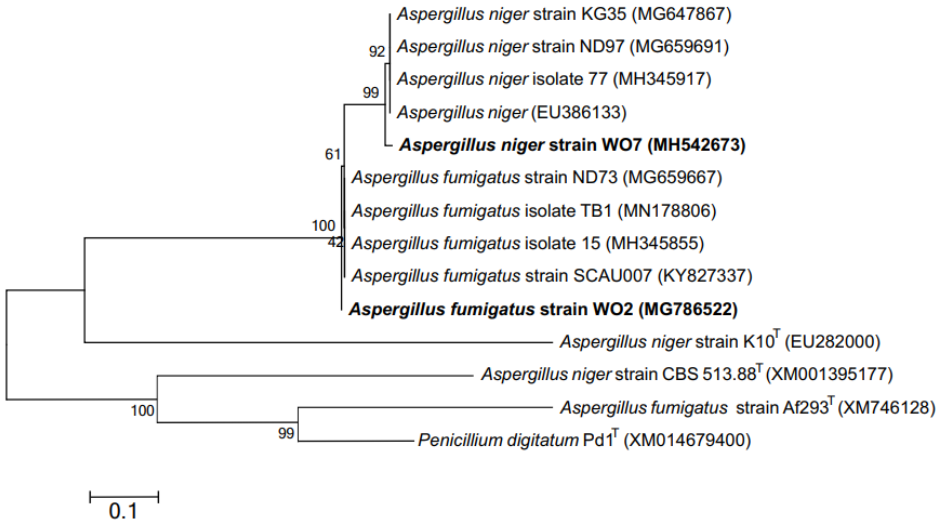

Figure 2: Phylogenetic tree (dendrogram) of nitrile-degrading *A. fumigatus* and *A. niger* ITS gene sequences using the neighbor-joining method (Saitou and Nei, 1987). Bootstrap test= 1000 replicates. The evolutionary distances were computed using the Tamura-Nei model. Analysis involving 14 nucleotide sequences was computed using Mega 5 software.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 16(3) 2020, pp. 153-158
DOI: http://dx.doi.org/10.21161/mjm.180234

phylogenetic tree showed that *Aspergillus* species are likely to have evolved from the same ancestor; however, belonged to two different subgroups (Figure 2). The cultural characteristics, as well as the ITS gene sequences, confirmed the association of WO2 and WO7 to the genus *Aspergillus*. The characteristic features of the strains were similar to those reported previously by Nierman *et al.* (2005), Machida *et al.* (2005) and Fedorova *et al.* (2008).

To the best our knowledge, this is the first report on identification of tropical *Aspergillus* sp. isolates obtained without any precedent in Lagos, Nigeria from solid waste leachates through morphological characters and ITS gene sequence analysis. The adequate growth for the evaluation of colony characteristics and microscopic features are required for identification of *Aspergilli* depending on morphological method. The results of the growth experiment showed that the dried weights of *A. fumigatus* strains WO2 and *A. niger* strain WO7 increased from 7.0 to 17.8 g/L and 6.8 to 23.8 g/L respectively within 16 days of incubation. The pH of the culture medium declined from 7.2 to 4.5 and 7.2 to 6.2, respectively for strains WO2 and WO7 (Figure 3). Strains WO2 and WO7 recorded maximum nitrilase activities of 0.0150 and 0.0161 mg/mL/min respectively (Figure 4).

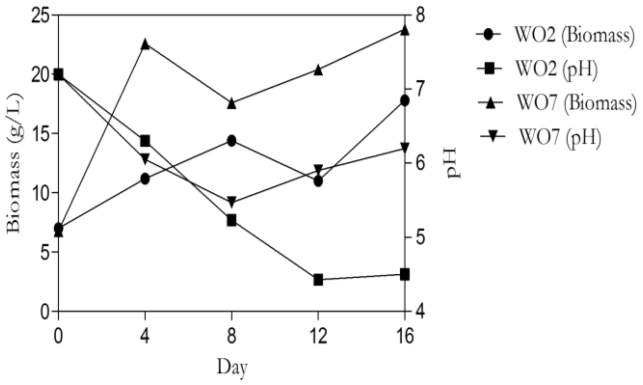

Figure 3: Growth profiles and pH changes in the culture fluids of pure cultures of *A. fumigatus* strain WO2 (MG786522) and *A. niger* strain WO7 (MH542673).

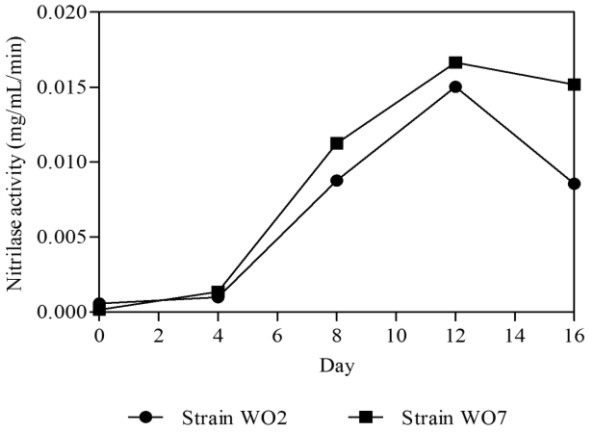

Figure 4: Nitrilase activities of *A. fumigatus* strain WO2 and *A. niger* strain WO7 on glutaronitrile.

CONCLUSION

This study has identified genetically distinct strains of *A. fumigatus* (MG78622) and *A. niger* (MH542673) as nitrilase producers from two selected dump sites in Lagos, Nigeria. This support their potentials as sources of biological agents of nitrile detoxification.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Prince Ata of Nigerian Institute of Medical Research for his technical support.

REFERENCES

Almatawah, C. A., Cramp, R. and Cowach D. A. (1999). Characterization of an inducible nitrilase from a thermophilic *Bacillus*. *Extremophiles* **3, 283-291.**

Banerjee, A., Sharma, R. and Banerjee, U. C. (2002). The nitrile-degrading enzymes: Current status and future prospects. *Applied Microbiology and Biotechnology* **60, 33-44.**

Barnett, H. L. and Hunter, B. B. (1972). Illustrated Genera of Imperfect Fungi. Minneapolis: Burgess publishing company, Minneapolis, MN. **pp 241.**

de Hoog G. S., Guarro, J., Gene, J. and Figueras, M. J. (2000). Atlas of Clinical Fungi (2 edn.). Centraalbureau voor Schimmelcultures (CBS), Netherlands.

Diba, K., Kordbacheh, P. and Mirhendi, S. H. (2007). Identification of *Aspergillus* species using morphological characters. *Pakistan Journal of Medical Sciences* **23, 867-872.**

Fedorova, N. D., Khaldi, N., Joardar, V. S., Maiti, R., Amedeo, P. and Nierman, W. C. (2008). Genomic islands in the pathogenic filamentous fungus *Aspergillus fumigatus*. *PLoS Genetics* **4(4), e1000046.**

Goldlust, A. and Bohak, Z. (1989). Induction, purification, and characterization of the nitrilase of *Fusarium oxysporum* f. sp. melonis. *Biotechnology and Applied Biochemistry* **11, 581-601.**

Gong, J. S, Li, H., Zhu, X. Y., Lu, Z. M., Wu, Y., Shi, J. S. and Xu, Z. H. (2012a). Fungal His-tagged nitrilase from *Gibberella intermedia*: Gene cloning, heterologous expression and biochemical properties. *PLoS One* **7(11), e50622.**

Gong, J. S., Lu, Z. M., Li, H., Shi, J. S., Zhou, Z. M. and Xu, Z. H. (2012b). Nitrilases in nitrile biocatalysis: Recent progress and forthcoming research. *Microbial Cell Factories* **11, 1-18.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Gupta, V., Gaind, S., Verma P. K. and Srivastava, A. K. (2010). Purification and characterization of intracellular nitrilases from *Rhodococcus* sp. – Potential role of periplasmic nitrilases. *African Journal of Microbiology Research* **4(11), 1148-1153.**

Harper, D. B. (1977). Fungal degradation of aromatic nitriles. Enzymology of C-N cleavage by *Fusarium solani*. *Biochemical Journal* **167, 685-692.**

Kaplan, O., Bezouska, K., Plihal, O., Ettrich, R., Kulik, N., Vanek, O., Kavan, D., Benada, O., Malandra, A., Sveda, O., Vesela, A., Rinagelova, A., Slamovaml, K., Cantarella, Felsberg, J., Duskova, J., Dohnalek, J., Kotik, M., Kren, V. and Martinkova, L. (2011). Heterologous expression, purification and characterization of nitrilase from *Aspergillus niger* K10. *BMC Biotechnology* **11, 2.**

Kaplan, O., Nikolaou, K., Pišvejcová, A. and Martínková, L. (2006a). Hydrolysis of nitriles and amides by filamentous fungi. *Enzyme and Microbial Technology* **38, 260-264.**

Kaplan, O., Vejvoda, V., Plíhal, O., Pompach, P., Kavan, D., Bojarová, P., Bezouška, K., Macková, M., Cantarella, M., Jirků, V., Křen, V. and Martínková L. (2006b). Purification and characterization of a nitrilase from *Aspergillus niger* K10. *Applied Microbiology and Biotechnology* **73, 567-575.**

Khandelwal, A. K., Nigam, V., Choudhury, B., Medicherla, K. M. and Ghosh, P. (2007). Optimization of nitrilase production from a new thermophilic isolate. *Journal of Chemical Technology and Biotechnology* **82(7), 646-651.**

Kim, D. H, Kim, S. H., Kim, Y. K., Kim, S. O. and Hong, S. B. (2009). Reidentification of *Aspergillus* spp. Isolated from clinical specimens of patients suspected as pulmonary Aspergillosis in Korea. *Korean Journal of Medical Mycology* **3, 133-144.**

Machida, M., Asai, K., Sano, M., Tanaka, T., Kumagai, T., Terai, G. *et al*. (2005). Genome sequencing and analysis of *Aspergillus oryzae*. *Nature* **438, 1157-1161.**

Martínková, L., Vejvoda, V., Kaplan, O., Kubáč, D., Malandr, A., Cantarella, M., Bezouška, K. and Křen, V. (2009). Fungal nitrilases as biocatalysts: Recent developments. *Biotechnology Advances* **27, 661-670.**

Nierman, W. C., Pain, A., Anderson, M. J., Wortman, J. R., Kim, H. S. and Denning, D. W. (2005). Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* **438, 1151-1156.**

Ogunyemi, A., Amund, O., Okpuzor, J., Adeiga, A., Idika, N. and Ahmed, O. (2010). Physicochemical properties of municipal refuse in Lagos metropolis and cellulolytic activities of resident microorganisms associated with organic matter degradation. *International Journal of Biological and Chemical Sciences* **4 (1), 209-217.**

O'Reilly, C. and Turner, P. D. (2003). The nitrilase family of CN hydrolysing enzymes – A comparative study. *Journal of Applied Microbiology* **95, 1161-1174.**

Samapundo, S. F., Devlieghere, B. D., Meulenaer and Debevere, J. (2007). Growth kinetics of cultures from single spores of *Aspergillus flavus* and *Fusarium verticilliodes* on yellow dent corn neal. *Food Microbiology* **24, 336-345.**

Santoshkumar, M., Veeranagouda, Y., Kyoung, L. and Karegoudar, T. B. (2011). Utilization of aliphatic nitrile by *Paracoccus* sp. SKG isolated from chemical waste samples. *International Biodeterioration and Biodegradation* **65, 153-159.**

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4(4), 406-425.**

Singh, R., Sharma, R., Tewari, N., Geetanjali and Rawat, D. S. (2006). Nitrilase and its application as a 'green' catalyst. *Chemistry and Biodiversity* **3, 1279-1287.**

Šnajdrová, R., Kristová-Mylerová, V., Crestia, D., Nikolaou, K., Kuzma, M., Lemaire, M., Gallienne, E., Bolte, J., Bezouška, K., Křen, V. and Martínková, L. (2004). Nitrile biotransformation by *Aspergillus niger*. *Journal of Molecular Catalysis B: Enzymatic* **29(1-6), 227-232.**

Tamura, K., Dudley J., Nei, M. and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24, 1596-1599.**

Thimann, K. V. and Mahadevan S. (1964). Nitrilase. I. Occurrence, preparation, and general properties of the enzyme. *Archives of Biochemistry and Biophysics* **105, 133-141.**

Vejvoda, V., Kaplan, O., Bezouska, K., Pompach, P., Sulc, M., Cantarella, M., Benada, O., Uhnáková, B., Rinágelová, A., Lutz-Wahl, S., Fischer, L., Kren, V. and Martínková, L. (2008). Purification and characterization of a nitrilase from *Fusarium solani* O1. *Journal of Molecular Catalysis B: Enzymatic* **50, 99-106.**

Vejvoda, V., Kubác, D., Davidová, A., Kaplan, O., Sulc, M., Sveda, O., Chaloupková, R. and Martínková, L. (2010). Purification and characterization of nitrilase from *Fusarium solani* IMI196840. *Process Biochemistry* **45, 1115-1120.**

Winkler, M., Kaplan, O, Vejvoda, V, Klempier, N. and Martínková, L. (2009). Biocatalytic application of nitrilases from *Fusarium solani* O1 and *Aspergillus niger* K10. *Journal of Molecular Catalysis B: Enzymatic* **59, 243-247.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538