RESEARCH Open Access

Nitrilase gene detection and nitrile metabolism in two bacterial strains associated with waste streams in Lagos, Nigeria

Adewale Kayode Ogunyemi^{1*}, Olanike Maria Buraimoh¹, Bukola Caroline Ogunyemi², Titilola Aderonke Samuel³, Matthew Olusoji Ilori¹ and Olukayode Oladipo Amund⁴

Abstract

Background: The use of nitrile compounds is usually high, particularly in chemical industries, which calls for serious concern because of their relevance to the environment. The essential role of nitrilases in the bioremediation of harmful nitriles from environmental wastes cannot be overemphasized. The study aimed to unveil the biodegradative potentials of bacterial strains associated with the degradation of nitrile pollutants.

Methods: Bacterial strains capable of utilizing glutaronitrile as the sole source of carbon and nitrogen were isolated from solid waste leachates by a selective enrichment culture technique. The test organisms were grown in mineral salts medium (MSM), and the metabolic products were determined using gas chromatography-flame ionization detection (GC-FID). The nitrilase gene was amplified by polymerase chain reaction (PCR) and by using appropriate primers.

Results: The growth studies showed that the test organisms grew on the two nitriles. The doubling times of 12.16 d and 9.46 d (specific growth rate, μ =0.082 d⁻¹, 0.106 d⁻¹) were obtained for each pure culture of *Bacillus* sp. srain WOD8 and Corynebacterium sp. srain WOIS2 on glutaronitrile (as single substrate), respectively. While the same strains had doubling times of 11.11 d and 10.00 d (μ =0.090 d⁻¹, 0.100 d⁻¹) on benzonitrile (as single substrate). However, the mixed culture (comprising the two strains) had doubling times of 7.40 d and 7.75 d (μ =0.135 d⁻¹, 0.129 d⁻¹) on glutaronitrile (as single and mixed substrates), respectively. While doubling times of 8.09 d and 8.71 d (μ =0.124 d^{-1} , 0.115 d^{-1}) were obtained for the same mixed culture on benzonitrile (as single and mixed substrates). Based on gas chromatographic analysis, the residual glutaronitrile concentrations at day 16 for strains WOD8 and WOIS2 were 35.77 g L⁻¹ (72.2%) and 9.30 g L⁻¹ (92.5%), respectively, whereas the residual benzonitrile concentrations for the same strains were 27.39 g L^{-1} (78.8%) and 13.79 g L^{-1} (89.2%), respectively. For the mixed culture, residual glutaronitrile and benzonitrile concentrations at day 16 were 13.40 g L^{-1} (88.5%) and 10.42 g L^{-1} (91.5%), respectively, whereas for the mixed substrates (glutaronitrile and benzonitrile), 7.21 g L^{-1} (91.7%) and 4.80 g L^{-1} (94.2%) of residual glutaronitrile and benzonitrile concentrations were obtained by the same consortium. The gene for nitrilase involved in nitrile degradation was detected in the genome of the bacterial strains. The amplified nitrilase gene gave PCR products of sizes 1400 bp and 1000 bp, as expected for strains WOD8 and WOIS2, respectively. 4-Cyanobutyric acid (4CBA), glutaric acid (GA), and benzoic acid (BA) were obtained as metabolites following nitrile degradation in vitro.

Full list of author information is available at the end of the article



^{*}Correspondence: waleogunyemi2002@yahoo.com

T Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria

Conclusion: These results revealed that strains WOD8, WOIS2 and the mixed culture (consisting of the two strains) have proven to have the capacity to metabolize nitriles (glutaronitrile and benzonitrile) as the carbon and nitrogen sources. However, the mixed culture had higher nitrile degradation rate as compared to each pure culture of the two test organisms. These results also provide insight into the evolutionary genetic origin of a nitrilase gene that encodes an enzyme that catalyzes nitrile degradation in these strains. Hence, the bacterial strains that harbor this kind of gene may be used as promising biological agents for the remediation of sites polluted with nitriles, thereby opening new perspectives for encouraging data for a bioremediation bioprocess.

Keywords: Bacterial strains, Nitriles, Nitrilases, Metabolites, Mixed culture, Solid waste leachates, Bioremediation bioprocess

Background

Bacterial nitrilases are responsible for the degradation of nitriles into corresponding carboxylic and ammonia. Some of the benefits, such as working under mild response conditions, being regioselective, and enantioselective, are reasons behind the wide usage of nitrilases over the usual chemical procedures (Wang et al. 2014; Vejvoda et al. 2010). Eventually, nitrilases are considered a reasonable candidate to drive the age of numerous useful carboxylic acids. Nitrilases, as important biocatalysts, convert a variety of nitriles to carboxylic acids, thereby facilitating more environmentally friendly chemical and pharmaceutical production (Ramteke et al. 2013). Nitrilases are found all over the world and are critical components of metabolic pathways as well as a response to environmental stimuli such as nitriles, whether natural or man-made. Nitrilases are frequently found in gene clusters on a genome that represent their metabolic roles (Podar et al. 2005). Nitrilase in bacteria and fungi has been studied (Kaplan et al. 2006; Vejvoda et al. 2006, 2008; Martínková et al. 2009; Gong et al. 2012; Egelkamp et al. 2017). Layh et al. (1998) reported that nitrilase producing bacteria use nitriles as a nitrogen source for growth as a result of nitrilase expression. In bacteria, however, nitrilases are uncommon, with only 10 nitrilase genes discovered in over 150 genomes analyzed (Podar et al. 2005). Sharma et al. (2017) discovered just 138 nitrilases in 2000 bacterial genomes after using genome mining data. According to these findings, nitrilases are expressed by just 6.8% of bacteria.

Recently, nitrilases have drawn general attention after the recognition of their enormous prospective uses in industry and bioremediation activities (Sharma et al. 2017). Usually, most nitriles are extremely toxic and have become an environmental issue that requires serious concern. Consequently, viable action towards reducing the nitrile-based wastes delivered from a few manufacturing processes is required. However, cyanogenic compounds have surfaced in some medical problems, including other primary central nervous system (PCNS) symptoms.

Moreover, cyanide anions formed from nitriles could be harmful by prompting the deactivation of respiration brought about by a strong affinity to cytochrome-c-oxidase (Kaul et al. 2007). With the inherently destructive and unfavorable effect of cyanide-based compounds, they can cause damage to living organisms and the environment (Kaul et al. 2007; Gupta et al. 2010; Banerjee et al. 2002). A few nitrile compounds are made and used as solvents, plastics, polymers, and herbicides (Kobayashi et al. 1990), while a few nitriles are used as raw constituents or biosynthetic precursors in generating pharmaceuticals, fine chemicals, and other useful products (Kaul et al. 2007). The exuberant increase in nitrile biotransformation (Mathew et al. 1988) and degradation of nitrilederived herbicides (Stalker et al. 1988) have confirmed the investigation regarding bacterial strains as the basis of these events. Lagos is a metropolitan city in Nigeria where few dumpsites exist.

This places an unexpected population of 180 million individuals in danger of cyanide toxicity. The present study aimed to reveal the bioremediation potentials of bacterial strains associated with nitrile degradation in Nigeria in order to strongly decrease health-risk factors in the ecosystem.

Methods

Source of metabolizing bacterial strains

Metabolizing bacterial strains, *Bacillus* sp. WOD8 (KX774193) and *Corynebacterium* sp. WOIS2 (KX774194) used was previously described by Ogunyemi et al. (2019). Bacteria capable of metabolizing glutaronitrile as the sole carbon and nitrogen source were isolated from solid waste leachates by a selective enrichment culture technique (Ogunyemi et al. 2019). Previously, these strains have shown ample ability to use glutaronitrile and benzonitrile for growth (Ogunyemi et al. 2019). The bacterial strains were revived and ready for further studies.

Evaluation of growth potentials on nitriles

This experiment was conducted to examine the ability of the bacterial species as pure and mixed culture to utilize nitriles. The laboratory tests were carried out in duplicate under aerobic conditions in Erlenmeyer flasks (250 ml). The constituents of MSM (g/L) included NaCl, 0.20; MgSO₄.7H₂O, 0.050; K₂HPO₄, 2.0; KNO₃, 2.0; CaCO₃, 0.020; and FeSO₄. 7H₂0, 0.010; 1000 mL distilled water, pH, 7.0 ± 0.2), fortified with 1.0 mL of sterile trace element solution (Santoshkumar et al. 2011). The medium was supplemented with 0.2% (v/v) nitrile as the sole carbon and energy source. The bacteria inoculum for the major biodegradation studies was prepared by growing each test organism in MSM and incubated at 48 h to allow the cells to fully grow to mid log phase. While the mixed culture inoculum, the cells harvested were in equal proportion with a corresponding cell density of 10⁸ cfu/ml for each. The Erlenmeyer flasks (250 mL) containing 100 mLof MSM was inoculated with 1.0 mLof inoculum (pure and mixed culture) and incubated (30 °C, 150 rpm) for 16 days. To monitor contamination and the effects of abiotic factors, two flasks of mineral salts medium acted as negative and positive controls. Flasks were incubated for 16 days. The growth experiments were set up in triplicate. The culture (5.0 mL) from the sets of flasks was harvested every 4 days to determination of turbidity. The increase in turbidity of the culture broth was used to monitor the growth of both pure and mixed culture (Bacillus sp. strain WOD8 and Corynebacterium sp. strain WOIS2) at a wavelength of 600 nm using a spectrophotometer (Thermo-scientific[™] Spectronic GENESYS 8, Thermofisher Scientific, USA). Generation times and the specific growth rate of the pure and mixed

Table 1 Nucleotide primers used for amplification of nitrilase gene

Primers	Sequence	Tm (°C)	%GC	Σ base (bp)
Bac-Nit-F	5'-GAC AAG TAT TTA CCC GAA GTT TCG-3'	55	24	24
Bac-Nit-R	5'-GAT ATT GAA TAG CCT CGT ATG-3'	55	38	21

culture on nitrile substrate were determined with non-linear regression of growth curves using GraphPad software, Prism version 6.

Biodegradation of nitriles by test organisms

Biodegradation of both glutaronitrile and benzonitrile in the media during the period of incubation was carried out using gas chromatography [Hewlett Packard (HP) 5890 series II (California, USA) with a flame ionization detection (FID)]. The GC column oven was operated with an initial temperature of 50 °C to 100 °C for 1 min. While operating conditions: an OV-3 glass column pack with internal diameter of 3.2 mm and length of 30 m packed with porapak N, 60/100, a column temperature: 220 °C, injector temperature: 200 °C, a detector temperature: 250 °C, flow rate: 450 mL/min with N₂ carrier gas and H₂ and temperature/ramping rate: 10 °C/min. A standard profile was first obtained with which the test sample was analyzed.

DNA extraction and nitrilase gene amplification

Jena Bioscience DNA preparation kits (Germany) were used for the extraction and purification of genomic DNA. Polymerase chain reaction was used for nitrilase gene amplification under standard conditions using the designed nitrilase-specific primer pair (Table 1). The following PCR program was used for the amplification of the DNA fragment: 5 min 94 °C followed by 30 cycles of 45 s 94 °C, 45 s 60 °C, 1 min 72 °C, and a final elongation of 10 min 72 °C. Millipore water (blank) was used as a negative control. The PCR product was gel purified and sequenced with an automated sequencer (ABI PRISM 377, PE Biosystems Inc.). BLAST (http://www.ncbi.nlm.nih.gov/) was used to find closely related nitrilase gene sequences.

ResultsGrowth potentials of nitrile-metabolizing bacteria on nitriles

Bacillus sp. and *Corynebacterium* sp. had doubling times of 12.16 and 9.46 (μ = 0.082, 0.106) on glutaronitrile (single substrate) (Table 2), while doubling times

Table 2 Growth potentials of nitrile-metabolizing bacteria species in form of pure and mixed culture grown on Glutaronitrile and Benzonitrile

	G			G+B/G	В			G+B/B
	WOD8	WOIS2	WOD8+WOIS2		WOD8	WOIS2	WOD8+WOIS2	
$\mu (d^{-1})$	0.082	0.106	0.135	0.129	0.090	0.10	0.124	0.115
$T_d(d)$	12.16	9.46	7.40	7.75	11.11	10.00	8.09	8.71

WOD8-Bacillus sp., WOIS2-Corynebacterium sp., WOD8 + WOIS2-Bacillus sp. plus Corynebacterium sp., G-Glutaronitrile, B-Benzonitrile, G+B-Glutaronitrile plus Benzonitrile, G*+B-Equal mix of Glutaronitrile with Benzonitrile, G+B M,/4/B*-Equal mix of Benzonitrile with Glutaronitrile, μ -specific growth constant, T_d -doubling time

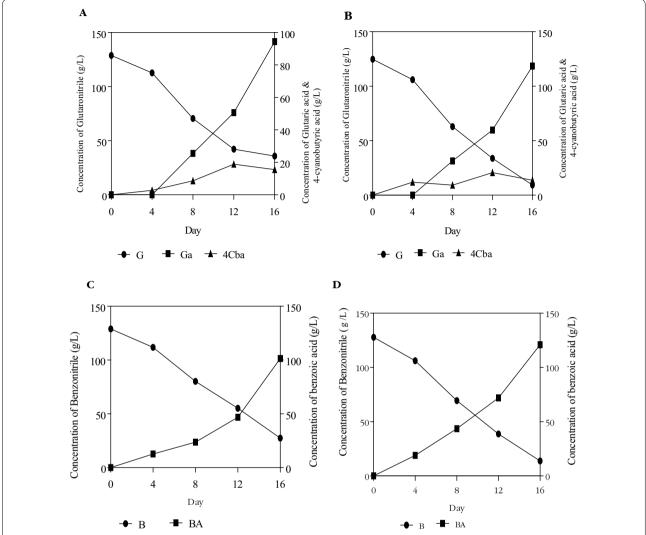


Fig. 1 Degradation of glutaronitrile and benzonitrile by *Bacillus* sp. strain WOD8 and *Corynebacterium* sp. strain WOIS2. **A** *Bacillus* sp. strain WOIS2 on glutaronitrile; **B** *Corynebacterium* sp. strain WOIS2 on glutaronitrile; **C** *Bacillus* sp. strain WOD8 on benzonitrile; **D** *Corynebacterium* sp. strain WOIS2 on benzonitrile

of 7.40 and 7.75 (μ =0.135, 0.129) were recorded for the mixed culture—on glutaronitrile (single substrate) and mixed substrates—(glutaronitrile and benzonitrile) (Table 2). Similarly, *Bacillus* sp. and *Corynebacterium* sp. had doubling times of 11.11 and 10.00 (μ =0.090, 0.100) on benzonitrile (single substrate), while doubling times of 8.09 and 8.71 (μ =0.124, 0.115) were recorded for the mixed culture—on benzonitrile (single substrate) and mixed substrates (Table 2). The results showed that the mixed cultures of the bacterial species had better growth in each of the nitrile compounds both as single and mixed forms (Table 2).

Biodegradation of nitriles by test organisms

The enzyme products formed during the degradation of nitriles were determined by gas chromatography (GC). The GC results revealed that strain WOD8 degraded glutaronitrile from the initial concentration of 128.75 gL⁻¹ to 35.77 gL⁻¹, while 15.54 gL⁻¹ of 4-cyanobutyric acid (4CBA) and 94.35 gL⁻¹ glutaric acids (GA) were obtained as enzyme products, though strain WOIS2 degraded 124.87 gL⁻¹ to 9.3 gL⁻¹ of glutaronitrile alongside the formation of 13.69 gL⁻¹ of 4-cyanobutyric acid (4CBA) and 118.59 gL⁻¹ of glutaric acid (GA) (Fig. 1). However, the mixed cultures degraded glutaronitrile from

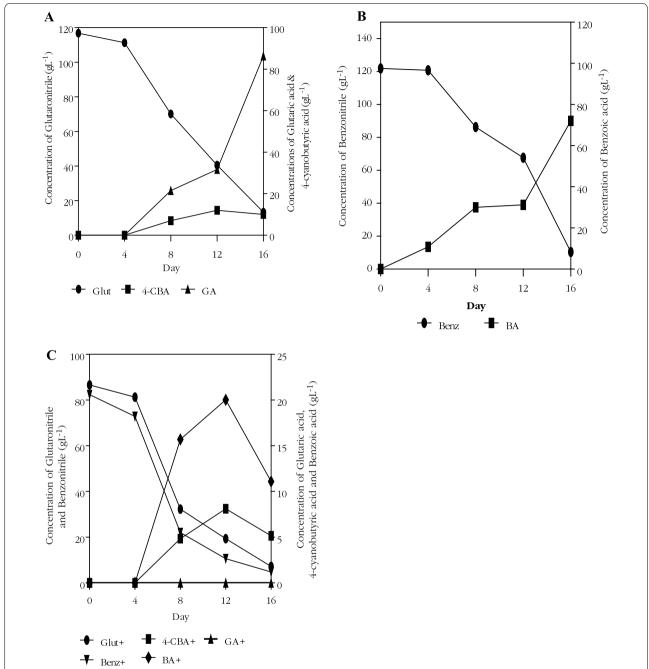


Fig. 2 Degradation of glutaronitrile and benzonitrile by mixed cultures in the culture fluid. A Glut-Glutaronitrile. B Benzonitrile, 4-CBA-4-cyanobutyric acid, GA-Glutaric acid, Benz-Benzonitrile and BA-Benzoic acid

initial concentration of 116.89 gL $^{-1}$ to 13.40 gL $^{-1}$ and 10.04 gL $^{-1}$ of 4CBA and 86.38 gL $^{-1}$ of GA had accumulated Finally, the mixed culture degraded glutaronitrile portion in the mixed substrates from initial concentration of 86.63 gL $^{-1}$, to 7.21 gL $^{-1}$ and 5.15 gL $^{-1}$ CBA had accumulated, while the same mixed culture degraded

benzonitrile portion from initial concentration of 82.40 gL $^{\!-1}$, to 4.80 gL $^{\!-1}$ and 11.10 gL $^{\!-1}$ BA had formed.

Furthermore, strain WOD8 cultured in mineral salts medium (MSM) containing benzonitrile used benzonitrile from an initial concentration of 128.99 gL⁻¹ to 27.39 gL⁻¹ and 101.44 gL⁻¹ benzoic acid was formed (Fig. 1) while strain WOIS2 degraded 127.75 gL⁻¹ to 13.79 gL⁻¹ and

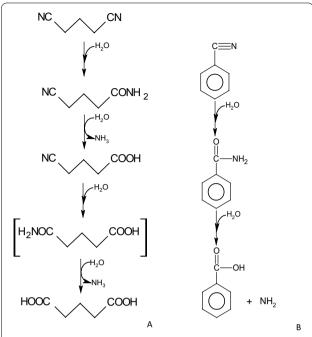


Fig. 3 Proposed pathway for the degradation of Glutaronitrile (**A**) and Benzonitrile (**B**) by *Corynebacterium* sp. strain WOIS2 and *Bacillus* sp. strain WOD8

120.97 gL $^{-1}$ benzoic acid (BA) was obtained (Fig. 1). The results of nitrile metabolism by the mixed cultures are presented in Fig. 2. The mixed cultures degraded benzonitrile from initial concentration of 121.98 gL $^{-1}$ to 10.42 gL $^{-1}$ and 90.01 gL $^{-1}$ of BA had accumulated.

Whereas on the mixed substrates, the mixed culture degraded 86.63 gL⁻¹ to 7.21 gL⁻¹ and 5.15 gL⁻¹ 4-CBA had accumulated while initial concentration of benzonitrile of 82.40 gL⁻¹ was degraded to 4.80 gL⁻¹ and 11.10 gL⁻¹ of BA had accumulated. The results indicated that we had reached 88.5% biodegradation of glutaronitrile and 91.5% for benzonitrile (Fig. 2).

Metabolic pathway of assimilation of glutaronitrile and benzonitrile by test organisms

From the investigations carried out, the metabolic pathway for the assimilation of nitrile compounds was elucidated in *Corynebacterium* sp. strain WOIS2 and *Bacillus* sp. strain WOD8.

Glutaronitrile was metabolized into 4-cyanobutyric acid and glutaric acid through the monoenzymatic pathway, while benzonitrile was similarly metabolized into benzoic acid just through the monoenzymatic pathway (Fig. 3). Considering the biodegradation investigation, the proposed monoenzymatic pathway was followed by

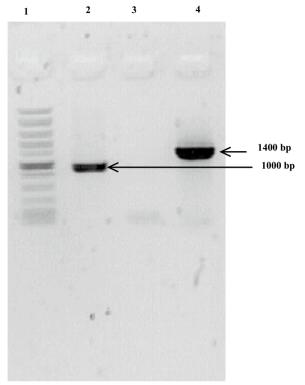


Fig. 4 Amplification of the DNA sequence encoding nitrilase. Lane 1—DL2000 molecular marker; lane 2—nitrilase gene from strainWOIS2; lane 3—millipore water negative control; lane 4—nitrilase gene from strain WOD8

Table 3 Genotypic identities of nitrilases from test bacterial isolates

Strain code	GAN	Closest nitrilase	% identity	GAN
WOD8	MG993210	Bacillus pumilus strain 8A3	97	AF492814
WOIS2	MG993211	Bacillus safensis strain U41	97	EU713463

WOD8 Bacillus sp. strain., WOIS2 Corynebacterium sp. strain, GAN GenBank Accession number

strains in the generation of enzyme products from glutaronitrile and benzonitrile (Fig. 3).

The culture broth tested positive for ammonia, which further confirmed the breakdown of nitrile compounds catalytically acted upon by nitrilase from the test organisms.

Nitrilase gene and evolutionary relatedness

Upon amplification, band sizes of 1400 bp and 1000 bp were obtained from nitrilases from strains WOD8 and WOIS2, respectively (Fig. 4). The extra domain gained

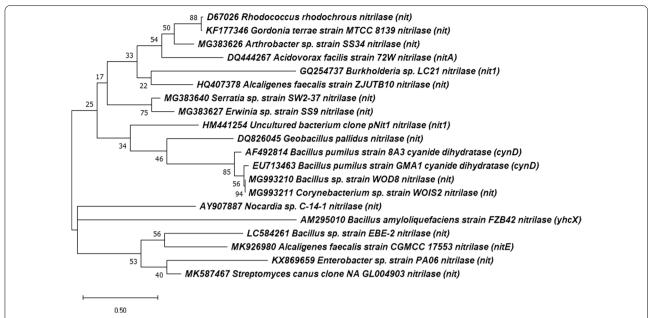


Fig. 5 Phylogenetic tree of nitrilase and cyanide dihydratase sequences (using Mega software version 5). The following sequences with their relevant NCBI registration numbers were used. The sequences were aligned by using the program CLUSTAL W (version 1.8), the dendrograms calculated by bootstrap neighbor joining, and the phylogenetic tree drawn

by the nitrilase gene from strain WOD8 caused a sharp difference in the expected band sizes. The nitrilase gene sequences have been submitted to the National Center for Biotechnology Information (NCBI) and have been assigned MG993210 and MG993211 as accession numbers (Table 3).

MEGA version 5 was used for phylogenetic and molecular evolutionary analyses (Tamura et al. 2007). The neighbor-joining evolutionary model was chosen for phylogenetic tree construction. The homology search showed that the nitrilase gene of *Bacillus* sp. strain WOD8 and *Corynebacterium* sp. WOIS2 both had 97% similarity to nitrilase gene of *Bacillus pumilus* strain 8A3 AF492814 and *Bacillus safensis* strain U41 EU713463, respectively (Fig. 5; Table 3).

Discussion

This investigation shows the main findings on nitrile metabolic potentials and nitrilase gene discovery in test species isolated from leachates in solid waste in Lagos, Nigeria.

Additionally, the gene responsible for nitrilase production in *Bacillus* sp. and *Corynebacterium* sp. were amplified and sequenced. Nitrilases with degradation potential that degraded nitriles to corresponding carboxylic acid and ammonia have been characterized (Gong et al. 2012; Thimann et al. 1964; Hook and Robinson 1964; Harper 1977a, b).

In this context, there are several reports on the metabolism of nitriles in the form of aliphatic and aromatic nitrile compounds. Numerous microorganisms, especially bacteria, have been reported to degrade both aliphatic and aromatic nitriles in the literature, and a few of these are *Arthrobacter* sp. I-9 (Yamada et al. 1980), *Bacillus pallidus* (Cramp et al. 1997), Mucolate Actinomycetes (Brandao and Bull 2003), *Microbacterium Imperiale* CBS 498-74 (Cantarella et al. 2006), *Klebsiella oxytoca* (Kao et al. 2006), *Cryptococcus* sp. UFMG-Y28 (Rezende et al. 2000), *Bacillus* sp. UG-5B (Ludmila et al. 2008), *Isoptericola variabilis* RGT01 (Gurdeep et al. 2014), *Klebsiella* sp. (Nawaz et al. 1992). *Acidovorax* sp. (Chen et al. 2009). *Acidovorax facilis* 72W (Hann et al. 2003).

The substrate choices of nitrilase produced by different organisms were discovered to be diverse. *R. rhodochrous* K22, for example, hydrolyzes aliphatic nitriles, but the nitrile concentration was very low (0.2 mmol/L). Similarly, *Bacillus pallidus* nitrilase hydrolyzed aliphatic, aromatic, and heterocyclic nitriles (Dennett and Blamey 2016), whereas *Acidovorax* sp. SK1 had a substrate selectivity for mandelonitrile as well as 4-methoxy, 2-nitrobenzonitrile. In the present study, the strains displayed wide specificities for both aliphatic and aromatic nitriles.

In this work, glutaronitrile was metabolized to 4-cyanobutyric acid and glutaric acid through the monoenzymatic pathway, while benzonitrile was

relatively metabolized to benzoic acid only through the monoenzymatic pathway. The results from this investigation showed that glutaronitrile and benzonitrile were degraded to enzyme products by nitrilases obtained from strains WOD8 and WOIS2, respectively. However, glutaronitrile was preferably degraded over benzonitrile.

Therefore, the fundamental analysis of nitrile degradation at the protein and gene levels could lead to biological environmental improvement.

According to Kiziak et al. (2005), nitrilase obtained from *Alcaligenes faecalis* JM3 has been described as arylacetonitrilase, simplifying its substrate specification, and it appeared to be acceptable and considered an accomplishment to also group the nitrilase obtained from *Corynebacterium* sp. strain WOIS2 and *Bacillus* sp. strain WOD8 in this study as glutaronitrilase. Along these lines, there were no strong connections between the enzymic action and the size or polarity of the substituents (Kiziak et al. 2005).

This was an unexpected observation because it has been previously suggested that enrichments with specific nitriles usually result in the isolation of bacteria with nitrile hydrolyzing enzyme systems demonstrating high activities toward the nitriles used as enrichment substrates (Layh et al. 1997). Kiziak et al. (2005) reported that nitrilase enzyme from *Pseudomonas fluorescens* EBC191 nitrilase validated a strong priority and best interest for valeronitrile as substrate out of all the tested aliphatic nitriles, which has a specific activity of approximately 4 U mg-1 The occurrence and location of the aromatic ring with the sort of alkyl side chain is a key factor which distinguishes suitable substrates.

Strains WOD8 and WOIS2 degraded glutaronitrile at degradation rates of 72.2% and 92.6%, respectively. The mixed cultures of strains WOD8 and WOIS2 degraded glutaronitrile at 88.5%. However, WOD8 and WOIS2 degraded benzonitrile at 78.8% and 89.2%, respectively. However, cocultures of two (2) strains degraded benzonitrile at 91.5%. This high percentage of nitrile degradation, as observed when using the mixed cultures of the two studied bacteria, can certainly be considered one of the findings of this study.

The nitrilases obtained from *Bacillus* sp. strain OxB-1 (Kato et al. 2000), *A. faecalis* JM3 (Kobayashi et al. 1993), *C. testosteroni* (Layh et al. 1997), and *R. rhodochrous* strains K22 and J1 (Kato et al. 2000; Kobayashi et al. 1993) have been sequenced. The bacterial nitrilases show remarkably less conservation inside the group. Approximately 53% homology was recorded between the aliphatic nitrilases of *C. testosteroni* (Levy-Schil et al. 1995) and *Bacillus* sp. strain OxB-1 nitrilase, which is linked with aldoxime degradation (Kobayashi

et al. 1992a). The base sequence of 957 bp from the nitrilase gene fragment from soil samples as shown by Riffani et al. (2015) and Kobayashi et al. (1992b), has high similarity with *Rhodococcus rhodochrous* strain tg1-A6 nitrilase. Asano and Kato (1998) found identical nitrilase genes in various bacterial strains and real-time PCR detection of the genes in soils demonstrates horizontal gene transfer. They discovered that bacterial enzymes that can hydrolyze nitrile have a lot of industrial potential.

Similarly, in the present study, band size values of 1400 and 1000 bp were obtained by nitrilases from strains WOIS2 and WOD8 and showed a high similarity to other nitrilases recently described. It was of remarkable interest that there was a contrast in the band sizes of the PCR products from the two strains isolated in this study, which might be caused by the domain acquired by the nitrilase gene from strain WOD8.

The results of this present study has led to a knowledge-based study of nitrile-degrading organisms, setting the stage for comparative genomics between Nigeria's nitrilase-producing organisms and their counterparts from other industrialized nations of the world. Likewise, this work revealed that in tropical environments, nitrilase-producing *Bacillus* sp. and *Corynebacterium* sp. that showed biodegradative capabilities for the degradation of nitrile. Therefore, these strains may be prospecting biological agents for the detoxifying of nitrile-based wastes from polluted environments.

A further investigation of these bacteria nitrilases for the large-scale bioremediation of nitrile compound is suggested.

Conclusions

In our opinion, the outcome of this study and an account of the various capabilities of these cultures (pure and mixed form) is a strong pointer that these tropical nitrilemetabolizing bacteria isolated from solid waste leachates in Lagos may be promising tools to control nitrile pollution as well as cyanogenic glucosides from polluted environments. Hence, nitrile-metabolizing bacteria are worth further pursuits for biotechnological purposes.

Acknowledgments

The Technologists of Department of Microbiology, Nigeria Institute of Medical Research, Yaba, Lagos, are well appreciated for their technical contribution to the work.

Author contributions

AKO, OMB, and BCO collected samples, carried out the experiment and analyzed the data; TAS, MOI, and OOA supervised the study; AKO, OMB, BCO, TAS, MOI, and OOA were responsible for the drafting and final version of the manuscript. All authors read and approved the final manuscript.

Funding

This study was self-funded by all the authors.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This work does not involve sampling from human or animal subject so there is no informed consent from any patient.

Consent for publication

This study has nothing to do with patient samples or data but purely laboratory analysis of environmental samples. We further recommend that the published article is disseminated only to the involved researchers from whom the data was drawn. All authors consented that the manuscript should be submitted and published.

Competing interests

The authors declared that there was no conflict of interest.

Author details

¹Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria. ²Department of Chemical Science, Science Laboratory Technology, Yaba College of Technology, Yaba, Lagos, Nigeria. ³Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. ⁴Department of Biological Sciences, Faculty of Basic and Applied Sciences, Elizade University, Ilara-Mokin, Ondo State, Nigeria.

Received: 19 January 2022 Accepted: 10 April 2022 Published online: 26 May 2022

References

- Asano Y, Kato Y (1998) Z-Phenylacetaldoxime degradation by a novel aldoxime dehydratase from *Bacillus* sp. strain QXB-1. FEMS Microbiol Lett 158:185–90
- Banerjee A, Sharma R, Banerjee U (2002) The nitrile-degrading enzymes: current status and future prospects. Appl Microbiol Biot 60:33–44
- Brandao PFB, Bull AT (2003) Nitrile hydrolyzing activities of deep-sea and terrestrial mycolate actinomycetes. Anontie Von Leeuwenhoek 84:89–98
- Cantarella M, Cantarella L, Gallífuoco A (2006) Nitrile bioconversion by *Micro-bacterium imperiale* CBS 498–74 resting cells in batch and ultrafiltration membrane bioreactors. J Ind Microbiol Biotechnol 33:208–214
- Chen J, Zheng RC, Zheng YG, Shen YC (2009) Microbial transformation of nitriles to high-value acids or amides. Adv Biochem Eng Biotechnol 113:33–77
- Cramp R, Gilmour M, Cowan DA (1997) Novel thermophilic bacteria producing nitrile-degrading enzymes. Microbiol 143:2313–2320
- Dennett GV, Blamey JM (2016) A new thermophilic nitrilase from an Antarctic Hyperthermophilic microorganism. Front Bioeng Biotechnol 4:5
- Egelkamp R, Schneider D, Hertel R, Daniel R (2017) Nitrile-degrading bacteria isolated from compost. Front Environ Sci 5:56. https://doi.org/10.3389/fenvs.2017.00056
- Gong JS, Lu ZM, Li H, Shi JS, Zhou ZM, Xu ZH (2012) Nitrilases in nitrile biocatalysis: recent progress and forthcoming research. Microb Cell Fact 11:142–160
- Gupta N, Balomajumder C, Agarwal VK (2010) Enzymatic mechanism and biochemistry for cyanide degradation: a review. J Hazard Mater 176(1–3):1–13
- Gurdeep K, Pankaj S, Rupinder T, Rohit S (2014) Isolation and characterization of a nitrile-hydrolysing bacterium *Isoptericola variabilis* RGT01. Indian J Microbiol 54:232–238
- Hann EC, Sigmund AE, Fager SK, Cooling FB, Gavagan JE, Ben-Bassat A, Chauhan S, Payne MS, Hennessey SM (2003) Dicosimo R Biocatalytic hydrolysis

- of 3-Hydroxyalkanenitriles to 3-Hydroxyalkanoic acids. Adv Synth Catal 345:775–782
- Harper DB (1977a) Fungal degradation of aromatic nitriles: enzymology of C-N cleavage by *Fusarium solani*. Biochem J 167:685–692
- Harper DB (1977b) Microbial metabolism of aromatic nitriles. Biochem J 165:309–319
- Hook RH, Robinson WG (1964) Ricinine nitrilase: II. Purification and properties. J Biol Chem 239:4263–4267
- Kao CM, Chen KF, Liu JK, Chou SM, Chen SC (2006) Enzymatic degradation of nitriles by Klebsiella oxytoca. Appl Microbiol Biotechnol 71:228–233
- Kaplan O, Nikolaou K, Pišvejcová A, Martínková L (2006) Hydrolysis of nitriles and amides by filamentous fungi. Enzyme Microb Technol 38:260–264
- Kato Y, Ooi R, Asano Y (2000) Distribution of aldoxime dehydratase in microorganisms. Appl Environ Microbiol 66:2290–2296
- Kaul P, Banerjee A, Banerjee UC (2007) Nitrile hydrolases. In: Polaina J, MacCabe AP (eds) Industrial enzymes. Springer, India, pp 531–547
- Kiziak C, Conradt D, Stolz A, Mattes R, Klein J (2005) Nitrilase from *Pseudomonas fluorescens* EBC191: cloning and heterologous expression of the gene and biochemical characterization of the recombinant enzyme. Microbiology 151:3639–3648
- Kobayashi M, Yanaka N, Nagasawa T, Yamada H (1990) Purification and characterization of a novel nitrilase of *Rhodococcus rhodochrous* K22 that acts on aliphatic nitriles. J Bacteriol 172(9):4807–4815
- Kobayashi M, Yanaka N, Nagasawa T, Yamada H (1992a) Primary structure of an aliphatic nitrile-degrading enzyme, aliphatic nitrilase, from *Rhodococcus rhodochrous* K22 and expression of its gene and identification of its active site residue. Biochemistry 31:9000–9007
- Kobayashi M, Komeda H, Yanaka N, Nagasawa T, Yamada H (1992b) Nitrilase from *Rhodococcus rhodochrous* J1. Sequencing and overexpression of the gene and identification of an essential cysteine residue. J Biol Chem 267:20746–51
- Kobayashi M, Izui H, Nagasawa T, Yamada H (1993) Nitrilase in biosynthesis of the plant hormone indole-3-acetic-acid from indole-3-acetonitrile—cloning of the *Alcaligenes* gene and site-directed mutagenesis of cysteine residues. Proc Natl Acad Sci USA 90:247–251
- Layh N, Hirrlinger B, Stolz A, Knackmuss HJ (1997) Enrichment strategies for nitrile-hydrolyzing bacteria. Appl Microbiol Biotechnol 47:668–674
- Layh N, Parratt J, Willets A (1998) Characterization and partial purification of an enantioselective arylacetonitrilase from Pseudomonas fluorescens DSM 7155. J Mol Catal B Enzym 66:417–424
- Levy-Schil S, Soubrier F, Crutz-Le Coq AM, Faucher D, Crouzet J, Petre D (1995) Aliphatic nitrilase from a soil-isolated *Comamonas testosteroni* sp.: gene cloning and overexpression, purification and primary structure. Gene 161:15–20
- Ludmila K, Plamen D, Ivanka B, Stefan E, Elena D, Elka E (2008) Nitrile degradation by free and immobilized cells of the thermophile *Bacillus* sp. UG-5B, isolated from polluted industrial waters. World J Microbiol Biotechnol 24:2383–2388
- Martínková L, Vejvoda V, Kaplan O, Kubáč D, Malandr A, Cantarella M, Bezouška K, Křen V (2009) Fungal nitrilases as biocatalysts: recent developments. Biotechnol Adv 27:661–670
- Mathew CD, Nagasawa T, Kobayashi M, Yamada H (1988) Nitrilase catalyzed production of nicotinic acid from 3-cyanopyridine in *Rhodococcus rho-dochrous* J1. Appl Environ Microbiol 54:1030–1032
- Nawaz MS, Heinze TM, Cerniglia CE (1992) Metabolism of benzonitrile and butyronitrile by *Klebsiella pneumonia*. Appl Environ Microbiol 58:27–31
- Ogunyemi AK, Samuel TA, Amund OO, Ilori MO (2019) Nitrile metabolizing bacterial strains associated with municipal waste tips in the Lagos metropolis, Nigeria. Pol J Nat Sci 34(2):257–272
- Podar M, Eads JE, Richardson TH (2005) Evolution of a microbial nitrilase gene family: a comparative and environmental genomics study. BMC Evol Biol 5:42. https://doi.org/10.1186/1471-2148-5-42
- Ramteke PW, Maurice NG, Joseph B, Wadher BJ (2013) Nitrile-converting enzymes: an eco-friendly tool for industrial biocatalysis. Biotechnol Appl Biochem 60(5):59–481
- Rezende RP, Teixeira A, Dias JC, Ferraz V, Linardi VR (2000) Metabolism of benzonitrile by *Cryptococcus* sp. UFMG-Y28. J Basic Microbiol 40:389–92
- Riffani R, Sulistinah N, Sunarko B (2015) Gene encoding nitrilase from soil sample of lombok gold mine industry using metagenomics approach. In: The 4th international conference on biological science

- Santoshkumar M, Veeranagouda Y, Kyoung L, Karegoudar TB (2011) Utilization of aliphatic nitrile by *Paracoccus* sp. SKG isolated from chemical waste samples. Int Biodeterior Biodegrad 65:153e9
- Sharma N, Thakur N, Raj T, Savitri T, Bhalla TC (2017) Mining of microbial genomes for the novel sources of nitrilases. BioMed Res Int 2017, Article ID 7039245. https://doi.org/10.1155/2017/7039245
- Stalker DM, Malyj LD, McBride KE (1988) Purification and properties of a nitrilase specific for the herbicide bromoxynil and corresponding nucleotide sequence analysis of the bxn gene. J Biol Chem 263:6310–6314
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–9
- Thimann KV, Mahadevan S, Nitrilase I (1964) Occurrence, preparation, and general properties of the enzyme. Arch Biochem Biophys 105:133–41
- Vejvoda V, Kaplan O, Klozova J, Masák J, Cejková A, Jirků V, Stloukal R, Martínková L (2006) Mild hydrolysis of nitriles by *Fusarium solani* strain O1. Folia Microbiology 51:251–256
- Vejvoda V, Kaplan O, Bezouska K, Pompach P, Sulc M, Cantarella M, Benada O, Uhnakova B, Rinagelova A, LutzWahl S, Fischer L, Kren V, Martinkova L (2008) Purification and characterization of a nitrilase from *Fusarium solani* O1. J Mol Catal B Enzym 50:99–106
- Vejvoda V, Kubác D, Davidová A, Kaplan O, Sulc M, Sveda O, Chaloupkovác R, Martínkováa L (2010) Purification and characterization of nitrilase from Fusarium solani IMI196840. Process Biochem 5:1115–1120
- Wang H, Li G, Li M, Wei D, Wang X (2014) A novel nitrilase from *Rhodobacter* sphaeroides LHS-305: cloning, heterologous expression and biochemical characterization. World J Microbiol Biotechnol 30(1):245–252
- Yamada H, Asano Y, Tani Y (1980) Microbial utilization of glutaronitrile. J Ferment Technol 6:495–500

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- ► Convenient online submission
- ► Rigorous peer review
- ▶ Open access: articles freely available online
- ► High visibility within the field
- ► Retaining the copyright to your article

Submit your next manuscript at ▶ springeropen.com