# ANNEXURES

# Annexure 1A:

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	acterium radiodurans strain K12016 16S ribosomal RNA gene, partial	Customize view	
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LOCUS DEFINITION	MF600628 708 bp DNA linear BCT 12-AUG-2017 Microbacterium radiodurans strain K12016 165 ribosomal RNA gene,	Highlight Sequence Features	
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KEYWORDS SOURCE	Microbacterium radiodurans	Related information	
ORGANISM	Microbacterium radiodurans	Taxonomy	
	Bacteria; Actinobacteria; Micrococcales; Microbacteriaceae; Microbacterium.		
REFERENCE AUTHORS	1 (bases 1 to 708) Chatterjee,C., Sarkar Biswas,S. and Bhattacharyya,R.		G
TITLE	Screening Heavy metal resistance and PGPR like traits of soil	LinkOut to external resources SILVA SSU Database	le le
JOURNAL	bacteria, isolated from Tolly Nullah Unpublished	SILVA GGO Database	[SILV/
REFERENCE AUTHORS	2 (bases 1 to 708) Chatterjee,C., Sarkar Biswas,S. and Bhattacharyya,R.		
TITLE	Direct Submission	Recent activity	
JOURNAL	Submitted (07-AUG-2017) LIFE SCIENCES, PRESIDENCY UNIVERSITY, 86/1 COLLEGE STREET, KOLKATA, WEST BENGAL 700073, India	,	Turn Off Clear
COMMENT	##Assembly-Data-START## Sequencing Technology :: Sanger dideoxy sequencing	Microbacterium radiodurans st	
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EATURES source	Location/Qualifiers 1708	Bacillus xiamenensis strain 26 ribosomal RNA gene, partial se	
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# Annexure 1B:

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ocus	MK353500 1403 bp DNA linear BCT 08-JAN-2019	Pick Primers	
EFINITION	Bacillus xiamenensis strain 1E0018 16S ribosomal RNA gene, partial sequence.	Highlight Sequence Features	
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EXWORDS	MK353500.1	Find in this Sequence	
OURCE	Bacillus xiamenensis		
ORGANISM	Bacillus xiamenensis	Related information	6
REFERENCE	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus. 1 (bases 1 to 1403)	Taxonomy	
AUTHORS	Chatterjee, C., Bhattacharyya, R. and Sarkar Biswas, S.		
TITLE	Analyse chromium tolerant mechanisms of soil bacteria isolated from different sewage sites of kolkata		
JOURNAL	Unpublished	Recent activity	6
AUTHORS	2 (bases 1 to 1403) Chatterjee,C., Bhattacharyya,R. and Sarkar Biswas,S.		Turn Off Clea
TITLE	Direct Submission	📙 Bacillus xiamenensis strain 1E	0018 165
JOURNAL	Submitted (02-JAN-2019) DEPARTMENT OF LIFE SCIENCES, PRESIDENCY UNIVERSITY, 86/1 COLLEGE STREET, KOLKATA, WEST BENGAL 700073, India	ribosomal RNA gene, partial se	equen Nucleoti
OMMENT	##Assembly-Data-START## Sequencing Technology :: Sanger dideoxy sequencing	Bacillus xiamenensis strain 26 ribosomal RNA gene, partial se	
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#### Annexures

# Annexure 1C:

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LOCUS DEFINITION	MK353157 1418 bp DNA linear BCT 08-JAN-2019 Bacillus xiamenensis strain 26K018 165 ribosomal RNA gene, partial	Pic	k Primers	
	sequence.	Hig	hlight Sequence Features	
ACCESSION VERSION	MK353157 MK353157.1	Fin	d in this Sequence	
KEYWORDS SOURCE	Bacillus xiamenensis			
	Bacillus xiamenensis	Pa	lated information	
REFERENCE	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus. 1 (bases 1 to 1418)		conomy	
AUTHORS TITLE	Chatterjee,C., Bhattacharyya,R. and Sarkar Biswas,S. Analyse heavy metal tolerant mechanisms of soil bacteria isolated			
	from different sewage sites of kolkata			6
JOURNAL	Unpublished 2 (bases 1 to 1418)	Re	cent activity	Turn Off Clear
AUTHORS	Chatterjee,C., Bhattacharyya,R. and Sarkar Biswas,S. Direct Submission	冃	Bacillus xiamenensis strain	
JOURNAL	Submitted (02-JAN-2019) DEPARTMENT OF LIFE SCIENCES, PRESIDENCY		ribosomal RNA gene, partia	
COMMENT	UNIVERSITY, 86/1 COLLEGE STREET, KOLKATA, WEST BENGAL 700073, India ##Assembly-Data-START##	Ð	Bacillus xiamenensis strain	
	Sequencing Technology :: Sanger dideoxy sequencing ##Assembly-Data-END##		ribosomal RNA gene, partia	STR. Strengton
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#### Annexure 2:

International Journal of Engineering, Science and Mathematics Vol. 7 Special Issue 4(1), April 2018, ISSN: 2320-0294 Impact Factor: 6.765 ISSN: 2520-0294 impact factor, 6, 65 Journal Homepage: <u>http://www.ijmra.us</u>, Email: editorijmie@gmail.com Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed Listed at: Ulrich's Periodicals Directory C, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A. 04 &

#### Characterize heavy metal tolerant rhizospheric sewage bacteria isolated from Tolly nullah (with special emphasis on strain Microbacterium radiodurans K12016)

#### Chitrita Chatterjee\* Rabindranath Bhattacharyya\*\* Shampa Sarkar (Biswas)\*\*\*

#### Abstract

# ADSITACT Because of rapid industrialization and urbanization, heavy metals are deposited in the environment in frightening quantity. Literature survey manifested, that the heavy metal contaminated soil born microbes can be a potent mode of environmental heavy metal degradation. In the present study, to reveal microbial heavy metal logrant activity thizosperie microbes were isolated from sewage sludge of circular canal (Folly Nullah) and characterize. Microbial heavy metal resistance pattern was checked against four heavy metal salts (CrO), CrO, CdCL and CoCL) individually and in consortium. Plant growth promoting rhizobacterial (PGPR) traits of isolated microbes were evaluated. One interesting isolated strain (Microbacterium radiodurus strain K12016), identified by 16s rDNA, shown total tolerance against 100 mM CrO (Cr<sup>2+</sup>) and also mentionable Cobalt and hexavalent Chromium (Ct<sup>2+</sup>) tolerance, but didn't produce a significant amount of plant growth promoting LAA whereas the most of the rest isolates produced adequate amount of IAA. 16s rDNA of that particular isolate was analyzed and phylogenic tree was constructed using the neighbor-joining method to ensure its taxonomic position. Furthermore experimentation and gene study should require to determining heavy metal resistance mechanism of Microbacterium radiodurums K12016. Heavy Metal tolerance; Biochemical analysis: Antibiotic resistance; Indole Acetic Acid (IAA);

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#### Author correspondence:

Chitrita Chatterjee,

Keywords:

16s rDNA

PhD Student, Department of Life Sciences, Presidency University, 86/1 College Street, Kolkata, West Bengal, India Email: chitrita87@gmail.com; chitrita.rs@presiuniv.ac.in

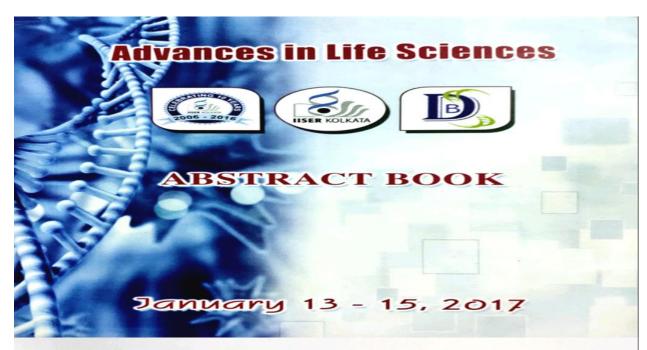
#### 1. Introduction

As a result of different unrestricted anthropogenic activities, different concerning heavy metals like Chromium (Cr), Lead (Pb), Cadmium (Cd), Cobalt (Co), Nickel (Ni), Iron (Fe) are alarmingly increased in the environment, particularly the areas where waste materials from different industrial sectors, tannery,

#### <u>Annexure 3A:</u>



#### Annexure 3B:



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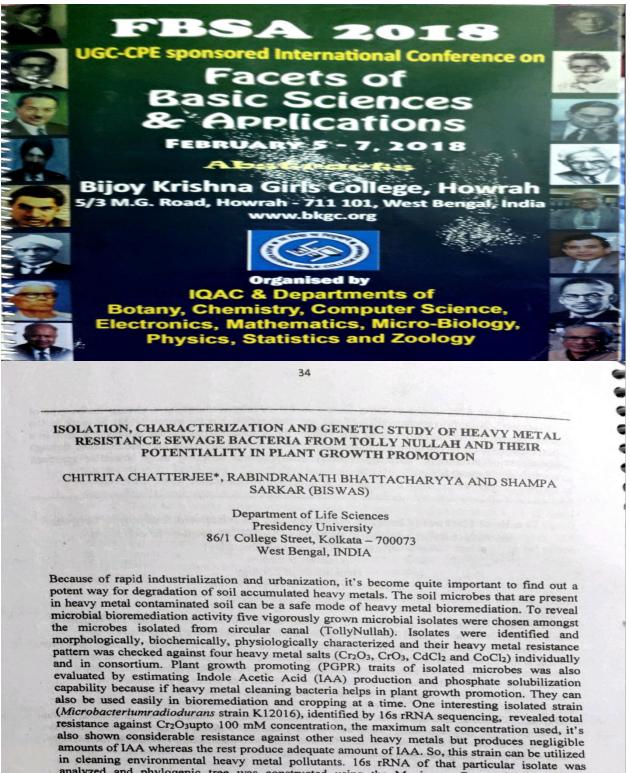
#### Isolation and partial characterization of heavy metal resistance sewage bacteria from tolly nullah and their potential in the plant growth promotion

Chitrita Chatterjee, Rabindranath Bhattacharyya and Shampa Sarkar (Biswas)

Department of Life Sciences Presidency University, Kolkata

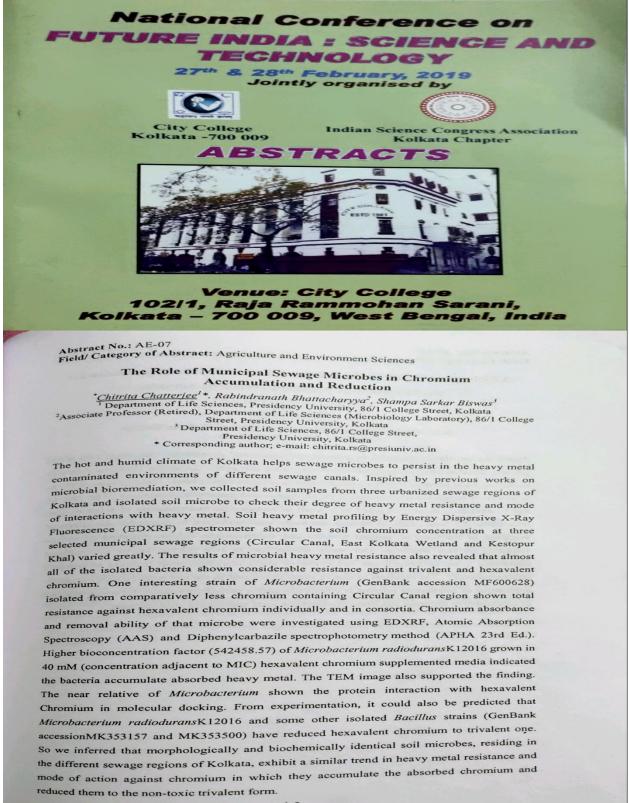
In the present circumstances, it's quite important to find out a potent technique for degradation of soil accumulated heavy metals. The soil microbes that are present in heavy metal contaminated soil can be a safe mode of heavy metal bioremediation. To reveal microbial bioremediation activity primarily chosen five vigorously grown microbial isolates amongst the microbes isolated from circular canal (Tolly Nullah), identified them biochemically and their heavy metal resistance pattern was checked against four heavy metal salts ( $Cr_2O_3$ ,  $CrO_3$ ,  $CdCl_2$  and  $CoCl_2$ ) in different concentrations (10 nM, 10  $\mu$ M, 10 mM, 50 mM and 100 mM). Biochemical characterization was evaluated by estimating Amylase, Catalase, Gelatinase production and mixed acid fermentation capability. Plant growth promoting potential of isolated microbes was also evaluated because if heavy metal cleaning bacteria helps in plant growth promotion also they can be used easily in bioremediation and cropping at a time. PGPR like traits were measured by estimating Indole Acetic Acid (IAA) production and phosphate solubilization capability. One isolate showed high susceptibility against up to 10 mM of CdCl2 and other interesting isolate (shown similarity with Microbacterium arborescens as per NCBI BLAST result) revealed total resistance against Cr2O3 till 100 mM concentration, the maximum salt concentration used, but produce negligible amounts of IAA whereas the rest produce adequate amount of IAA. 16s rRNA of one isolate was sequenced and analyzed. Further experimentation and gene study should be needed to conclude any role of isolated microbes in heavy metal bioremediation and plant growth promotion.

Annexure 3C:



analyzed and phylogenic tree was constructed using the Maximum Composite Likelihood method to calculate evolutionary distances. In silico analysis of different microbial heavy metal attaching regions and degrading pathways is also helpful to predict bacterial signal transduction in response to heavy metal. Furthermore experimentation and gene study should require to standardizing bacterial heavy metal resistance pattern.

#### Annexure 3D:



#### Annexure 3E:



The Role of Heavy Metal Resistance Indole Producing Municipal Sewage Microbes in Plant Heavy Metal Absorption and Accumulation

<sup>1\*</sup>Chitrita Chatterjee, <sup>2</sup>Rabindranath Bhattacharyya, <sup>2</sup>Shampa Sarkar Biswas <sup>1</sup>Department of Life Sciences, Presidency University, Kolkata, <sup>2</sup>Department of Life Sciences, Presidency University, Kolkata

The hot and humid climate of Kolkata helps sewage microbes to persist in the heavy metal contaminated environments of different sewage canals. Chromium resistant sewage microbes isolated from slightly alkaline sewage soil of different municipal sewage, showed Chromium absorption and accumulation. Chromium absorbeance capability of microbes was investigated using Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer and Diphenylcarbazile spectrophotometry method (APHA 23rd Ed.). Molecular characterization of the microbes shown that most of them are different species of Bacillus (GenBank accession numbers JN392001–JN3920013, MK353157, MK353500). Only one interesting Microbacterium species (GenBank accession MF600628) was identified which shown considerable resistance against hexavalent Chromium. Higher (542458.57) microbial bioconcentration factor of Microbacterium radiodurans K12016 grown in 40 mM (concentration adjacent to MIC) hexavalent Chromium supplemented media indicated the bacteria accumulate absorbed heavy metal. The near relative of Microbacterium shown the protein-ligand interaction with hexavalent Chromium in molecular docking. But the Microbacterium didn't produce a significant amount of Indole compound whereas HPTLC analysis showed most of the other strain isolated from Kestopur and East Kolkata Wetland produce plant growth promoting hormone IAA and IBA. But Plants grown in heavy metal contaminated regions absorb metal from rhizospheric soils; through food chain the absorbed metal reached to human being, magnified and affect human health. Instead of the alarming soil Chromium concentration, the considerably low Plant bioconcentration factor, BCF (East Kolkata Wetland, Circular Canal and Kestopur Khal were 0.036, 0.028 and 0.085) respectively indicated that they couldn't absorb a significant amount of soil metal. Less than one bioconcentration factor also indicates that the plant didn't accumulate absorbed metal within the cell. Then it could be predicted from the analyzed data that the soil microbes play a remarkable role in plant Chromium absorption control.

Keywords: EDXRF; HPTLC; docking; BCF

### Annexure 3F:



Abstract under theme: Environmental Economics

# *Microbacterium radiodurans* and *Bacillus xiamenensis* isolated from different sewage microbial communities of Kolkata, perform chromium bioremediation and cropping

Chitrita Chatterjee<sup>\*</sup>, PhD Student; Shampa Sarkar (Biswas)<sup>\*\*</sup>, Assistant Professor; Rabindranath Bhattacharyya<sup>\*\*\*</sup>, Professor (Retired) Presidency University, 86/1, College Street, Kolkata 700073 \*Presenting Author, Email: <u>chitrita87@gmail.com</u>, chitrita.rs.presiuniv.ac.in, Phone: 9830935178 \*\*Email: <u>shampa.dbs@presiuniv.ac.in</u>, Phone: 9903870923 \*\*\* Email: <u>rabindranathbpc@gmail.com</u>, Phone: 9433050316

The amount of dissolved oxygen (DO) and chemical oxygen demand (COD) of different sewage sites revealed that Kolkata's sewage sites studied herein are highly polluted with organic materials, but have not yet crossed the borderline, which is why different pollutant tolerant plants, animals, and microbes are still able to grow and survive.

The chromium resistant aerobic microbes isolated from the heavily polluted rhizospheric wetland soils of Kolkata could accumulate absorbed Cr(VI) within their cells and converted them to less hazardous Cr(III). 16S rRNA based analysis exhibited that different species of *Microbacterium* and *Bacillus* were highly abundant in chromium contaminated regions. Sequence comparison study displayed that different geochemically important chromium tolerant and chromium resistant genes which translate numerous stress-induced proteins were native in *Microbacterium* and some species of *Bacillus*. Soil microbes can bind heavy metals in the soil by producing siderophores and microbial acids. Additionally, the microbial isolates were found to produce the plant growth promoting hormones like IBA and IAA which alters the endogenous IAA pool in plants and increases the length and surface area of plant roots.

The isolated chromium removing, eco-friendly strains have a great impact on agro-economic society as they could be applied to eliminate environmental chromium, as well as improve plant growth in heavy metal contaminated, poorly cultivable soil and decrease the risk of metal accumulation and biomagnification by the edible plants that are consumed regularly. Further field application should be required to clarifying their bioremediation and plant interaction strategies.

# Annexure 4A:

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Director, BSI	Convener	Convener	Convener & HoD

## Annexure 4B:



# <u>Annexure 4C:</u>

# Bijoy Krishna Girls' College, Howrah 5/3 M.G. Road, Howrah - 711 101, West Bengal, India www.bkgc.org





UGC-CPE sponsored International Conference on Facets of Basic Sciences & Applications (FBSA)

Organised by IQAC & Departments of Botany, Chemistry, Computer Science, Electronics, Mathematics,Micro-Biology, Physics, Statistics and Zoology



Certificate of Participation

Certified that Ms. Chitrita Chatterjee



Presidency University, Kolkata

of



has attended and presented a paper entitled "ISOLATION, CHARACTERIZATION AND GENETIC STUDY OF HEAVY METAL RESISTANCE SEWAGE BACTERIA FROM TOLLY NULLAH AND THEIR POTENTIALITY IN PLANT GROWTH PROMOTION"

in the Conference held on February 5 - 7, 2018

Blanda

Convenor FBSA 2018

anising Secretary

FBSA 2018

Sweta Juha Coordinator

IQAC

Principal BKGC & **Conference** Chair **FBSA 2018** 











#### Annexure 4D:



# Annexure 4E:

National Conference on           Future India: Science and Technology
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27th & 28th February, 2019
Jointly Organised by
City College Indian Science Congress Association
Kolkata - 700 009         Venue         Kolkata Chapter           www.sciencecongress.nic.in         City College         www.sciencecongress.nic.in
102/1 Raja Rammohan Sarani, Kolkata – 700 009, West Bengal, India
This is to certify that Mr. Ms. Chitrita Chatterfee of Prusidency University participated &
of Presidency University participated & presented a paper in the National Conference on 'Future India: Science and
Technology' on 27th and 28th February, 2019 held at City College, Kolkata - 700 009.
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Prof. Tushar Kanti Ghosh, Dr T. K. Ghosh & Dr K. K. Chaki Dr S. P. Chattopadhyay Convener Jt. Organizing Secretary Principal, City College, Kolkata ISCA, Kolkata Chapter

## Annexure 5:

WEB 2020 <web2020zoology@gmail.com>

Mar 25, 2020, 8:46 PM 🏠 🔦

to me 👻 Dear Madam.

Thank you for sending the abstract entitled "Microbacterium radiodurans and Bacillus xiamenensis isolated from different

#### sewage microbial communities of Kolkata, perform chromium bioremediation and cropping".

We are to inform you that as per the present situations and Govt. Directives the conference has to be postponed until the situations normalize. We will contact the speakers once our college opens and as they confirm their availability to deliver the talk, we will intimate the revised dates to all participants and paper presenters. Please wait for some time and be safe.

Best regards,

WEB-2020 Organizing Committee

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