International Journal of Advanced Scientific Research
www.multidisciplinaryarticle.com
ISSN: 2456-0421
Received: 04-11-2023, Accepted: 19-11-2023, Published: 05-12-2023
Volume 8, Issue 2, 2023, Page No. 45-51

Isolation and identification of zinc resistant and isolates from the soil dump side of electronic waste

S Sivanantham[1], B Indhu[2], C Jayalakshmi[3], S.Praveen[4]
[1] Department of Microbiology, Kandaswami Kandar's College, Velur, Namakkal, Tamil Nadu, India
[2] Department of Zoology, Kandaswami Kandar's College, Velur, Namakkal, Tamil Nadu, India
[3] Department of Botany, Government Arts College for Women, Salem, Tamil Nadu, India
[4] Department of Microbiology, Kandaswami Kandar's College, Velur, Namakkal, Tamil Nadu, India

Abstract

The pharmaceutical, food, chemical, metallurgical, and textile production industries are responsible for the release of high volumes of waste into the environment that contains harmful compounds. According to a report on the environment, it is evident that almost seventy percent of the heavy metals that are discovered in landfills originate from industrial plants and electronic waste sites. We took a soil sample from the location where we dispose of metal and electrical debris. The *Bacillus amyloliquefaciens* isolate is one of the most promising bacterial isolates because it has the ability to withstand zinc and make it less effective.

Keywords: Electronic waste soil, *Bacillus amyloliquefaciens*, Zinc

Introduction

The challenges associated with Municipal Solid Wastes (MSW) are especially severe in developing nations. (Gronow *et al.*, (1988), Wilson and Balkau (1990), and UN-DESA (2011) [10, 32, 31]. This is because the socio-economic transformations that have occurred in these countries have not been met with advancements in waste management technologies. Many cities in emerging countries were experiencing environmental degradation and pollution as a result of the increase in the amount of garbage load. During the year 2006, it was anticipated that the total amount of municipal solid waste (MSW) generated worldwide reached 2.02 billion tonnes. This figure represents a 7% annual increase since the year 2003, according to the Global Waste Management Market Report published in 2007. Further, the United Nations Environment Programme (2009) predicted that between 2007 and 2011, the global generation of municipal waste increased by 37.3%, which is equivalent to an increase of almost 8% each year. There is a total of 21275 tonnes of rubbish that is produced every single day in India by the five largest metropolitan cities that are all combined.

Previous research has also confirmed that the levels of heavy metals found in municipal solid wastes are higher than the limitations that have been stated (Merian 1991; Cebula et al., 1995; Ciba *et al.,* 1999; Thangaraj, Sivanantham, 2015) [17, 6, 7, 30]. For instance, Ciba *et al.*, (1999) described that municipal solid waste compost contained trace amounts of cadmium, cobalt, manganese, nickel, lead, and zinc. When the concentration of these elements exceeds a particular threshold, they have hazardous effects on living creatures, but they are unaffected by the decomposition process of organic waste. Some heavy metals are vulnerable to bioaccumulation when the compost from municipal solid waste is utilized as manure. These heavy metals may pose a threat to human health if they are passed to the food chain. It has been suggested that exposure to heavy metals can result in blood and bone diseases, damage to the kidneys, a reduction in mental capacity, and damage to the nervous system (NIEHS, 2002).[19] For this reason, heavy metals require careful consideration prior to the application of compost that is generated from municipal solid waste.

A number of different physical and chemical techniques are available for the removal of heavy metals like zinc from their respective surroundings. However, it has been reported that these methods are not practical because of the high operational costs and the subsequent generation of solid waste, which is difficult to treat and difficult to apply on large surfaces. Furthermore, these methods are financially prohibitive and environmentally ecofriendly because they sometimes require toxic reagents in the process of remediation. A significant number of microbes have been shown to collect high amounts of metals, according to research conducted in recent years (Ramteke, 2000) [22].

Heavy metal resistant bacteria are a superior alternative for heavy metal decontamination, and they have already been effectively employed for such reasons in the industrialized world (Sinha *et al.*, 2009; Sivanantham *et al.*, 2023) [27, 28]. Heavy metals are pollutants that are associated with heavy metals. A number of microorganisms that are able to tolerate zinc have been described. These microorganisms include *Pseudomonas spp.* (Mondaca *et al.*, 1998), [18] *Bacillus spp.* (Camargo *et al.*, 2003) [5] and a number of additional bacterial isolates (Holman *et al.*, 1999; Indhu, *et al.*, 2014) [13, 14].

Microorganisms are essential components for recycling nutrients and heavy metals, which places a persistent strain on the decomposer subsystem. As a result, a wide range of experimental systems and regimes have been explored. There are some of these creatures that have the capability of altering the physicochemical conditions of their surrounding environment. This can be accomplished through detoxification, metal homeostasis, precipitation or solubilization, redox transformations, or metabolic exploitation. (Bruneel *et al.,* 2006; Hetzer *et al.,* 2006; Guine *et al.,* 2007; Indhu *et al.,* 2017) [14, 12, 9, 15].

The conventional chemical and physical treatment technologies have a number of advantages over the bioremediation approaches that are utilized as an in situ treatment (Indhu *et al.,* 2023; Sasikala *et al.,* 2018) [16, 2018]. These advantages are especially beneficial for diluted and extensively disseminated pollutants.

The purpose of this study is to isolate and identify the bacteria that are present at the municipal waste disposal site in the Namakkal area as well as the foundry soil area. In addition to identifying the bacteria physically and biochemically, the potential of the bacteria to resist the heavy metal zinc was also assessed. Additionally, a decrease of zinc was applied to a greater number of possible isolates.

Materials and Methods

Sample Collection

An electronic waste and metal dumping site provided the location for the collection of soil samples. The soil samples were taken from the location where the foundry production was taking place. In total, 4 distinct soil samples were gathered for the purpose of this investigation. The soil samples were all collected in sterile bottles and transferred to the laboratory in a refrigerated environment. Each sample weighed 200 gm.

Determination of moisture content (%) and pH of waste samples

Filter paper was used to store newly acquired samples, and the starting weight of each sample was recorded. After that, these samples were stored in an incubator with heated air at a temperature of 110ºC. The samples were weighed multiple times until a weight that was consistent was reached. With the assistance of a pH meter and a combination glass electrode, the pH was calculated using the electrometric method.

Isolation of bacteria from soil samples

Blanks for sterile dilution were marked in a sequential order, beginning with stock and going from 10-1 to 10-4. Using a new, sterile pipette, one ml of the stock was transferred to the blank for the 10-1 dilution. During each successive stage, one ml of the 10-1 dilution was moved to the 10-2 tube. After that, the 10-2 tube was transferred to the 10-3 tube, and finally, the 10-3 tube was transferred to the 10-4 tube. Following the transfer of 0.1 ml of dilution fluid from each dilution tube into Nutrient Agar culture media, the mixture was then incubated at 37ºC for a period of 24 hours. The culture medium known as Nutrient Agar (NA) was composed of 0.5% peptone, 0.3% yeast extract, 0.5% NaCl, 0.25% glucose, 1.5% agar, and distilled water. The pH was adjusted to 7 at room temperature.

Isolation of Metal tolerance isolates

The agar plate method was utilized in order to investigate the bacterial strains' capacity to tolerate heavy metals, specifically zinc. For bacterial growth, the sterile agar medium was made by adding 10 gm of beef extract, 10 gm of peptone, 5 gm of sodium chloride, 1 gm of glucose, and 20 gm of agar to 1 l of distilled water at a pH of 7.5. Zinc at a concentration of 0.5 mM was introduced into the plates. Utilizing the streak plate approach, the plates were infected with a bacterial suspension. Every single plate was subjected to an incubation period of 48 hours at a temperature of 37±2ºC.

Heavy metal Bioaccumulation assay

The biodegradability test was carried out using a total of six possible isolates in this particular investigation. A shake flask containing LB broth medium was used to culture bacterial isolates for one hour in a rotary shaker at a speed of 150 revolutions per minute. The pH and temperature were kept at 7.0 and 37ºC, respectively. Following the achievement of an optical density (OD) of 0.6, 14 mM of sterile zinc (Zn) was added in a distinct manner to each culture flask, and the flasks were then incubated once more for a period of 24 hours as before. Following that, the entire culture was centrifuged for fifteen minutes at a speed of 5000 rpm. Afterwards, the supernatants were separated and combined to a volume of concentrated HNO3 that was twice as powerful. When the extract was collected into a volumetric flask, it was filtered using Whatman 42 filter paper to eliminate any insoluble material. After that, the extract was diluted.

16S rRNA Analysis

Isolation of genomic DNA

The overnight culture was taken in a micro centrifuge tube with a capacity of 2 ml. It took five minutes to centrifuge the culture at 8000 rpm. After the pellet was suspended, it was mixed by vortexing with 200µl of 1X TE buffer and 100µl of 10% SDS at a concentration of 10%. After keeping the tubes in a water bath at a temperature of 60ºC for 20 minutes, 300 ml of a combination consisting of phenol, chloroform, and isoamyl alcohol (24:25:1) was added to extract the DNA. The mixture was thoroughly mixed by vortexing. The DNA was then pelleted by centrifuging the tubes at a speed of 10,000 rpm for 10 minutes. The pellet was collected, and the supernatant was thrown away immediately after. Following the addition of 200 ml of 70% ethanol to the pellet, it was subjected to centrifugation at a speed of 10000 rpm for a duration of 10 minutes. After the ethanol was entirely drained, the pellet was allowed to air-dry in order to obtain DNA that had been purified. Using tapping, the DNA pellet that had been dried was re-suspended in 20 ml of TE buffer and then dissolved. In preparation for further work, DNA solutions were kept at a temperature of 4ºC.

Methodology for automated DNA sequencing:

The following universal primers were used for the amplification of 16S rRNA gene:

F 5'AGA GTT TGA TCC TGG CTC AG'3 and R 5'ACG GCT ACC TTG TTA CGA CTT'3.

A Big Dye terminator V3.1 cycle sequencing Kit that contained AmpliTac DNA polymerase was utilised in order to carry out the cycle sequencing process. This kit was purchased from Applied Biosystems and has the product number 4337457. In order to prepare the sequencing reaction mix, 1 ml of Big Dye version 3.1, 2 ml of 5x sequencing buffer, and 1 ml of 50% DMSO were added. After adding 4 Pico moles of primer (2 µl) to 4µl of Sequencing reaction –mix, 10 µl of amplified DNA was also contained inside the mixture. Following the loading of the purified reaction onto the 96 capillary ABI 3700 DNA analyzer, electrophoresis was performed for a period of 455 minutes.

Data Analysis

Using the BLASTn programme found in the NCBI, the sequences of the 16S rRNA gene of the isolates were compared to the sequences found in the non-redundant sequences database known as Gene Bank. Multiple sequence alignment was carried out for homologous sequences, and the neighbour joining method was utilised in order to generate a phylogenetic tree.

Results

Physiochemical characterization

In the Namakkal region, a total of four soil samples that included metals were carried out. Numerous levels of wetness are suitable for the growth of bacteria. It was discovered in this research that the moisture content and pH of the soil sample that was collected from Municipality solid waste 1 and 2 sites, as well as foundry soil 1 and 2 sites, were around 54.2%, 55.0%, 51.3, and 53.5% correspondingly. Every sample was subjected to the pH test in this investigation, and the results showed that the pH of the samples ranged from 7.3 to 7.1 to 7.4 to 7.0 table.1.

Isolation of bacterial isolates

Based on the data shown in table 2, the total bacterial counts (TBC) of each soil sample varied from 2.21 x 109 colony forming units (cfu) per gramme of soil to 2.12 x 109 cfu/g of soil to 1.12 x 109 cfu/g of soil to 2.05 x 109 of Municipality solid waste 1 and 2 and foundry soil 1 and 2, respectively.

Isolation of zinc resistance bacterial isolates

All of the isolates were subjected to a zinc-containing medium with a concentration of 0.5 mM in order to isolate zinc-resistant isolates. Only 5 of the 20 isolates were developed on media that included metals. These 5 isolates were designated as S1, S5, A2, G5, and AP4. In this particular investigation, neither *Serratia marcescens* nor *Klebsiella pnemoniae* were able to become established on the medium that contained metals (Table 3).

Heavy metal bioaccumulation assay

A total of two possible isolates, S1 and AP4, were chosen for this investigation on the basis of their zinc tolerance. Atomic absorption spectrometer analysis was performed on the treated samples, and the results were compared to the control in order to determine the total heavy metal (Zn) biodegradation capability. The S1 isolate demonstrated higher levels of activity compared to the AP4 isolate used in the study. 1.2 mM (0.0078 gm) of zinc is degraded by the S1 isolate, as shown in Table 4.

16sRNA sequencing

The BLAST similarity search revealed that the isolate had a maximum score of 2082 bits and a similarity of 98% to the 16S ribosomal RNA gene partial sequence of *Bacillus amyloliquefaciens* strain (Accession no. MH144273.1). This was determined by comparing the isolate to the sequences in the database. The homologous sequences that were found through the BLAST search that demonstrated a connection to *Bacillus amyloliquefaciens* strains were used to generate a distinct phylogenetic tree of the isolate. This collection of findings provides conclusive evidence that the isolate is a member of the *Bacillus cereus* genus. Observations were made in the following sequence.

ACTTGGCTGACATTACTACCCGCGGTTGCTTATACA
TTGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCT
TATGAAGTTAGCGGCGGACGGGTGAGTAACACGTG
GGTAACCTGCCCATAAGACTGGGATAACTCCGGGA
AACCGGGGCTAATACCGGATAACATTTTGAACCGC
ATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCAC
TTATGGATGGACCCGCGTCGCATTAGCTAGTTGGT
GAGGTAACGGCTCACCAAGGCAACGATGCGTAGCC
GACCTGAGAGGGTGATCGGCCACACTGGGACTGAG
ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC
AACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTA
AAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTG
AATAAGCTGGCACCTTGACGGTACCTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGC
GTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATG
TGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGG
AAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTG
GAATTCCATGTGTAGCGGTGAAATGCGTAGAGATA
TGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGT
CTGTAACTGACACTGAGGCGCGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGC
CCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCG
CCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAA
GGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA
TGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CAGGTCTTGACATCCTCTGACAACCCTAGAGATAG
GGCTTCTCCTTCGGGAGCAGAGTGACAGTGTGCAT
GGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCACCCTGATCTAGTGCCAT
CATAAGTGGCACTCTAGTGACTGCGGTGACAACCG
AGAGTGGGATGACGTCAATCATCATGGCCCTATGA
CTGGCTACCACACGTGCTACAATGACGTTACAAAA
GAGCAACCTACGCTAACTT.

Discussion

The heavy metals that are being removed from the contaminated areas are currently being disposed of using a variety of different procedures. Among them, microorganism-based remediation strategies, such as bioremediation, have demonstrated the capability to breakdown and detoxify specific pollutants (Sherameti & Varma 2011). [25] Such treatments include bioremediation.

Microorganisms are the first recyclers in nature. They turn harmful chemical substances into safe products, most commonly carbon dioxide and water. According to Atkinson (1998),[3] the conventional procedures that are often used to remove heavy metals from waste water and contaminated soil include chemical methods such as precipitation and neutralizations, as well as physical methods such as ion exchange, membrane separation, electro dialysis, and activated carbon adsorption. A further point to consider is that these processes could not be viable at low doses. Moreover, these procedures are not only costly but also harmful to the environment.

The present study presents the isolation and characterization of a few bacteria that were isolated from garbage from the municipality that contained soil samples from the Namakkal area. There is a wide variety of moisture levels in which bacteria are able to grow. According to the findings of this research, the moisture content of the soil samples that were obtained from the Namakkal area, as well as those that were gathered from waste dump sites and foundry soils such as metal casting work place, were around 54.2%, 55.0%, 51.3, and 53.5% correspondingly. The bacterial population of different soils has a strong relationship with the amount of moisture that they contain. The highest bacterial density is found in areas with a relatively high moisture content, and the optimal level for the activities of aerobic bacteria is typically between 55% and 75% of the soil's capacity to hold moisture (Amrita *et al.*, 2014).[2]

The microbial populations of each soil sample were measured in this study, and they varied from 2.21 x 109 colony forming units (cfu) per gramme of soil to 2.12 x 109 cfu/g of soil to 1.12 x 109 cfu/g of soil to 2.05 x 109 of foundry soil sample and 2.05 x 109 of municipal waste soil sample, respectively. According to Amir and Pineau (1998) [1], these numbers were also within the range that was reported by other employee.

As Yusof *et al.*, (2009) [33]. point out, the primary source of pollution, particularly that caused by heavy metals, is typically associated with regions that are characterized by high industrial activity and the use of automobiles. On account of the fact that soil organisms are in close proximity to soluble metals for an extended period of time, the soil ecosystems are more susceptible to the negative effects of heavy metal pollution, Shoeb (2006) [26]. Microbial organisms, on the other hand, have developed mechanisms that allow them to tolerate the presence of heavy metals. These mechanisms include the efflux pump, the complication or reduction of metal ions, or the utilization of heavy metals as terminal electron acceptors in anaerobic respiration (Gadds, 1992; Nies and silver, 1995).[8,20] As a result of the fact that the cell is required to accumulate some of these cations at trace levels while simultaneously reducing cytoplasmic concentrations from potential toxic levels, the cell is faced with a unique challenge when heavy metals are resistant to increased or toxic concentrations of essential metals like copper, zinc, nickel, and chromium (Nies, 1992).[21]

A variety of processes are responsible for the poisonous effect that zinc has on bacteria. (Samuel *et al.,* 2013; Sobariu *et al.,* 2016)[23,29] Zinc-resistant bacteria have been utilized for the purpose of bioremediation of contaminated locations. These bacteria are capable of overcoming the harmful effects of zinc (II). Researchers Hansda *et al.,* (2014)[11] found that various metal-resistant PGP Rhizobacteria boost metal absorption efficiency and enhance metal translocation.

Conclusion

The removal of hazardous heavy metals from industrial effluents and heavy metal-contaminated soil can be accomplished with the help of microorganisms, which are the most readily available and cost-effective technique of bioremediation or biodegradation. Microorganisms can also be utilized to remove heavy metals from soil. These dangerous metals are utilized as nutrients by the microbes, which have the ability to either absorb the metals or convert them into forms that are not toxic. *Bacillus amyloliquefaciens* isolate was found to be one of the most promising bacterial isolates that could withstand and lower zinc levels, as demonstrated by the findings of this investigation. Therefore, the discovery of a bacterium that is capable of reducing 22.85% of the initial concentration of zinc without the addition of any carbon source could be of considerable benefit in the process of bioremediation of zinc contaminated soil wastes.

Table 1: Physicochemical analysis of soil

S. No	Sample name	Physicochemical	
		pH	Moisture
1.	Municipality solid waste 1	7.3	54.2
2.	Municipality solid waste 2	7.1	55.0
3.	Foundry soil 1	7.4	51.3
4.	Foundry soil 2	7.0	53.5

Table 2: Determination of total population of municipality waste containing soil samples

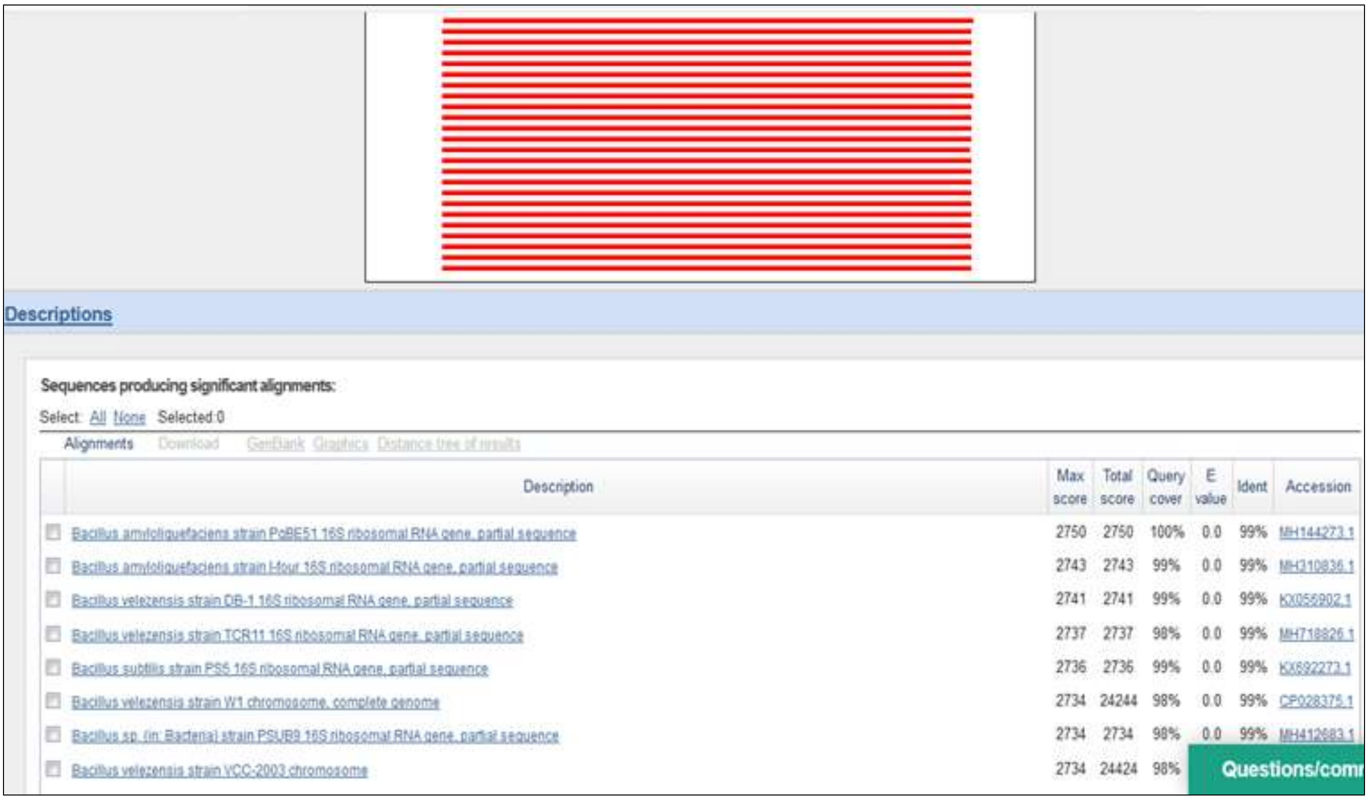
S. No	Sample name	No. of colonies on 10^{-5}	No. of colonies on 10^{-7}
1.	Municipality solid waste 1	TNTC	2.21 X 10^9
2.	Municipality solid waste 2	TNTC	2.12 X10^9
3.	Foundry soil 1	2.86 X 10^7	1.12 X 10^9
4.	Foundry soil 2	TNTC	2.05 X 10^9

Table 3: Isolation of metal tolerance isolates

S. No	Isolates name	Result
1.	S1	+
2.	S2	-
3.	S3	-
4.	S4	-
5.	S5	+
6.	A1	-
7.	A2	+
8.	A3	-
9.	A4	-
10.	A5	-
11.	G1	-
12.	G2	-
13.	G3	-
14.	G4	-
15.	G5	+
16.	AP1	-
17.	AP2	-
18.	AP3	-
19.	AP4	+
20.	AP5	-

Table 4: Heavy metal biodegradability assay

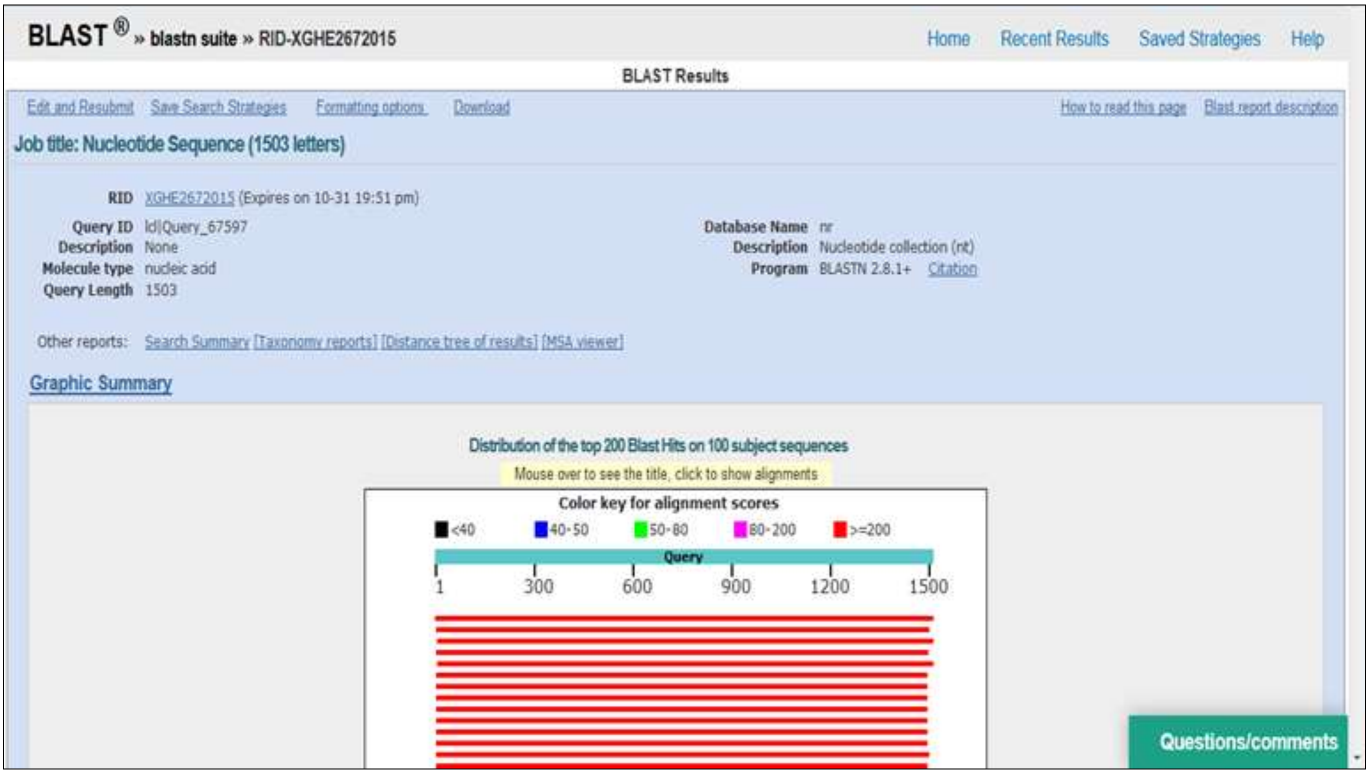
S. No	Isolates name	Initial amount mM (wt-gm)	After process-mM (gm)	Heavy metal utilized mM (gm)	Percentage
	S1	14 (0.091)	12.8 (0.0832)	1.2 (0.0078)	8.57
	AP4	14 (0.091)	13.00 (0.0849)	1 (0.00653)	7.14



Sequences producing significant alignments:

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Bacillus amyloliquefaciens strain PoBE51 16S ribosomal RNA gene, partial sequence	2750	2750	100%	0.0	99%	MH144273.1
☐	Bacillus amyloliquefaciens strain l-four 16S ribosomal RNA gene, partial sequence	2743	2743	99%	0.0	99%	MH310836.1
☐	Bacillus velezensis strain DB-1 16S ribosomal RNA gene, partial sequence	2741	2741	99%	0.0	99%	KX056902.1
☐	Bacillus velezensis strain TCR11 16S ribosomal RNA gene, partial sequence	2737	2737	98%	0.0	99%	MH718826.1
☐	Bacillus subtilis strain PS5 16S ribosomal RNA gene, partial sequence	2736	2736	99%	0.0	99%	KX692273.1
☐	Bacillus velezensis strain W1 chromosome, complete genome	2734	24244	98%	0.0	99%	CP028375.1
☐	Bacillus sp. (in: Bacteria) strain PSUB9 16S ribosomal RNA gene, partial sequence	2734	2734	98%	0.0	99%	MH412683.1
☐	Bacillus velezensis strain VCC-2003 chromosome	2734	24424	98%			

Fig 1: Blast analysis of *Bacillus amyloliquefaciens*



Job title: Nucleotide Sequence (1503 letters)

RID XGHE2672015 (Expires on 10-31 19:51 pm)
Query ID lcl|Query_67597
Description None
Molecule type nucleic acid
Query Length 1503

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.8.1+ Citation

Other reports: Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer]

Fig 2: Phylogenetic analysis of *Bacillus amyloliquefaciens*

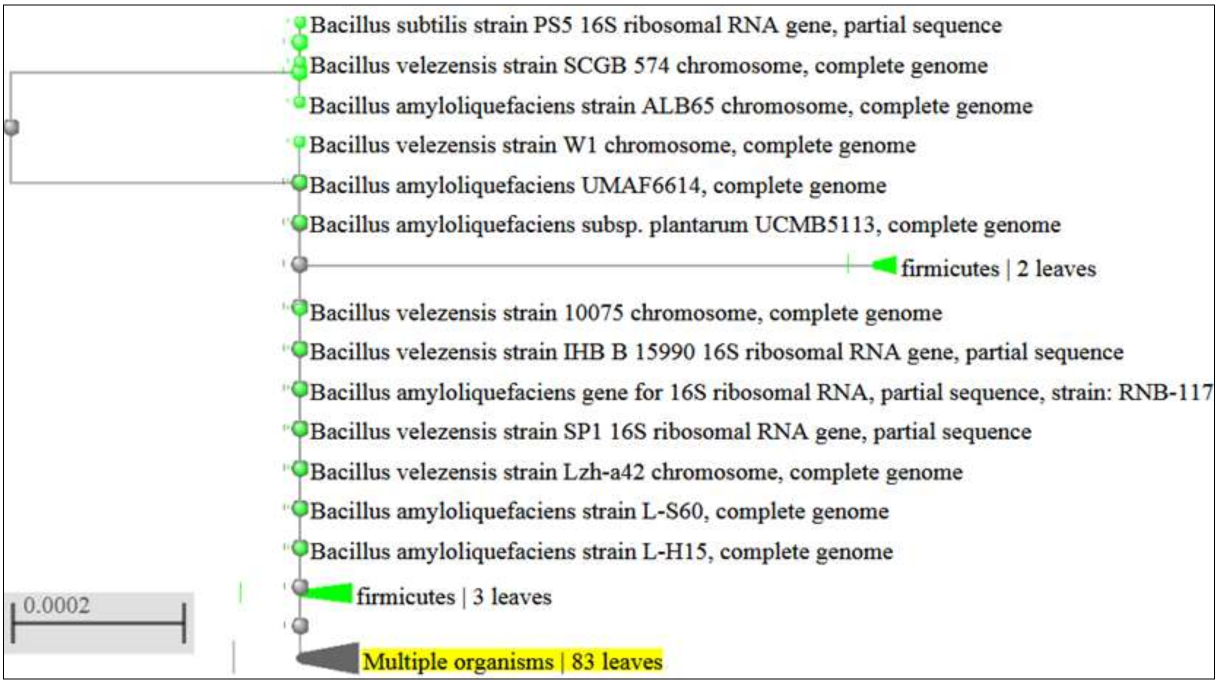


Fig 3

Acknowledgement

The authors would like to use this opportunity to convey their appreciation to the administration of the Department of Microbiology at Kandasawami Kandar's College in Velur, Namakkal, Tamilnadu, India for fulfilling their request to provide the essential facilities.

References

1. Amir H, Pineau R. Influence of plants and cropping on microbiological characteristics of some new Caledonian Ultramafic soils. *Aust. J. soil Res*.1998:36(n3):457-47.
2. Amrita Saha and Subhas Chandra Santra. Isolation and Characterization of Bacteria Isolated from Municipal Solid Waste for Production of Industrial Enzymes and Waste Degradation. *J Microbiol Exp*,2014:1(1):00003.
3. Atkinson, B.W., Bux, F and Kasan, H.C. Consideration for application of biosorption technology to remediate metal-contaminated industrial effluent. Wat. Saf.,1998:24:129-35.
4. Bruneel O, Duran R, Casiot C, Elbaz-Poulichet F, Personne JC. Diversity of Microorganisms in Fe-As-Rich Acid Mine Drainage Water of Carnoules, France. Applied and Environmental Microbiology,2006:72:551-556.
5. Camargo F.A.O., Bento F.M., Okeke B.C. and Frankenbarger W.T. Chromate reduction by chromium resistant bacteria isolated from soil contaminated with dichromate. Journal of Environmental Quality,2003:32:1228-1233.
6. Cebula J, Bodzek M, Loska K. Contaminated Soil, Kluwer Academic Publishers, Dordrecht, 1995, 95.
7. Ciba J, Korolewicz T and Turek M. The occurrence of metals in composted municipal wastes and their removal, Water Air Soil Pollution,1999:111:159-170.
8. Gadds GM. Metals and Microorganisms: A problem of definition. FEMS Microbiol. Lett.,1992:100:197-204.
9. Guine V, Martins JMF, Gaudet JP. Facilitated transport of heavy metals by bacterial colloids in sand columns. Journal de Physique,2003:4(107):593-596.
10. Gronow J, Schofild A, Jain R. Land disposal of Hazardous Waste. Engeneering and Environmental Issues, Ellis Horwood Ltd., Chichester, 1988.
11. Hansda A, Kumar V, Anshumali A, Usmani Z. Phytoremediation of heavy metals contaminated soil using plant growth promoting rhizobacteria (PGPR): a current perspective. *Recent Res. Sci. Technol*,2014:6:131-134.
12. Hetzer A, Daughney CJ, Morgan HW. Cadmium Ion Biosorption by the Thermophilic Bacteria Geobacillus stearothermophilus and G. thermocatenulatus. Applied and Environmental Microbiology,2006:72:4020-4027.
13. Holman HYN, Perry DL, Martin MC, Mcking WR, Huntercwera JC. Real time characterization of biogeochemical reduction of Cr (vi) on basal surfaces by SRFTIR imaging. Geomicrobiology Journal,1999:16:307-324.
14. Indhu B, Muthusami S,Thirunavukkarasu N. “Studies on microflora and their role on egg shell contamination and infection.” International Journal of Pharmaceutical, Chemical and Biological Sciences,2014:4(3):518-521.
15. Indhu B, Muthusami S Thirunavukkarasu N. ”Study of Plankton Diversity of Cauvery River from Jedarpalayam to Velur, Namakkal District,2017:5(1):28-30.
16. Indhu B, Sivanantham S, Thangaraj N, Thirunavukkarasu N. “Effect of different levels of cow manure on phytoplankton productivity in culture.” International Journal of Zoology Studies,2023:8(4):32-35.
17. Merian E. Metals and their compounds in the Environment, VCH, New York, 1991.
18. Mondaca MA, Gonzalez CL, Zaror CA. Isolation, characterization and expression of a plasmid encoding chromate resistance and reduction in *Pseudomonas putida* KT2441. Letters in Applied Microbiology,1998:26:1-10.
19. NIEHS. Source: http://www.niehs.nih.gov/external/faq/landfill.htm 2002.

20. Nies DH, Silver S. Ion efflux systems involved in bacterial metal resistances. Ind Microbiol,1995:14:186-199.
21. Nies DH. Resistance to cadmium, cobalt, zinc, and nickel in microbes. Plasmid,1992:27:17-28.
22. Ramteke PW. Biosorption of nickel (ii) by *Pseudomonas stutzeri*. Environmental Biology,2000:21:219-224.
23. Samuel J, Paul ML, Ravishankar H, Mathur A, Saha DP, Natarajan C. The differential stress response of adapted chromite mine isolates Bacillus subtilis and Escherichia coli and its impact on bioremediation potential. Biodegradation,2013:24:829–842.
24. Sasikala P, Indhu B, Pugazhendy K, Sattanathan G. “Freshwater plankton culture using different organic and inorganic fertilizer.” Indo American Journal of Pharmaceutical Sciences. 2018, 05 (07), 6275-6282.
25. Sherameti, Irena, Varma. Detoxification of Heavy Metals. Springer-Verlag Berlin Heidelberg. 2011, 30.1.
26. Shoeb E. Genetic basis of heavy metal tolerance in bacteria. Pak. Res. Repository,2006:11:389-490.
27. Sinha KR, Valani D, Sinha S, Singh S, Herat S. Bioremediation of contaminated sites: A low-cost nature biotechnology for environmental clean-up by versatile microbes, plants and earthworms. In: Solid waste management and environmental remediation. Timo Faerber and Johann Herzog,2009:4(1):761-3.
28. Sivanantham S, Thangaraj N, Indhu B, C Jayalakshmi C. “Efficacy of *Glycyrrhiza glabra* as an antibacterial agent against isolates that produce biofilm and beta-lactamases.” International Journal of Multidisciplinary Education and Research,2023:8(4):81-86.
29. Sobariu DL, Fertu DIT, Diaconu M, Pavel LV, Hlihor RM, Dragoi EN. Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. N. Biotechnol,2016:10:10-16.
30. Thangaraj N, Sivanantham S. Phytochemical Screening, Characterization, compound identification and separation from *Daucus carota*. INT .JI. Curr. Res. Biosci. Plant. Biol,2015:2(7):168-172.
31. UN-DESA (United Nations Department of Economic and Social Affairs). Trends in solid waste management. Issues, challenges, and opportunities. International Consultative Meeting on Expanding Waste Management Services in Developing Countries (Final Draft), 18-19 March 2010, 2011, Tokyo, Japan.
32. Wilson, D. C. and Balkau, F. Adopting hazardous waste management to the needs of developing countries- an overview and guide to action, Waste Management and Research,1990:8:87-97.
33. Yusuf AJ, Galadima A, Garba ZN, Nasir I. Determination of some Heavy Metals in Soil Sample from Illela Garage in Sokoto State, Nigeria. Research Journal of Chemical Sciences,2015:5(2):8-10.