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Molecular characterization of heavy metal resistant *Proteus species*

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Heavy metals are silent killer of mankind and the cause of environmental pollution. The ability of some microorganisms to resist heavy metals makes them useful in bioremediation. The aim of this study was to molecularly characterize heavy metal-resistant *Proteus* species isolated from the soil of a cement factory. *Proteus* species were tested for resistance to lead, chromium, copper and iron at concentrations 0.00, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mg/L. Minimum inhibitory concentration (MIC) was determined at mg/mL. Plasmid profiling was done. Genomic DNA was extracted using DNA Kit by Zymo Research USA. The concentration of genomic DNA was determined using NanoDrop Spectrophotometer. Twenty-five microlitre was used for polymerase chain reaction. Amplicons were electrophoresed and sequenced. Nucleotide sequences were blasted at the NCBI website. *Proteus* species showed resistance to the test heavy metals. MIC was determined for lead, copper, partly for iron and not for chromium. Plasmid profiling showed that six *Proteus* species harbor high molecular weight plasmids. Concentration of genomic DNA ranged between 1.88 and 2.03 ng/µl. Electrophoresis revealed 16S rRNA genes amplified at 1500 base pair. Blast analysis revealed that six was *Proteus mirabilis* and one was *Proteus terrae*. Phylogeny constructed and study revealed that these *Proteus* species may be useful as bioremediation agents.

Key words: *Proteus* species, resistance, heavy metal, 16S rRNA gene, bioremediation.

INTRODUCTION

Heavy metals are regarded as one of the environmental pollutants due to their toxic effects on plants, animals, human beings and even microorganisms. According to Bharti (2012), heavy metals such as arsenic, lead, cadmium, nickel, mercury, selenium, cobalt, antimony, vanadium, zinc, platinum, palladium and rhodium are highly toxic even in small amount. Heavy metal pollution of the environment and exposure to heavy metals such as mercury, cadmium and lead is a serious growing problem throughout the world. Human exposure to heavy metals has risen dramatically in the last few decades, as a result of an exponential increase in the use of heavy metals in industrial processes and products. Microorganisms that are able to survive well in high concentration of heavy metals are of great interest as bioremediation agents because they can achieve different transformation processes (Adamis et al., 2004).

Heavy metals are increasingly found in microbial

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habitats due to natural processes and anthropogenic activities. Hence, microorganisms have acquired a variety of mechanisms of adaptation and biotransformation responses to heavy metals which include intracellular and extracellular sequestration, compartmentalization, complex formation, synthesis of binding proteins such as metallothioneins, reduction of the heavy metal ions to a less toxic state and to use them as terminal electron acceptors in anaerobic respiration.

Gustav Hauser first described the genus *Proteus* in 1885. The genus *Proteus* belongs to the family Enterobacteriaceae and the tribe Proteeae together with the genera *Morganella* and *Providencia*. *Proteus* species are differentiated from other genera by their ability to swarm across agar surfaces of solid media. Currently, the genus is divided into *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri*, *Proteus hauseri* and three unnamed genomospecies. *Proteus* bacteria are widely found in the natural environment, occurring in polluted water, soil and manure (Różalski et al., 2012). It is an age-long fact that microorganisms are detrimental and beneficial. *Proteus* bacteria are no exception in that they have these two sides of the coin. In medical microbiology, the genus *Proteus* exhibits varied clinical significance in humans and animals as opportunistic pathogens. This is one side of the coin but the other side of the coin is different because of its beneficial effects in the natural environments. Environmental microbiologists have explored the innate ability of *Proteus* bacteria, most especially *Proteus mirabilis* in natural environments and discovered that they exhibit more positive aspects of their existence in natural environments.

There were reports on *Proteus* species as bioremediators of heavy metals, hydrocarbons, pesticides, insecticides, herbicides, aromatic compounds and azo dyes in contaminated environments. Hassen et al. (1998) isolated many Gram-negative bacteria from wastewater in Tunisia with *Proteus* mirabilis as the dominating strain. He reported that the *Proteus mirabilis* were highly resistant to several heavy metals such as copper, chromium, lead, iron cobalt, cadmium, zinc, silver and mercury. Ibrahim et al. (2013) worked on soil samples collected from the rhizosphere of legumes planted on crude-oil contaminated soil in Kaduna, Nigeria and reported that *Proteus mirabilis* and *Proteus vulgaris* were the most active crude oil degraders among the several isolated species. *Proteus* species isolated from the rhizosphere of rice in West Bengal, India, used hexachlorocyclohexane (HCH) pesticide (Das et al., 1995) and phorate insecticide (Das and Mukherjee, 2000; Das et al., 2003) as a source of carbon and energy, and the addition of these chemicals to soil promoted the growth of bacteria. Correa and Steen (1995) found *Proteus mirabilis* strain to be the fastest degrader of a commonly used herbicides called propanil among the natural microflora inhabiting a pristine lake in northeast Georgia, USA. *Proteus* mirabilis strain identified in wastewater samples from Casablanca City, Morocco, exhibited resistance to naphthalene and anthracene (Filali et al., 2000). Olukanni et al. (2010) isolated *P. mirabilis* from municipal dump site soil near Lagos, Nigeria. The isolated *P. mirabilis* was used to degrade a Reactive Blue 13 azo dye to phyto-non-toxic products. The aim of this study was to investigate the ability of *Proteus* species isolated from the soil of a cement factory to resist heavy metals namely lead, chromium, copper and iron.

MATERIALS AND METHODS

List of chemicals and reagents

All chemicals and reagents used were of analytical grade. Chemicals used were manufactured by Merck Specialties Pvt. Ltd., Mumbai, India. The MacConkey and nutrient agar used were manufactured by HiMedia Laboratories Pvt. Ltd., Mumbai, India.

Sample collection

Forty (40) soil samples were collected at the depth of 0-30 cm with the aid of soil auger from the control site and Dangote cement factory located at Tse-Kucha Gboko, Benue State. The soil samples were collected from the mining, waste disposal and industrial sites of Dangote cement factory.

Isolation of *Proteus* species from soil samples

Streak plate technique was used for isolating *Proteus* species from the soil samples. Ten (10) grams of soil sample was added to 90 ml sterile diluent. A loopful from the suspension was streaked on MacConkey and blood agar plates. Plates were incubated at 30°C for 24 h. After incubation, distinct colonies were randomly picked, sub-cultured severally to obtain pure cultures and then preserved on agar slants in the refrigerator.

Heavy metals resistance assay for *Proteus* species

Heavy metal resistance assay was carried out on *Proteus* species according to the method described by Mgbemena et al. (2012). Heavy metals used for this assay were lead (Pb), chromium (Cr), copper (Cu) and iron (Fe) at concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mg/L respectively. The concentration 0.00 mg/L containing no heavy metal served as the control. Nutrient agar medium was supplemented with different salts of heavy metals namely: Lead nitrate Pb $(NO_3)_2$, potassium dichromate $K_2Cr_2O_7$, copper sulphate pentahydrate $CuSO_4.5H_2O$ and iron sulphate heptahydrate $FeSO_4.7H_2O$. Pure isolates were spot inoculated on the heavy metal supplemented medium. The petri- dishes were incubated at 30°C for 48 h. After the period of incubation, the plates were examined for growth and results recorded.

Determination of minimum inhibitory concentration (MIC) of heavy metals on *Proteus* species

Determination of the minimum inhibitory concentration (MIC) of heavy metals on *Proteus* species was carried out according to the method described by Amalesh et al. |(2012). Heavy metals used for MIC were lead (Pb), chromium (Cr), copper (Cu) and iron (Fe) at concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mg/mL

Table 1. Lead resistance assay on *Proteus* species.

Isolate	Concentration mg/L						
	0.00	0.25	0.50	0.75	1.00	1.25	1.50
Proteus mirabilis (MSS7)	+++	+++	+++	+++	+++	+++	++
Proteus mirabilis (ISS10)	+++	+++	+++	+++	+++	++	+
Proteus terrae (WSS7)	+++	+++	+++	+++	+++	++	++
Proteus mirabilis (WSS14)	+++	+++	+++	+++	+++	+++	++
Proteus mirabilis (WSS16)	+++	+++	+++	+++	+++	++	++
Proteus mirabilis (CSS10)	+++	+++	+++	+++	+++	+++	++
Proteus mirabilis (CSS11)	+++	+++	+++	+++	+++	+++	++

MSS = Mining Site Soil, ISS = Industrial Site Soil, WSS = Waste Site Soil, CSS = Control Site Soil, + = Scanty Growth, ++ = Moderate Growth, +++ = Exuberant Growth.
Source: Authors.

respectively. Control experiment was 0.00 mg/mL with no heavy metal. Pure isolates were spot inoculated on nutrient agar supplemented with different salts of lead nitrate $Pb(NO_3)_2$, potassium dichromate $K_2Cr_2O_7$, copper sulphate pentahydrate $CuSO_4.5H_2O$ and iron sulphate heptahydrate $FeSO_4.7H_2O$ of heavy metals. The Petri- dishes were incubated at 30°C for 48 h. After the period of incubation, the plates were examined for growth and results were recorded.

Plasmid extraction and profiling of *Proteus* species

Plasmid extraction was carried out by alkaline lysis method described by Sumathy and Lekha (2017). The plasmid DNAs were run in Tris-Boric EDTA (TBE) 0.8% agarose gel stained with ethidium bromide and was visualized in UV Transilluminator.

Extraction of genomic DNA, PCR and 16S rRNA gene sequencing

Extraction of genomic DNA was carried out according to the method described by Macherey-Nagel (2009). Each pure culture of *Proteus* species grown overnight in 1.5 ml Muller Hinton broth inside microcentrifuge tubes were centrifuged at 14000 rpm for 5 min to obtain pellets. Quick-DNA™ Universal Kit by Zymo Research USA was used for extraction according to the manufacturer's instructions. The concentration of extracted genomic DNA was checked using NanoDrop 2000 Spectrophotometer at 260/280 absorbance. Twenty-five (25) µl volume was used for polymerase chain reaction (PCR). The components of each reaction mixture were: Master mix 4 µl, forward primer 2 µl, reverse primer 2 µl, DNA template 2 µl and DNase free water 15 µl. The two universal primers used have the following sequence (27F:5′-AGAGTTTGATCCTGGCTCAG-3′) and (1492R: 5′-GGTTACCTTGTTACGACTT-3′). Amplification was done for 16S rRNA gene in the thermal cycler as follows: Initial denaturation at 95°C for 5 min, 30 cycles of denaturation, annealing and extension at 94, 52 and 72°C for 30, 30 and 85 s, respectively, followed by a final extension at 72°C for 10 min and kept at a hold temperature of 4°C. The PCR products were run in Tris-Boric EDTA (TBE) 2.0% agarose gel stained with ethidium bromide and amplicons were visualized in UV Transilluminator. Amplicons were purified for sequencing using Zymo PCR cleanup Kit according to the manufacturer recommendations. Both strands of the purified DNAs were sequenced using Applied Biosystems Seqstudio Genetic Analyzer at the Macrogen Laboratory in Maryland USA with the following address: 1330 Piccard Drive, Suite 205, Rockville, MD 20850. Forward and reverse nucleotide sequences of each 16S rRNA gene were aligned and edited using Geneious Sequence Alignment Editor and were subjected to blast (Basic Local Alignment Search Tool) at NCBI website to find the best match of sequences producing significant alignments in order to christen each of the isolates. Seven (7) 16S rRNA genes of the *Proteus* species were submitted at the website of NCBI (National Centre for Biotechnology Information) Genbank. They were accepted and accession numbers were issued for them.

Construction of phylogeny

Nucleotides sequences of other strains of *Proteus* and related genera were pulled from website of NCBI and together with each of the seven nucleotide sequences of *Proteus* strains in this study; phylogeny was constructed using Molecular Evolutionary Genetic Analysis (MEGA) 7.0 version.

RESULTS AND DISCUSSION

Resistance assay of *Proteus* species to lead, chromium, copper and iron is presented in Tables 1 to 4. The growth of all organisms at concentrations of 0.25 to 1.50 mg/L of heavy metals indicated resistance. Scanty growth was depicted by + while moderate growth was indicated by ++ and exuberant growth was indicated by +++.

The minimum inhibitory concentration (MIC) of lead, chromium, copper and iron on *Proteus* species is shown in Table 5. In order to determine the concentration of each heavy metal that hinders the growth of *Proteus* species, MIC was carried at higher concentration of 0.00, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mg/mL. MIC was determined for all *Proteus* species grown on lead and copper supplemented agar while MIC was only determined for three (*Proteus mirabilis* [MSS7], *Proteus terrae* [WSS7] and *Proteus mirabilis* [WSS16]) out of seven *Proteus* species incubated in iron supplemented agar. MIC was not determined for all *Proteus* species inoculated on chromium metal supplemented agar.

Plasmid electrophoresis profile of *Proteus* species is presented in Figure 1. The DNA ladder in the first well

Table 2. Chromium resistance assay on *Proteus* species.

Isolate	Concentration mg/L						
	0.00	0.25	0.50	0.75	1.00	1.25	1.50
Proteus mirabilis (MSS7)	+++	+++	+++	+++	+++	+++	+++
Proteus mirabilis (ISS10)	+++	+++	+++	+++	+++	+++	+++
Proteus terrae (WSS7)	+++	+++	+++	+++	+++	+++	+++
Proteus mirabilis (WSS14)	+++	+++	+++	+++	+++	+++	+++
Proteus mirabilis (WSS16)	+++	+++	+++	+++	+++	+++	+++
Proteus mirabilis (CSS10)	+++	+++	+++	+++	+++	+++	+++
Proteus mirabilis (CSS11)	+++	+++	+++	+++	+++	+++	+++

MSS = Mining Site Soil, ISS = Industrial Site Soil, WSS = Waste Site Soil, CSS = Control Site Soil, + = Scanty Growth, ++ = Moderate Growth, +++ = Exuberant Growth.
Source: Authors.

Table 3. Copper resistance assay on *Proteus* species.

Isolate	Concentration mg/L						
	0.00	0.25	0.50	0.75	1.00	1.25	1.50
Proteus mirabilis (MSS7)	+++	+++	+++	++	++	+	+
Proteus mirabilis (ISS10)	+++	+++	+++	++	++	+	+
Proteus terrae (WSS7)	+++	+++	+++	++	++	+	+
Proteus mirabilis (WSS14)	+++	+++	+++	++	++	+	+
Proteus mirabilis (WSS16)	+++	+++	++	++	+	+	+
Proteus mirabilis (CSS10)	+++	+++	+++	+++	+++	+++	++
Proteus mirabilis (CSS11)	+++	+++	+++	+++	+++	++	+

MSS = Mining Site Soil, ISS = Industrial Site Soil, WSS = Waste Site Soil, CSS = Control Site Soil, + = Scanty Growth, ++ = Moderate Growth, +++ = Exuberant Growth.
Source: Authors.

Table 4. Iron resistance assay on *Proteus* species.

Isolate	Concentration mg/L						
	0.00	0.25	0.50	0.75	1.00	1.25	1.50
Proteus mirabilis (MSS7)	+++	+++	+++	+++	+++	++	+
Proteus mirabilis (ISS10)	+++	+++	+++	+++	+++	+++	+++
Proteus terrae (WSS7)	+++	+++	+++	++	++	+	+
Proteus mirabilis (WSS14)	+++	+++	+++	+++	+++	+++	+++
Proteus mirabilis (WSS16)	+++	+++	+++	+++	++	++	++
Proteus mirabilis (CSS10)	+++	+++	+++	+++	+++	+++	+++
Proteus mirabilis (CSS11)	+++	+++	+++	+++	+++	+++	+++

MSS = Mining Site Soil, ISS = Industrial Site Soil, WSS = Waste Site Soil, CSS = Control Site Soil, + = Scanty Growth, ++ = Moderate Growth, +++ = Exuberant Growth.
Source: Authors

shows standard bands. The molecular weight of DNA ladder used are 23130, 9416, 6557, 4361, 2322 and 2027 kilobase pair (kpb). The plasmids found in the heavy metal resistant *Proteus* species at ng/µl concentration were in the region of high molecular weight plasmids (23130 kbp) of the DNA ladder. Plasmids habour resistant markers. The presence of these high molecular weight plasmids in *Proteus* species may be responsible for their heavy metal resistance.

Gel electrophoresis of amplified 16S rRNA genes of

Table 5. Minimum Inhibitory concentrations (mg/mL) on *Proteus species* for different heavy metals.

Isolate	Lead	Chromium	Copper	Iron
Proteus mirabilis (MSS7)	1.50	ND	0.75	1.25
Proteus mirabilis (ISS10)	1.25	ND	0.75	ND
Proteus terrae (WSS7)	1.25	ND	0.75	0.75
Proteus mirabilis (WSS14)	1.50	ND	0.75	ND
Proteus mirabilis (WSS16)	1.25	ND	0.50	1.00
Proteus mirabilis (CSS10)	1.50	ND	1.50	ND
Proteus mirabilis (CSS11)	1.50	ND	1.25	ND

MSS = Mining Site Soil, ISS = Industrial Site Soil, WSS = Waste Site Soil, CSS = Control Site Soil, ND- Not Determined.
Source: Authors.

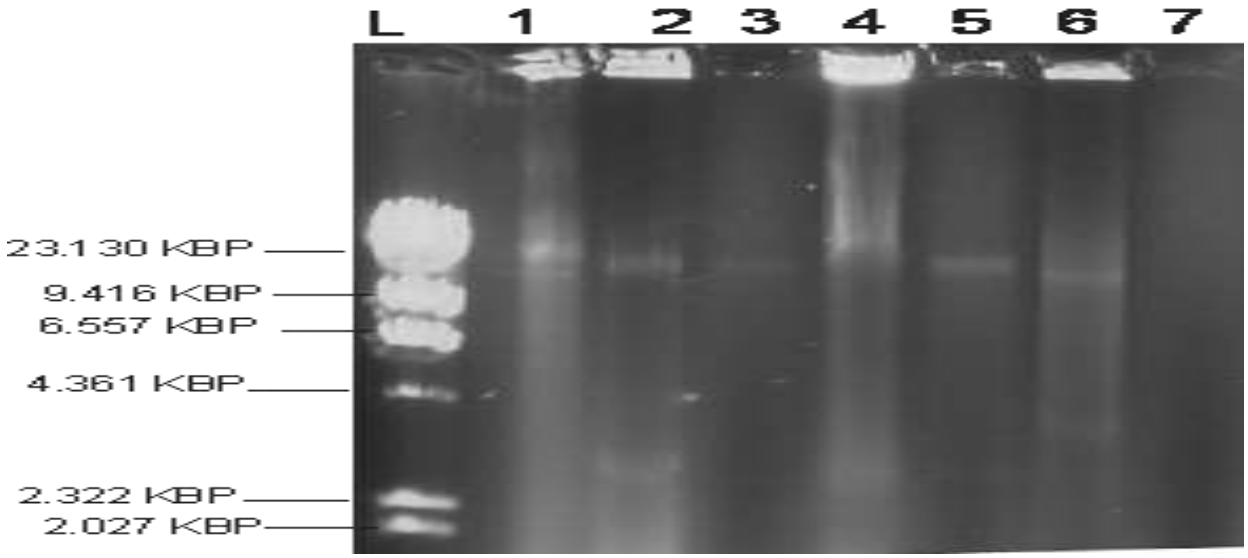

Figure 1. Gel Electrophoresis showing Plasmid Profile of *Proteus* species**.** L = Ladder, 1 = MSS7, 2 = ISS10, 3 = WSS7, 4= WSS14, 5 = WSS16, 6 = CSS10, 7 = CSS11.
Source: Authors

heavy metal resistant *Proteus* species is presented in Figure 2. The DNA ladder containing standard bands was loaded in the first well of the agarose gel. Control sample (nuclease free water) was loaded in well no 6. The highest molecular weight of DNA ladder used was 1500 base-pair (bp) and the lowest molecular weight of the ladder was 100 base-pair. Well no 1, 2, 3, 4, 5, 7 and 8 contained amplified 16S rRNA genes of *Proteus* species. The amplified 16S rRNA aligned at the corresponding region 1500 bp of the DNA ladder. This is because the molecular weight of 16S rRNA gene is 1500 bp.

Table 6 presents the molecular characterization of the *Proteus* species. The generic and specific names of the organisms were identified. The strain number and the accession number given for each organism by the national center for biotechnology information (ncbi) are also presented.

Figure 3 presents the phylogenetic tree showing genetic relatedness between other genera and *Proteus*. The linkage distance of 0.050 depicts that 5% percentage base substitution occurred in the nucleotide sequences of various organisms in the phylogeny. The base substitution brought about evolution of new organisms from parental organisms. The *Proteus* species in bold letters are isolates from this research. Phylogeny was constructed using nucleotides sequences of 16S rRNA genes of *Proteus* species in this study and related genera pulled from ncbi website. The software used for the construction was Molecular Evolutionary Genetic Analysis (MEGA) 7.0 version.

The results of heavy metal resistance assay for *Proteus mirabilis* and *P. terrae* revealed that *Proteus mirabilis* isolated from mininig, industrial and waste site soil of a cement factory and control site soil showed resistance to the four heavy metals (Pb, Cr, Cu and Fe) in this study (Table 1 to 4). A similar research by Adekanmbi et al.

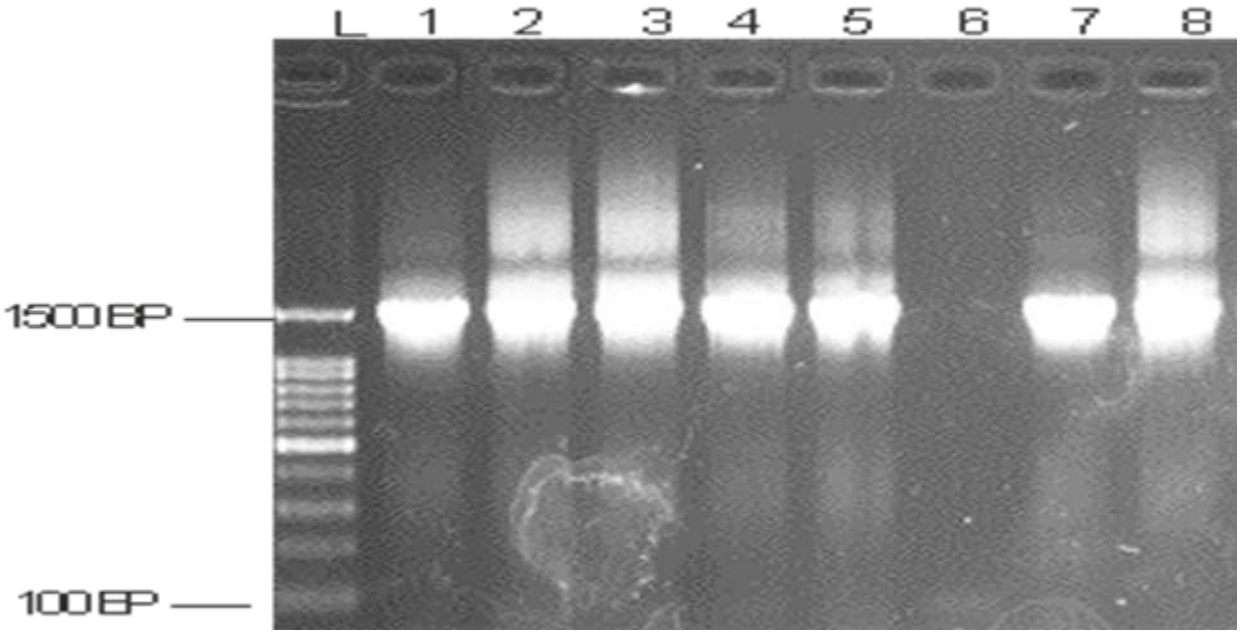

Figure 2. Gel Electrophoresis showing amplified 16S rRNA genes of *Proteus* species. Key: L = Ladder, 1= MSS7, 2= ISS10, 3= WSS7, 4= WSS14, 5= WSS16, 6= Control sample, 7= CSS10, 8=CSS11.
Source: Authors.

Table 6. Molecular characterization of the *Proteus* species.

Isolate	Organism identified	Blast best match	%Similarity	Strain number	Accession number
MSS7	*P. mirabilis*	*Proteus mirabilis* strain ALK418	99	ADY 13	MH084801
ISS10	*P. mirabilis*	*Proteus mirabilis* strain ALK418	99	ADY23	MH084809
WSS7	*P. terrae*	*Proteus terrae* strain N5/687	86	ADY17	MH119106
WSS14	*P. mirabilis*	*Proteus mirabilis* strain JCM 1669	99	ADY 19	MH084805
WSS16	*P. mirabilis*	*Proteus mirabilis* strain ATCC 29906	97	ADY 20	MH084806
CSS10	*P. mirabilis*	*Proteus mirabilis* strain JCM 1669	99	ADY 30	MH084814
CSS11	*P. mirabilis*	*Proteus mirabilis* strain JCM 1669	99	ADY 28	MH084813

MSS = Mining Site Soil, ISS = Industrial Site Soil, WSS = Waste Site Soil, CSS = Control Site Soil, BLAST = Basic Local Alignment Search Tool.
Source: Authors.

(2019) reported that *Proteus mirabilis* isolated from printeries wastewaters demonstrated resistance to lead, cadmium, chromium, copper, silver and zinc. Nwagwu et al. (2017) reported that *Proteus mirabilis* isolated from Panteka stream showed resistance to lead, iron, cadmium, zinc and nickel. Also *Proteus mirabilis* isolated from industrial wastewater displayed resistance to zinc (Owolabi and Hekeu, 2015). Mgbemena et al. (2012) found that a strain of *Proteus mirabilis* isolated from Otamiri River showed resistance to lead, iron and zinc. *Proteus terrae* isolated from waste site soil of a cement factory in this study demonstrated resistance to lead, chromium, copper and iron. Based on literatures at our disposal, the heavy metal resistance ability of *Proteus terrae* has not been determined prior to this time and this is likely to be the first research reporting the heavy metal resistance ability of this organism.

Badar et al. (2000) opined that contamination with a specific metal is known to increase the level of resistance of the bacterial community to that metal. Bacteria adapt to metal stress in their environment and respond to it by developing several resistances or coping mechanisms to its toxicity (Adekanmbi et al., 2019). The 100% resistance (growth) of *Proteus* species to the heavy metals at various concentrations (mg/L) for resistance assay (Tables 1 to 4) suggests that the microbes have adapted to, tolerate and grow in the presence of these heavy metals in their metal-stressed natural soil habitats. Plasmid profiling (Figure 1) of the *Proteus* species revealed that the *Proteus terrae* and five out of the six

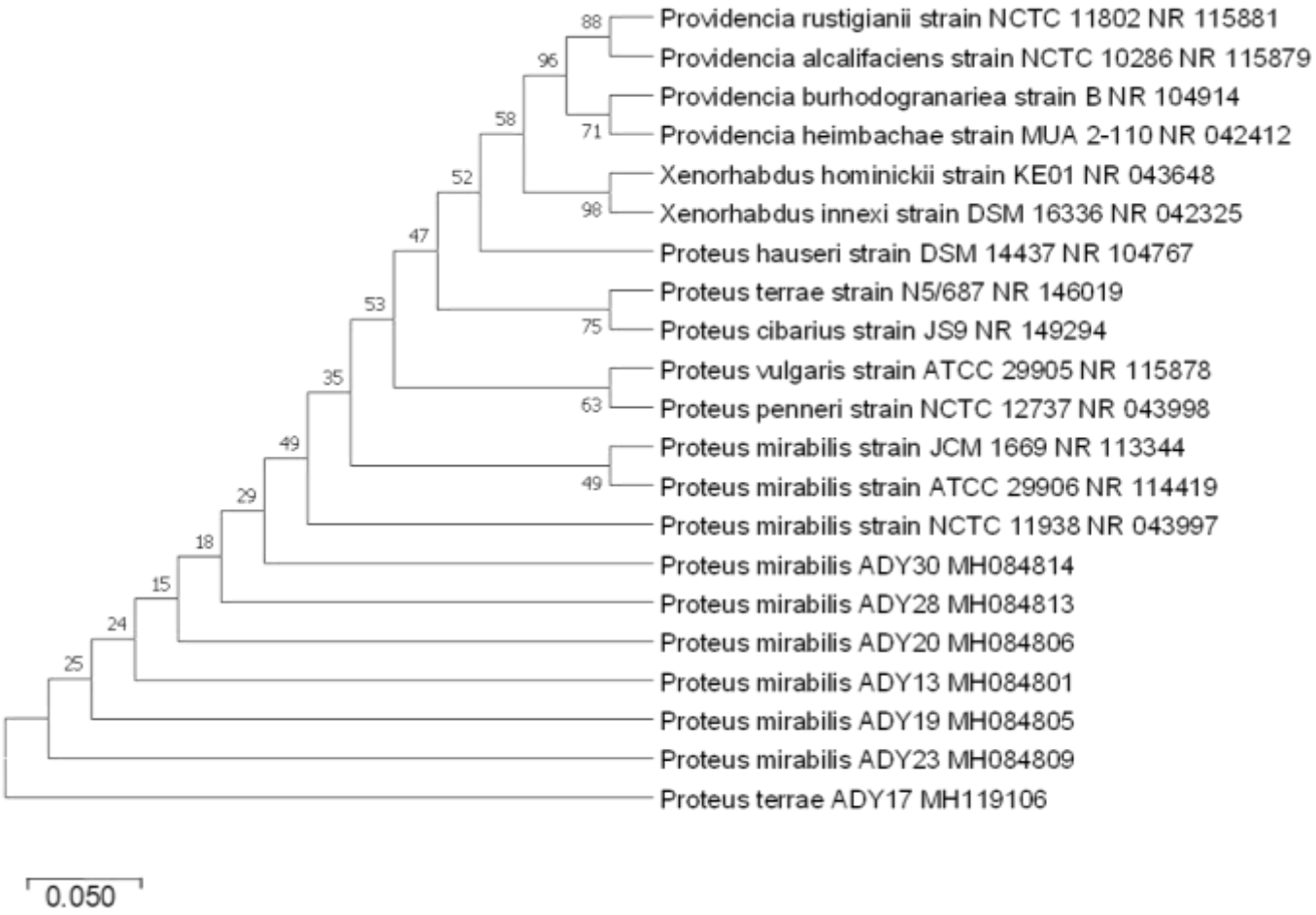

Figure 3. Dendrogram showing the phylogenetic relationship of different strains of *Proteus* species and related genera. The organisms with letters ADY in their strain number are the *Proteus species* isolated in this study.
Source: Authors.

Proteus mirabilis habour high molecular weight plasmid (23000 bp). The resistance determinants to the test metals are likely located on the plasmids. Abou-Shanab et al. (2007) reported that genes encoding heavy metal resistance in bacteria can be located within bacterial plasmids, chromosomes, or on transposons. Plasmid genes may code for proteins involved in metal reduction, binding, sequestration, complex formation and for specific transport systems e.g. efflux pumps (Ghosh et al., 2000). Plasmid-borne resistance to copper has been reported in several species of bacteria and documented by several authors (Hansen et al., 2016; Buberg et al., 2020). Also chromosomal resistance to copper has also been reported in species of bacteria (Jones et al., 2005). This might be responsible for the resistance to copper and other metals by *Proteus mirabilis* strain ADY28 in this study, even without the possession of the plasmid-borne resistance determinants.

In addition, Adekanmbi et al. (2019) reported the detection of plasmid-borne chrA gene responsible for chromate resistance in *Proteus mirabilis* PW3a from printeries wastewater. The possession of plasmids encoding chromate resistance has also been reported in certain species of *Alcaligenes*, *Bacillus*, *Escherichia coli*, *Pseudomonas* and *Salmonella* by several authors (Ghosh et al., 2000; Verma et al., 2002; Kamala-Kannan and Lee, 2008: Das et al., 2014). Adekanmbi et al. (2019), detected chrB gene which regulates the chrA transporter in *Proteus mirabilis* PW4c and *Providencia vermicola* PWAP3 and this corroborated the report on the possession of the chr operon on the plasmids of Gram negative bacteria (Verma et al., 2009). The CBA-transport systems responsible for export of metal ions, xenobiotics and drugs are found in Gram negative bacteria. This system safeguards cytoplasm of Gram negative cells through translocation of metals and other toxicants across their outer membrane (Adekanmbi et al., 2019). The pbr proteins are a group of proteins encoded in the widely studied metal-resistant *Cupriavidus metallidurans* CH34, and they include pbrT, pbrA, pbrB, pbrC, pbrD and pbrR. The pbrA is a PIB-type ATPase in *Cupriavidus metallidurans*, and is the main lead efflux

transporter (Borremans et al., 2001). The pbr proteins might be present in *Proteus* species in this study due to the appreciable resistance shown to lead.

In order to determine the minimum inhibitory concentration (MIC) of each heavy metal on *Proteus* species, the concentration of the heavy metals was increased from mg/L to mg/mL (Table 5). Despite this geometric increase, MIC was wholly determined for lead and copper, partly for iron but not for chromium at various concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mg/mL. The resistance shown to lead by *Proteus terrae* strain ADY17 and all variants of *Proteus mirabilis* (strains ADY13, ADY19, ADY20, ADY23, ADY28 and ADY30) up to 1.00 mg/mL and even at 1.25 mg/mL is highly commendable because lead is known to be a very poisonous heavy metal with high toxic effect on bacteria as reported by Eghomwanre et al. (2016). The minimum inhibitory concentration of lead on *Proteus mirabilis* (ISS10), *Proteus terrae* (WSS7) and *Proteus mirabilis* (WSS16) was 1.25 mg/mL and that of *Proteus mirabilis* (MSS7), *Proteus mirabilis* (WSS14), *Proteus* mirabilis (CSS10) and *Proteus* mirabilis (CSS11) was 1.50 mg/mL. It is noteworthy that these organisms survived high concentrations of lead and could be very advantageous as remediating agents in lead polluted soil and water. Furthermore, the growth of all these variants of *Proteus* at concentrations of 0.25 to 1.50 mg/mL of chromium depicts they are highly resistant to chromium which is an advantage in bioremediation of chromium in polluted environment. The very high resistance shown by *Proteus mirabilis* (CSS11) and (CSS10) strains ADY28 and ADY30 to copper up 1.00 and 1.25 mg/mL is of interest and indication of the effectiveness of these two strains as potential bioremediation agents in copper polluted environment. Minimum inhibitory concentration of copper was determined for other *Proteus* species. The minimum inhibitory concentration of copper on *Proteus mirabilis* (MSS7), *Proteus mirabilis* (1SS10) *Proteus terrae* (WSS7) and *Proteus mirabilis* (WSS16) was 0.75 mg/mL and that of *Proteus mirabilis* (WSS14), was 0.50 mg/mL. Eghomwanre et al. (2016) reported the toxic effect of copper on bacterial isolates from soil and sediment. Nonetheless, all these organisms thrived well in the presence of copper and could be exploited for remediative advantage in copper polluted areas. *Proteus terrae* strain ADY17 had the least MIC for iron compared to all other variants of *Proteus mirabilis.* This indicates that *Proteus mirabilis* had higher resistance to iron compared to *Proteus terrae* and this might be due higher need of iron for metabolic activities by *Proteus mirabilis than P*. *terrae*. In addition, it has been reported that *Proteus mirabilis* strains are able to produce siderophores which translocate iron from outside across the cell membrane into these organisms (Prescott et al., 2008). It is worthwhile to point out that *Proteus mirabilis* (ISS10), *Proteus mirabilis* (WSS14), *Proteus* mirabilis (CSS10) and *Proteus* mirabilis (CSS11) survived iron concentrations from 0.00 to 1.50 mg/mL. This finding corroborates the report of Mgbemena et al. (2012) and Nwagwu et al. (2017). It can be inferred that these strains: ADY23 ADY19 ADY30 and ADY28 of *Proteus* can be used to get rid of excess iron in both human and environmental systems. In summary, the MIC results revealed that copper had highest toxic effect on the growth of *Proteus* species, followed by lead, then iron and chromium had no toxic effect at these concentrations.

Conclusion

On a general note, *Proteus* species are known for their detrimental activities such as being opportunistic pathogens in humans and animals, indicator of fecal pollution, marine food-borne pathogens etc. Contrariwise, this study reveals that *Proteus* species are also beneficial to humans in our natural and industrial environment. *Proteus species* investigated for heavy metal resistance in this study, turned out to be potential bioremediation agents for the test heavy metals [lead (Pb), chromium (Cr), copper(Co) and iron(Fe)]. These organisms should be explored more to harness their full potential in bioremediation of toxic heavy metals in polluted environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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