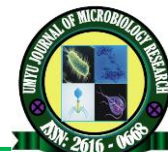
UJMR, Vol. 7 No. 2, December, 2022, pp. 70 -78 E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2272.011



Received: 24th June, 2022 **Accepted:** 29th June, 2022

Identification of Iron Reducing Bacteria from Kaolin of Different Mining Sites in Northern Nigeria for Potential Bioleaching Activity

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Abstract

Kaolin is a naturally occurring raw material for several industrial applications. Moreover, the economy of using Kaolin has some restrictions due to presence of iron-bearing impurities in form of oxides and hydroxides. This has a negative effect on the whiteness and refractoriness of some products and the production of high purity ceramics. The application of microbial leaching has been regarded as cheap and bio-friendly than conventional methods, hence this study investigates the isolation of potential iron reducing bacteria capable of providing efficient bioleaching. Bacteria isolation was carried out using serial dilution and plating techniques. Result of molecular, morphological and biochemical analyses reveal the presence of many bacterial isolates that includes Acinobacter spp., Aeromonas spp., Clostridium spp., Klebsiella spp., Pseudomonas spp. and Sporanaerobacter spp. Aeromonas and Sporanaerobacter species were identified as more closely related to the Pseudomonas and Geobacter species that are reported to be good iron reducing bacteria. This may suggest a possible candidate strain that could provide efficient bioleaching activity. Result of X-ray Fluorescence spectrometry of Kaolin prior to isolation and identification of indigenous bacteria was found to contain the following oxides; Fe_2O_3, SiO_2, $A1_2O_3$, MgO, P_2O_5, SO_3, TiO_2, MnO and CaO which confirmed the identity of Kaolin samples collected. The highest oxide was found to be SiO_2.

Key word: Kaolin, Silica sand, Oxides, Bacteria, Iron reducing bacteria

INTRODUCTION

Kaolin is one of the most extensively used raw materials for a wide range of industries. These raw materials include paper, filling and coating, ceramics, porcelain, dinnerware, tiles and enamels, catalyst for petroleum cracking and auto exhaust emission; catalytic control devices, paint, adhesive and ink pigment, and new ones are still discovered (Garcia-Valles, 2021). It is unique industrial mineral due to its chemical inertness over a relative wide pH range (Pinheiro *et al.*, 2020).

On the estimate, that the annual global demand for kaolin is 27,001.40 kilotons with a market value of over USD 4.33 billion (Grand View, 2016). Here in Nigeria, it was estimated that the national demand for kaolin is over 360,000 tones (Orosun *et al.*, 2021). This means that there is going to be increase in the use of ceramic products (including tiles and sanitary wares) and its use in cement industry as a supplementary material is expected to propel product demand over the next eight years. This is anticipated to positively impact the kaolin industrial growth (Grand View, 2016).

Before kaolin products can be used by manufacturing industry, the raw Kaolins have to be refined to meet commercial specifications (Styriakova and Styriak, 2000). One of the key problems for some industrial applications of Kaolins are the iron-bearing impurities. It discolors ceramic products, lowers the melting point of refractory materials and also affects quality in paper manufacture(Zegeye *et al.*, 2013). Even a low content in the mineral substantially affect the whiteness and refractoriness of the product (Zegeye *et al.*, 2013). Both Kaolin and Silica sand are considered as naturally occurring mineral with several applications especially in production of all types of glasses. Moreover, these minerals are widely distributed in Nigeria with over 26 States of kaolin deposits (RMRDC, 2010).

Recent developments in the fall of crude oil prices have led to economic diversification especially in oil dependent economies like Nigeria. This has heightened the interest of the federal government in the mining of solid minerals including Kaolin and Silica sand.

Moreover, their commercial value is greatly affected by the presence and content of iron impurities which can have a negative effect on the whiteness and refractoriness of manufactured products (Zegeye *et al.,* 2013). Leaching methods commonly used in removing iron content include chemical; oxalic acid, froth flotation, gravity separation and magnetic separation. Moreover, all of these methods are costly, very difficult to operate and have an adverse effect on the environment. Despite the isolation of some bacteria for use in iron reduction (Childers *et al.,* 2002; Lovley *et al.*, 1998; Tang *et al.,* 2016; Jalali *et al.*, 2019; Eisele and Gabby, 2012), still the reduction was not efficient. This therefore necessitates the need to isolate and identify indigenous microorganisms in fresh Kaolin and Silica sand samples as a cheap and efficient method capable of bioleaching of iron impurities.

MATERIALS AND METHODS

Sample collection area

Kaolin samples were collected from four different sources in Katsina, Kano and Bauchi States, Northern Nigeria. The sources were: Kankara (11.914559, 7.428591)^0, Kankara Local Government Area (LGA) Katsina State, Getso (11.883123, 7.969823)^0, Gwarzo LGA, Kano State, Danbakoshi (12.0462, 7.9515)^0, Shanono LGA, Kano State and Gwaram (10.259805, 10.312235)^0, Alkaleri LGA, Bauchi State.

Sample collection

Kaolin samples were collected from freshly exposed rock using a clean hammer and scrapper. The samples were immediately placed aseptically in anaerobic jars and Ziploc bagsto maintain an anaerobic environment for the isolation and identification of the indigenous microorganisms. In addition, separate kaolin samples were also collected for the purpose of bioleaching experiments using indigenous microorganisms. All samples collected were transported to the Central laboratory complex, Bayero University Kano, for subsequent analyses as described by Yahaya (2011).

Isolation of indigenous microorganisms

Pour plate method as described by Styriakova and Styriak, (2000) was adopted for this research. A small amount of inoculum from broth culture was added by a pipette to the center of a Petri dish. Molten, cooled and sterile [Trypticase SoyAgar/Broth and Nutrient Agar/Broth (Trypticase Soy and nutrient Agar/Broth TM Media Titan Biotech Ltd., Delhi, India) were for used for the isolation. The process was conducted aseptically. The dish was gently rotated to ensure that the culture and medium are thoroughly mixed and the medium covers the plate evenly. The plate was allowed to solidify and was incubated at 37^{0}C for 24 hours in an inverted position; Society for General Microbiology (SGM), (2006). Pour plates allow micro-organisms to grow both on the surface and within the medium discrete colonies chosen were purified by re-isolation on Nutrient agar plates to obtain pure strain cultures. Morphology was examined by light microscopy after Gram staining (SGM, 2006).

Gram staining procedure

Thin films of the isolates were made on clean glass slides, air dried and heat fixed. Smear was flooded with crystal violet stain for 60 seconds and gently washed off under slow running tap water. Smear was flooded with Grams' iodine for 60 seconds and gently washed off. Then it was flooded with 95% ethanol for 30 seconds and gently washed off. Lastly, smear was counterstained with safranin for 60 seconds, rinsed and air dried. Smear was examined under the microscope using oil immersion (Procop *et al.*, 2017).

Endospore test

A loopful of colony was heat fixed on a clean grease free slide. The smeared slide was flooded with malachite green stain, and then placed over a steaming water bath which was heated gently until steam rose for about 5 minutes. The slide was washed with distilled water. It was counter stained with Safranin, and allowed to stand for 2 minutes. The stain was washed off with distilled water and air dried. It was observed under the microscope. Cells that appear pink are endospore negative while green cells are endospore positive (Goyal *et al.*, 2012).

Biochemical analysis

Catalase Test

A microscope slide was placed inside a petri dish and placed on the black working surface. The petri dish cover was kept by the side. Using a sterile inoculating loop, a small amount of organism from a 24-hour colony was scooped and placed onto the microscope slide. Using a dropper, a drop of 3% $H_2O_2$was placed onto the organism on the microscope slide. It was not mixed. The petri dish was immediately covered with a lid to limit aerosols and observed for immediate bubble formation (O_2 + water = bubbles.) observing for the formation of bubbles against a dark background enhanced readability. Formation of bubbles is positive while the absence of bubble formation indicates a negative result (Procop *et al.,* 2017).

Oxidase test

A small piece of filter paper was soaked in 1% Kovacs oxidase reagent and allowed to dry.

A loop was used to pick a well isolated colony from a fresh (24-hour culture) bacterial plate and rubbed onto treated filter. It was observed for color changes. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color doesn't change or it takes longer than 2 minutes (Procops *et al.,* 2017).

Indole test

Isolates were inoculated into peptone broth and incubated at 35^{0}C for 24-hours. Three drops of Kovac's reagent was added and observed for color change (Procops *et al.,* 2017). A red coloration indicates a positive test while a negative test is colorless.

Voges-Proskauer test

Alpha-naphtol and 0.2ml KOH were added to test tube containing MRVP medium that had been inoculated with test organism, it was mixed gently and allowed to stand for 20 minutes to observe color change. Positive results were red while negative results were colorless (Madigan and Martinko, 2008).

Methyl red test

Two drops methyl red indicator was added to a test tube containing MRVP medium that had been inoculated with test organism and incubated for 48 hours. It was mixed gently and observed for color change. A red color is a positive reaction while no color change indicates negative reaction (Arora and Arora, 2012).

Citrate utilization test

Test isolates were inoculated into Simmon's citrate agar in McCartney bottles and incubated at 37^{0}C for 24-hours. Positive utilization gave blue coloration while an unchanged color (green)indicates negative citrate utilization (Arora and Arora, 2012).

Motility test

A colony of the young test organism was touched with a needle. It was stabbed to a depth of one third of a test tube containing semi solid agar medium. This was incubated at 35^{0}C. It was examined daily for seven days to observe for a diffuse zone of growth flaring out from the line of inoculation. A positive test displayed a diffuse, hazy growth that spread throughout the medium rendering it slightly opaque while a negative result showed a confined growth to the stab-line with sharp defined margins, leaving the surrounding medium clearly transparent (Tille and Forbes, 2004).

Glucose utilization test

Isolates ability to produce gas from glucose was realized in MRS-BCP broth containing 1% glucose. Inverted Durham tubes were placed in the test tubes to collect the produced gas. Gas production by lactic acid bacteria is used to distinguish the nature of metabolism exhibited by that particular organism. Homofermentative organisms do not produce gas from glucose utilization, while heterofermentative organisms produce gas during glucose metabolism (Guetouache and Guessas, 2015).

Preservation of isolates

All isolates were centrifuged at 4000 rpm for 20 minutes, washed twice with saline solution (0.9% NaCl) and preserved in a sterile 20% glycerol buffer (100% glycerol, KH_2PO_4: 3.0gL^{-1}, Na_2HPO_4: 7.0gL^{-1}, NaCl: 4.0gL^{-1}, $MgSO_4.7H_2O$: 0.2gL^{-1}) in a ratio of 50% glycerol: 50% bacterial buffer.

Genomic DNA extraction

The genomic DNA of the bacteria was extracted using Promega Wizard™ Genomic DNA Purification Kit (Promega) according to the protocol of the manufacturer. The concentration and purity of the DNA extracted were determined using Nanodrop™ ND-1000, and visualized on a 1% agarose gel.

Agarose gel Electrophoresis

To prepare the agarose gel, 5 g of agarose powder was weighed into 50 mL of TAE buffer dissolved in a microwave for 1 minute and cooled to the room temperature. Then 0.1 µL of ethidium bromide (EtBr) was added and poured onto the gel tray. The DNA sample (5 µL) was mixed with 2 µL of the loading dye and loaded into the well. The electrophoresis was run for 45 minutes at 80 volts. The DNA band was later viewed in a UV trans-illuminator (Mak-Mensah and Ahiakpah, 2013).

Polymerase Chain Reaction (PCR) amplification of the isolates

The PCR reaction was carried out using KAPATaq DNA polymerase. The total reaction volume was 25µL. Reaction mix comprise of 2µL each of the genomic DNA, 2.5 µL of 10 TaqA Buffer, ~0.4M (0.85 µL) each of forward and reverse primers, 1.25 mM (1.5 µL) of $MgCl_2$, 0.25 mM (0.2 µL) of dNTP mixes and 0.2 µL of Taq DNA polymerase, in ddH_2O. Amplification was carried out using the following conditions (Mak-Mensah and Ahiakpah, 2013): initial denaturation of 5 minutes at 95 ^{0}C, followed by 35 cycles each. Thirty (30) seconds at 94 ^{0}C (denaturation), 30 seconds at 60 ^{0}C (primer annealing) and 1 minute at 72 ^{0}C (extension). This was followed with 10-minute final extension at 72 ^{0}C. PCR products were separated in a 1.5% agarose gel stained with ethidium bromide and sent for sequencing to Inqababiotech^{TM}, Inqaba biotechnology industries South Africa.

16S rRNA Gene amplification and bioinformatics analysis

To amplify the 16S rRNA gene, the method of Mak-Mensah and Ahiakpah, (2013) was adopted. A universal forward and reverse primer was used. The gel was visualised on 1% agarose gel as described earlier. The PCR products were later sent to inqaba Biotec (South Africa) for purification and sequencing. Analysis was performed using BioEdit V7.2.5 software and sequence was compared with other sequences using online BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

MEGA 7.0 was used for the construction of phylogenetic tree using Neighbor joining method. The partial 16S rRNA sequence was submitted to Gene Bank and accession number obtained.

RESULTS AND DISCUSSION

Isolation of indigenous Bacteria

Bacteria isolation was preceded with mineralogical and elemental analyses of Kaolin using Fourier Transform Infrared (FTIR) Spectroscopy, Scanning Electron Microscopy (SEM), X-ray Diffraction spectrometry (XRD), and X-ray fluorescence spectrometry (XRF) (results not shown).

Bacteria isolation was successfully conducted and the results were summarized and presented in tables 1 and 2. The results indicated that most of the bacteria isolated from the samples are mostly Gram Positive Bacilli with some Gram Negative Bacilli and Gram Positive Cocci. Endospore staining indicated a pink-red with vegetative cells based on microscopic observation results. Table 1 shows the morphological features and Gram reactions of some of the indigenous bacteria isolated from samples. Table 2 showed the biochemical test and spore forming test of the indigenous bacteria.

In all of the 10 bacterial isolate obtained, all were found to be morphologically *Bacilli*, isolate 1 and 2 formed chain characteristic of cellular arrangement, sample 4, 5 and 9 were in bunch whereas sample 3, 6 and 7 were pairs. The results reveal only samples 8 have chain pairs. Spore was absent in all the isolate except sample 5, 6, 9 and 10 (Figure 3). Result for presence of capsule indicated that all the isolates does not have capsule (Table 1 and 2). Furthermore, the different colors of Kaolin collected from different mining sites, Gram reactions and cell arrangements of some of the isolates were shown in Plates 1 and 2.

Generally, rocks and minerals provides microbes with nutrients and living habitats while the microbes impacts the rocks and the minerals weathering and digenesis rates through their effect on mineral solubility and speciation with a consequence of either dissolving or precipitating minerals, in addition the organisms also gain energy and grow (Dong, 2010). The Gram reactions of the indigenous bacteria isolated from the samples (Plate 1) is in line with the findings of Styriakova and Styriak (2000) that earlier reported the isolation of different *Bacillus* spp. from Kaolin. As demonstrated in this study, results of biochemical test reveal the presence of *E. coli, Enterobacter* spp., *Aeromonas* spp., *Pseudomonas* spp., *Bacillus* spp, and *Clostridium* spp. Among them only *Aeromonas* spp. and *Clostridium* spp. was capable of producing hydrogen.

Literature reported that, other bacteria such *Geobacter lovleyi* isolated by Sung *et al*. (2006) at 30^{0}C under anaerobic condition was found to reduces ferric iron (ferric citrate); Derives energy from acetate oxidation coupled to polychloroethylenes dechlorination.

Table 1: Morphological Characteristics of the Indigenous Bacteria isolated from the samples

Sample ID	Description	*GR	Morphology	*CA	Spore	Capsule
Sample 1	KA I	+	Bacilli	Chain	Absent	Absent
Sample 2	KA II	+	Bacilli	Chain	Absent	Absent
Sample 3	KQ I	+	Bacilli	Pairs	Absent	Absent
Sample 4	KQ II	+	Bacilli	Bunch	Absent	Absent
Sample 5	SB I	+	Bacilli	Bunch	Present	Absent
Sample 6	SB II	+	Bacilli	Pairs	Present	Absent
Sample 7	SD 1	+	Bacilli	Pairs	Absent	Absent
Sample 8	SD II	+	Bacilli	Chain Pairs	Absent	Absent
Sample 9	SM I	-	Bacilli	Bunch	Present	Absent
Sample 10	SM II	-	Bacilli	Chain	Present	Absent

Key: *GR = Gram Reaction, *CA = Cell Arrangement.

Table 2: Biochemical Reactions of the Indigenous Bacteria isolated from the samples

Biochemical Reaction	*E. coli*	*Klebsiella* sp.	*Enterobacter* sp.	*Aeromonas* sp.	*Pseudomonas* sp.	*Bacillus* sp.	*Clostridium* sp.	*Acinetobacter* sp.
Gram Reaction	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve
Morphology	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Spore Formation	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve
Oxidase	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
Indole	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Citrate	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
VogesProskauer	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Methyl Red	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Glucose	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Lactose	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Sucrose	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
Motility	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
H_2S Production	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve

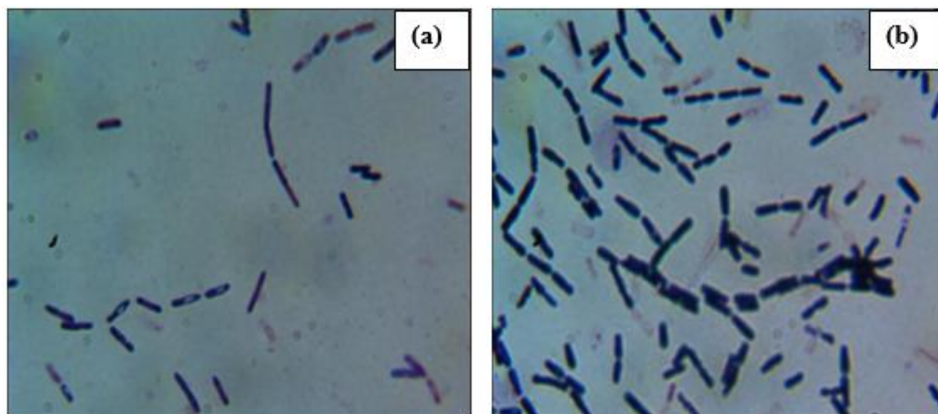


Plate 1: Results for gram reaction. (a) Gram positive Bacilli pair, (b) Gram negative Bacilli pair chain isolated from a sample

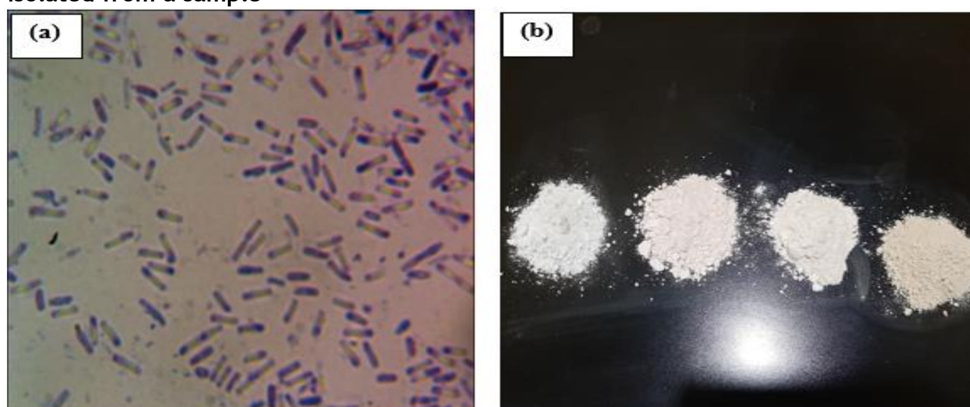


Plate 2: Results. (a) Gram positive Bacilli spore formers isolated from a sample, (b) Shades of Kaolin collected from a mining site

16S rRNA result

Molecularly identified bacteria isolated were found to include *Aeromonas dhakensis* strains VM32 and Blm 16S ribosomal RNA gene complete sequence with accession numbers MF95327.1 and MF 953247.1 respectively with a 95.09% identification for both strains. *Sporanaerobacter* strain 906C and *Sporanaerobacter* strain DSM 1310616S ribosomal RNA gene partial sequences. 906C had 97% query cover and 93.92% identity while DSM 13106 had 98% query cover and 93.19% identity. *Acinobacter* spp, *Clostridium, Klebsiella* spp. and *Pseudomonas* spp were also identified among others (Figures 4).

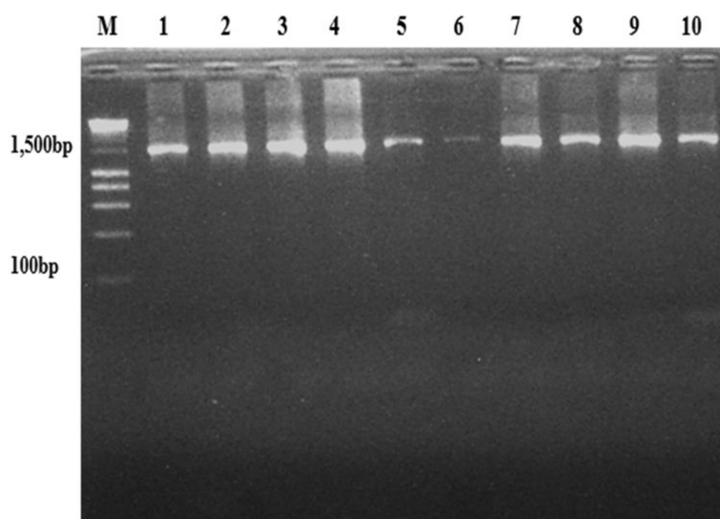
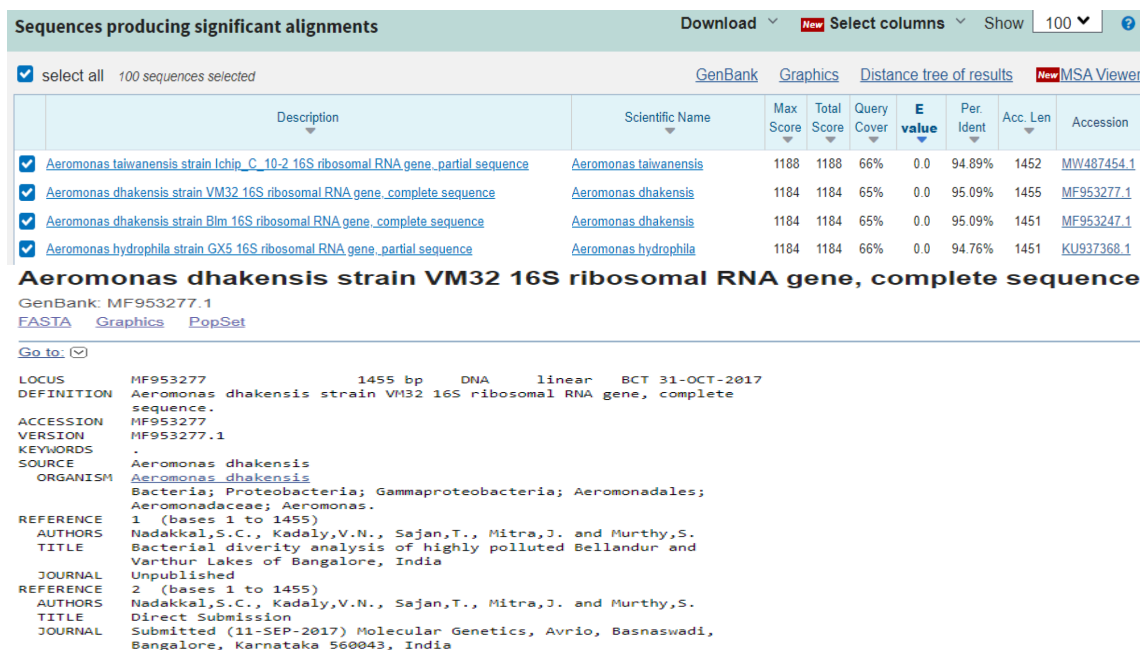


Figure 4: Agarose gel electrophoresis showing the PCR amplification of the 16S rRNA gene from the indigenous isolated bacterial species. M; 100 bp DNA marker 16S rRNA gene amplicon of 1 = *Aeromonas dhakensis*, 2, 3, 4 and 9 = *Pseudomonas* Spp, 5 = *Acinobacter* Spp, 8 and 10 = *Clostridium* Spp, 7 = *Sporanaerobacter* strain.

Insilico analysis of 16S rRNAgene

In order to identify the species of our isolate, the sequence obtained was subjected to an insilico analysis where Uniprot software was used to compare our sequence with sequences previously deposited in the gene bank and National Center for Biotechnology Information (NCBI). The results obtained indicate the highest scores of similarity index of our sequences. Therefore, our isolate are considered and identified based on the BLAST ran on NCBI after comparison of our sequence with other sequences in the database and to get the similarity percentage and query cover as well. Some of the BLAST results are shown on Figures 5 and 6.



Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aeromonas taiwanensis strain Ichip_C_10-2 16S ribosomal RNA gene, partial sequence	Aeromonas taiwanensis	1188	1188	66%	0.0	94.89%	1452	MW487454.1
Aeromonas dhakensis strain VM32 16S ribosomal RNA gene, complete sequence	Aeromonas dhakensis	1184	1184	65%	0.0	95.09%	1455	MF953277.1
Aeromonas dhakensis strain Blm 16S ribosomal RNA gene, complete sequence	Aeromonas dhakensis	1184	1184	65%	0.0	95.09%	1451	MF953247.1
Aeromonas hydrophila strain GX5 16S ribosomal RNA gene, partial sequence	Aeromonas hydrophila	1184	1184	66%	0.0	94.76%	1451	KU937368.1

Aeromonas dhakensis strain VM32 16S ribosomal RNA gene, complete sequence

GenBank: MF953277.1

FASTA Graphics PopSet

Go to:

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DEFINITION  Aeromonas dhakensis strain VM32 16S ribosomal RNA gene, complete
            sequence.
ACCESSION   MF953277
VERSION     MF953277.1
KEYWORDS    .
SOURCE      Aeromonas dhakensis
  ORGANISM  Aeromonas dhakensis
            Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales;
            Aeromonadaceae; Aeromonas.
REFERENCE   1  (bases 1 to 1455)
  AUTHORS   Nadakkal,S.C., Kadaly,V.N., Sajan,T., Mitra,J. and Murthy,S.
  TITLE     Bacterial diverity analysis of highly polluted Bellandur and
            Varthur Lakes of Bangalore, India
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1455)
  AUTHORS   Nadakkal,S.C., Kadaly,V.N., Sajan,T., Mitra,J. and Murthy,S.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-SEP-2017) Molecular Genetics, Avrio, Basnaswadi,
            Bangalore, Karnataka 560043, India
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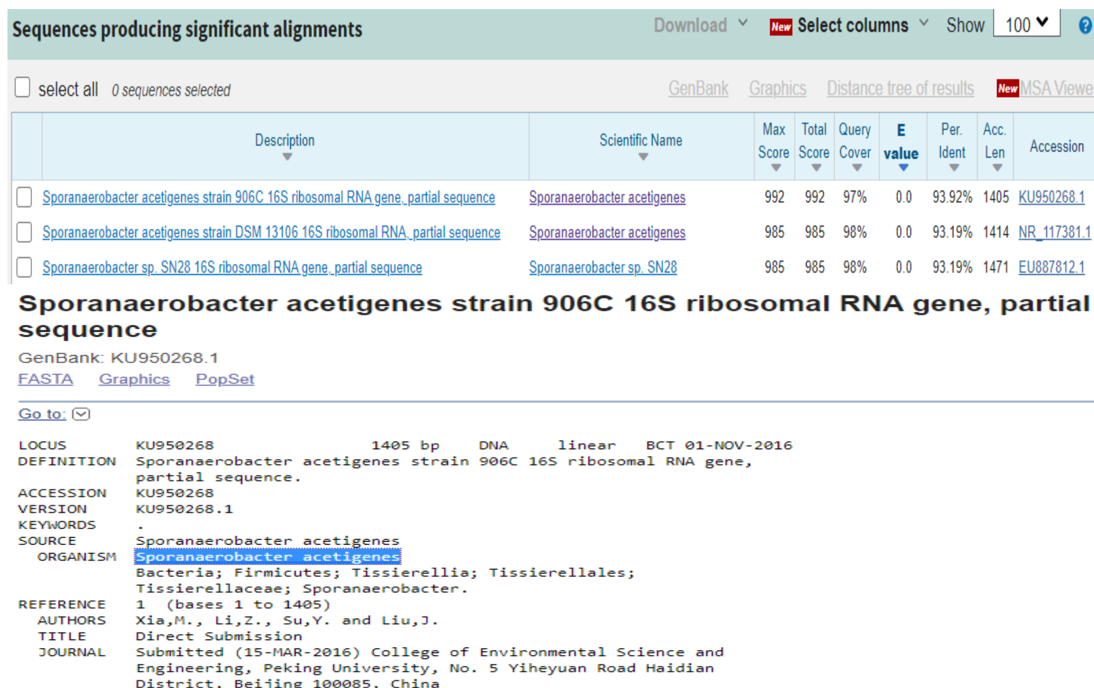
Figure 5: Nucleotide sequence blast *Aeromonas* species partial sequence



Sequences producing significant alignments — Download — Select columns — Show 100

select all 0 sequences selected — GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Sporanaerobacter acetigenes strain 906C 16S ribosomal RNA gene, partial sequence	Sporanaerobacter acetigenes	992	992	97%	0.0	93.92%	1405	KU950268.1
Sporanaerobacter acetigenes strain DSM 13106 16S ribosomal RNA, partial sequence	Sporanaerobacter acetigenes	985	985	98%	0.0	93.19%	1414	NR_117381.1
Sporanaerobacter sp. SN28 16S ribosomal RNA gene, partial sequence	Sporanaerobacter sp. SN28	985	985	98%	0.0	93.19%	1471	EU887812.1

Sporanaerobacter acetigenes strain 906C 16S ribosomal RNA gene, partial sequence

GenBank: KU950268.1

FASTA Graphics PopSet

Go to:

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ACCESSION   KU950268
VERSION     KU950268.1
KEYWORDS    .
SOURCE      Sporanaerobacter acetigenes
  ORGANISM  Sporanaerobacter acetigenes
            Bacteria; Firmicutes; Tissierellia; Tissierellales;
            Tissierellaceae; Sporanaerobacter.
REFERENCE   1  (bases 1 to 1405)
  AUTHORS   Xia,M., Li,Z., Su,Y. and Liu,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (15-MAR-2016) College of Environmental Science and
            Engineering, Peking University, No. 5 Yiheyuan Road Haidian
            District, Beijing 100085, China
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Figure 6: Nucleotide sequence blast *Sporanaerobacter* species partial sequence

After obtaining the above information through basic local alignment search tool (BLAST), the organism is aligned using Three (3) different templates and an Out-group (E. *coli*) in order to get the similarity index to which the organism is more similar to our isolates (Figure 7).

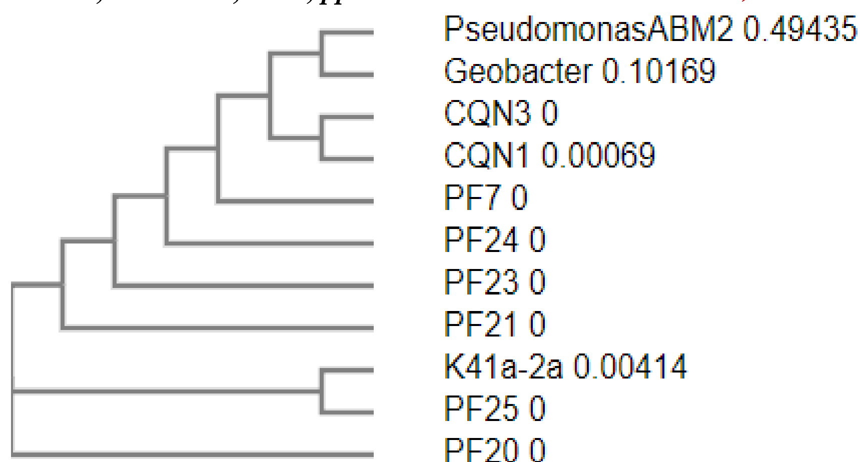

Figure 7. Phylogenetic tree showing evolutionary relationships of the isolates with other related bacteria

Table 3: X-ray fluorescence spectrometry of Kaolin samples

Composition (%)	KABS	KDGK	KGGK	KQKS
Fe_2O_3	0. 610	1.507	0. 352	0. 149
SiO_2	46.553	71.923	64.870	50.831
$A1_2O_3$	37.480	28.600	34.250	37.530
MgO	3.790	3.330	3.960	5.380
P_20_5	0.085	0.140	0.159	0.041
SO_3	0.178	0.162	0.194	0.175
TiO_2	1.386	0.190	0.094	0.012
MnO	0.002	0.019	0.143	0.003
CaO	0.078	0.014	0.134	0.533

CONCLUSION

X-ray Fluorescence spectrometry was used to identify Kaolin prior to isolation and identification of bacteria. It was found to contain many oxides including Iron oxide. The highest oxide was found to be SiO_2.Naturally occurring bacteria from Kaolin samples were successfully isolated. The indigenous bacteria identified in this study includes *Sporanaerobacter* strain906C accession number KU950268.1and*Sporanaerobacter* strain DSM 13106 accession number NR117381.1.*Acinetobacter baumannii* strain NWPKD with accession number MW720652.1. *Clostridium huakuii* strain LAM1030 with accession number NR_134006.1. *Klebsiella pneumonia* strain MS1.5 with accession number KY317923.1.*Aeromonas dhakensis* strain VM32 with accession numbers MF953277.1 and *Aeromonas dhakensis* strain Blm with accession number MF953247.1. *Pseudomonas fluorescens* strain Cr2 with accession number KX589061.1 and *Pseudomonas azotoformans* strain RBP31 with accession number MT556794.1. Among these bacteria identified, *Aeromonas* and *Sporanaerobacter* species were found to be more closely related to the known iron reducing bacteria and may therefore have the potential for bioleaching. Once tested and proved, these bacteria could be employed as robust biological agents for iron reduction.

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