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In silico Analysis of Copper Nanoparticles Synthesizing Bacteria Contributing Towards Big Data Bank

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A B S T R A C T

16S rRNA gene sequences are most commonly used for determination or studying the bacterial phylogeny and taxonomy because its present in almost all bacterial population. 16S rRNA gene sequence is remaining conversed during evolution so that 16S rRNA gene identification method is widely used for identification of bacterial diversity. In this study, bacterial population was isolated from soil sample for copper nanoparticles synthesis and maximum copper synthesizing bacteria further used for characterization. Bacteria firstly characterized by using microscopic and biochemical characters and then confirmed by using 16S rRNA gene technology. In this experiment, we have used blastn, Cluster W, MEGA6.0 software to find homology and phylogenetic analysis to identification of bacterial isolate. We have identified *Stenotrophomonas maltophilia* strain SCS1.1 with a noble trait such as to synthesizing copper nanoparticles.

Introduction

Isolation of bacteria from different kinds of environments such as soil, water, hot water springs, saline conditions, humid areas, dry and temperate regions is very important to study the new properties of microbial population. Populations in microbial communities or ecosystem may interact and cooperate to obtain nutrients from environment and allows waste products from one group of microbes to serve as nutrients for another. Microorganisms are known to be capable of synthesizing inorganic molecules that are deposited either intracellularly or extracellularly (Plaza *et al.*, 2014). Calamity of environment led to the survival of only those organisms which have meticulously adapted to the survival strategies designed by nature. The efficient natural nanomachineries in form of enzymes, organic molecules and the intracellular or extracellular shuttlers have been endowed with special characteristics for survival. Toxic metals spilled off by human civilization are contaminating the elixir of life i.e. water. Soil also cannot escape this

cataclysmic contamination (Wang *et al.,* 2011). Eradication of the toxicity of metals is the only other alternative for biological system to survive. Hence, living organisms with their proficient nanomachineries transform toxic metal ions into non toxic ones (Lu *et al.,* 2006). Copper nanoparticles synthesizing bacteria have been isolated from different sources such that as specific mines, municipal waste, marine samples etc.

In the last decade, sequencing of 16S rRNA gene and comparison between 16S rRNA gene used and phylogeny has confirmed (Spiegelman *et al.,* 2005). Advances in computational biology and bioinformatics has been remarkable in the last few decades, that established large scale sequencing, structure and function determination, gene prediction and specific landmarks on the genome as well as proteome analysis on strong foundations.

NCBI and EMBL are various online service used when homologous sequences are to be compared which are essential for further analyses. Use of these tools in multiple sequence computational phylogenetic analysis, multiple sequence alignment and proteomics has been carried out. Keeping in view above considerations, the present study was designed for isolation, biochemical characterization and molecular identification of copper nanoparticles synthesizing bacteria by use of 16S rRNA technology to determine evolutionary rates of different bacterial isolate and close match between them by using various bioinformatics.

Materials and Methods

Sample collection for isolation bacteria

A survey was conducted for selection of various sites of Solan District of Himachal Pradesh for the collection of samples. Only one site was selected for isolation of bacterial population for synthesis of copper nanoparticles. Different samples such as soil, pebbles and rock matting were collected from selected site in sterilized containers.

Isolation and screening of copper nanoparticles synthesizing bacteria

Nutrient agar enriched with 2mM CuSO4 solution using standard pour plate method (David *et al.,* 2014) by incubation at 37º C for 24 hrs. Copper nanoparticles synthesizing bacterial isolates were characterized morphologically and biochemically and confirmed using PCR- 16S rDNA technology.

DNA isolation and PCR amplification of rRNA gene

Genomic DNA of selected bacterial isolate was extracted using Genomic DNA extraction Mini kit (Real Genomics) used as template for amplification of the 16S *rrna* gene using universal primers for 16S *rrna* gene of bacteria. Amplifications were performed using thermal cycler and with a temperature profile standardized for 16S *rrna* gene amplification. PCR amplification was carried out in 0.2 ml PCR tubes with 20 µl reaction volume containing 5U/reaction of Taq DNA polymerase, 10X PCR buffer with 1.5 mM MgCl2, 10 nmol/reaction primers, 0.5mM deoxynucleotide triphosphate (dNTPs) and template DNA. Initial cycle of 3 min at 95°C followed by 35 cycles of 30 sec at 95°C, annealing temperature of 50°C for 30 sec, elongation step of 2 min at 72°C and a final extension step of 10 min at 72°C.

Gel electrophoresis

One percent agarose was used for analysis of PCR products (GeNei, Bangalore, India) dissolved in 1X TAE buffer containing (10 mg/ml) ethidium bromide and images were taken through Gel Documentation Unit

(Syngene, UK). Size of the amplified products was determined by 100bp standard molecular weight ladder or markers (GeNei, Bangalore, India). The selected bacterial isolate were further characterized using 16S *rrna* gene technology and genomic DNA extracted from these isolates were selectively amplified using PCR technology with Universal primers B27F and U1492R for 16S *rrna* gene were used.

16S rrna gene Sequencing

The 16S rRNA purified PCR products (100ng concentration) were subjected to sequencing using the chain termination method developed by Sanger and his coworkers in 1977 (Applied Biosystem Inc). Sequencing of 16S *rrna* gene fragments of selected bacterial isolates was done from both forward and reverse directions. The selected sequences obtained were subjected to BLASTn search to find homology. The percentages of sequence matching were also analyzed and the sequences were submitted to NCBI-Gen Bank and accession numbers were obtained for the same.

Results and Discussion

Bacterial identification is more accurate identified using molecular method as compared to the traditional techniques including morphological and biochemical characters. In recent times comparison of the bacterial 16S *rrna* gene sequence which are conserved in nature has emerged as a preferred genetic technique. Total 49 bacterial isolates were obtained only single isolate SCS1.1 synthesis maximum copper nanoparticles (Fig. 1). Morphologically and biochemically SCS1.1 identified as gram-negative bacteria. Total genomic DNA of selected bacterial isolates was extracted successfully then amplified using PCR technology and were characterized using 16S *rrna* gene technology. After 35 cycles of PCR amplification, universal primers for 16S *rrna* gene were able to successfully amplify 16S *rrna* gene and produced an amplicon of expected size i.e. 1500 On the basis of results obtained from 16S *rrna* gene analysis. On the basis of results obtained from 16S *rrna* gene analysis and in addition to G+C content analysis (Table 1), the selected bacterial isolate were found to belong to genera *Stenotrophomonas*. Further *in silico* analysis pertaining to the sequence, so obtained, was carried out using various bioinformatics tools available online. Analysis of 16S *rrna* gene of the selected bacterial isolates revealed homology with various other 16S *rrna* gene sequences. BLASTn search of selected bacterial sequences with the most similar 16S *rrna* gene sequences of the GenBank database (http://www.ncbi.nlm.nih.gov/blast) revealed the closest sequence identities from the sequence database (Marchler– Bauer *et al.*, 2000; Pruitt *et al.*, 2005).

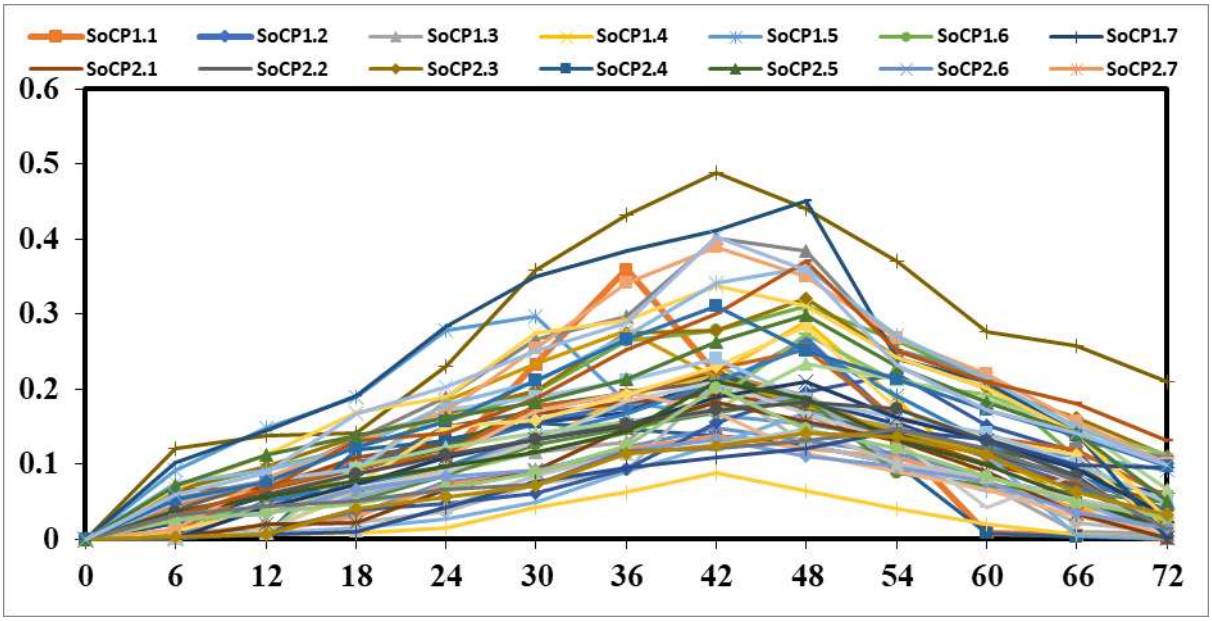
Table.1 Nucleotide base composition in the query sequence (SCS1.1 isolate)

Nitrogenous Base	**Nucleotide Count**	
	Total	**Percentage (%)**
Adenine (A)	270	**20**
Thymine (T)	321	**27**
Cytosine (C)	402	**30**
Guanine (G)	310	**23**
G+C	711	**54.62**
A+T	**592**	**45.40**

Table.2 Percent homology of nucleotide query sequence of SCS1.1 with other nucleotide sequences present in the database using BLASTn analysis

Accession Number	Closest match	Length (bp)	Query cover	Per cent Similarity
LN558615.1	*Stenotrophomonas maltophilia* isolate AAIII-2	1430	99%	97%
MH703447.1	*Stenotrophomonas* sp. strain Gamma-16	1481	95%	98%
MF942711.1	Uncultured bacterium clone 1_deep	1512	95%	98%
CP022053.2	*Stenotrophomonas maltophilia* strain FDAARGOS_325 chromosome, complete genome	4851512	95%	98%
MF354012.1	*Stenotrophomonas maltophilia* strain KUGK130 16S	1475	95%	98%
KY908461.1	*Stenotrophomonas* sp. strain M5	1320	95%	98%
KY407758.1	Uncultured *Stenotrophomonas* sp. clone KR 245	1445	95%	98%
KT034431.1	*Stenotrophomonas* sp.	1480	95%	98%
KT580582.1	*Stenotrophomonas maltophilia* strain CanL-56	1506	95%	98%
KP790032.1	*Stenotrophomonas maltophilia* strain SBB17	1492	95%	98%
KF059260.1	*Stenotrophomonas* sp. Db-1	1537	95%	98%
KC894543.1	Uncultured bacterium clone H96	1525	95%	98%
KC871534.1	*Pseudomonas* sp. An 18	1505	95%	98%
JQ291604.1	*Pseudomonas hibiscicola* strain HPG72	1507	95%	98%
JN644502.1	*Stenotrophomonas maltophilia* strain ROI_3A	1476	95%	98%
FR853777.1	Uncultured bacterium- clone 1661	1512	95%	98%
FR853763.1	Uncultured bacterium -clone 1643	1510	95%	98%
HQ407233.1	*Stenotrophomonas maltophilia* strain E56	1511	95%	98%
GU564359.1	*Stenotrophomonas* sp. Bt-45	1453	95%	98%
GU391033.1	*Stenotrophomonas maltophilia* strain CCF0025	1497	95%	98%
FJ193149.1	Uncultured *Stenotrophomonas* sp. clone GI6-10b-C05	1503	95%	98%

Fig.1 Screening of copper nanoparticles synthesizing bacterial isolates from Solan District



The percentages of sequence matching were also analyzed. The 16S *rrna* gene sequence analysis showed 97% similarity with *Stenotrophomonas maltophilia* isolate AAIII-2 (Table 2) and the sequence was submitted to NCBI-Gen Bank and accession numbers was MK183005. Similarly, 16S rRNA gene technology along with *in silico* analysis have been successfully used to identify metal nanoparticles synthesizing bacterial isolates, *Bacillus cereus* strain CS11 (Das *et al.*, 2014), *Stenotrophomonas maltophilia* strain OS4 (Oves *et al.*, 2013), *Bacillus pumilis* (Modi *et al.*, 2015) and *Bacillus subtilis* (Bhuvaneswari *et al.*, 2016). copper nanoparticles synthesizing bacteria has been isolated and identified by Kaur *et al.*, (2015) and Tiwari *et al.*, (2016) as *Kocuria flava* and *Bacillus cereus* respectively using 16S rRNA technology.

In conclusion, it has been found that isolate SCS1.1 which was isolated from Solan Chambaghat Soil, have maximum ability to synthesized copper nanoparticles. SCS1.1 isolate found to be gram negative by morphological and biochemical characteristics. In this study , universal primers B 27F 5́ -AGAGTTTGATCCTGGC TCAG-3́ and U1492R 5`-GGTTACCTTG TTACGACTT-3́ with an annealing temperature of 50℃ produced an amplified product near 1500 bp of 16S *rrna* gene of selected bacterial isolate. Molecularly SCS1.1 isolate partially identified as *Stenotrophomonas maltophilia* which show 97% similarity with *Stenotrophomonas maltophilia* isolate AAIII-2. In this study, this bacteria was reported first time in India for synthesis of copper nanoparticles.

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