# STUDIES ON TOLERANCE AND BIOTRANSFORMATION OF TELLURIUM BY SOIL BACTERIAcertificates

*Submitted in the partial fulfilment for the award of the degree of*

*Master of Science in Microbiology*

**By**

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**July 2013**

# Certificate

This is to certify that the thesis entitled, "**Studies on tolerance and biotransformation of Tellurium by soil bacteria**" submitted by Harneet Kaur in partial fulfillment of the requirement for the award of the degree of Master of Science in Microbiology, to Thapar University, Patiala, is an authentic record of her own work carried out by her during the period of six months from January 2013 to July 2013, under my supervision and guidance. This report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

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# Candidate's declaration

I hereby declare that the work which is being presented in the dissertation entitled "**Studies on tolerance and biotransformation of tellurium by soil bacteria**" in the partial fulfillment of the requirements for the award of degree of Master in Science in Microbiology from Department of Biotechnology and Environment Sciences, Thapar University, Patiala is an authentic record of my own work during a period of six months from January 2013 to June 2013, under the supervision of Dr. N. Tejo Prakash, Professor, School of Energy and Environment, Thapar University, Patiala. I have not submitted the matter embodied in this dissertation for the award of any other degree or diploma.

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This is to certify that the above statement given by the above candidate is correct and true to the best of my knowledge.

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*Harneet Kaur*

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# Summary

Nanotechnology is the emerging field in science and technology and is poised to bring in revolutionary changes across all spheres of life. It deals with the nano-meter sized objects. Nanotechnology can be applied across a wide variety of fields, right from medicine, textile, and education to defense and manufacturing. Nanomaterials are the leading edge of the rapidly developing field of nanotechnology. A decade ago, nanoparticles were studied because of their size-dependent physical and chemical properties. Now they have entered a commercial exploration period.

To avoid the use of toxic chemicals in the synthesis of nanoparticles there is a growing need to develop environmentally sound nanoparticle synthesis process that do not contain toxic chemicals. Biological systems, masters of ambient condition chemistry, synthesize inorganic materials that are hierarchically organized from the nano to macroscale. The use of microorganisms in synthesis of nanoparticles is very exciting area of research with considerable potential for development.

The use of bacteria to synthesize metal nanoparticles is not new but the rational biosynthesis strategy providing avenues for further more research as demonstrated by recent literature.

The work resulted in (a) determining the tolerance of the test bacterial strains to various concentrations of tellurium which indicated their tolerance up to 700µM of tellurium; (b) generation of nanoparticles in by the bacterial cultures; (c) characterization of resulting nanoparticles using differential light scattering and X-ray diffraction; and (c) evaluation of antimicrobial activity of synthesized nanoparticles.

# *Chapter 1*

# *Introduction*

# 1.0 Introduction

Nanotechnology has become a popular term representing the main efforts of science and technology. The field of nanotechnology has recently witnessed spectacular advances in the methods of nanomaterial fabrication and the utilization of their exotic physicochemical and optoelectronic properties. In its original sense, 'nanotechnology' refers to the projected ability to construct items using techniques and tools being developed today to make complete, high performance products. This field represents a rather broad interdisciplinary field of research and industrial activity involving particles less than 100 nanometers (nm) in diameter.

Numerous studies have been reported for the synthesis of nanoparticles of different size and shape. Engineered materials made of such small particles exhibit novel properties that are distinctively different from their conventional forms and can affect their physical, chemical, and biological behavior. These nanoscale particles can be tubular, spherical, irregularly shaped, and may also exist in aggregated formations. Both chemical and biological methods play a role in the synthesis of nanoparticles.

Ever-increasing pressure to develop environmentally benign nanoparticle synthesis has lead to a renewed interest in biotransformations as a route to growth of nanoscale structures. Many biological entities, such as bacteria, yeast, fungi etc., play an important role in the synthesis of nanoparticles.

Nanoparticles have different applications in different fields. In biology or medicine, there is an important role of nanoparticles such as in drug delivery, fluorescent biological labels, tissue engineering, probing of DNA structure, detection of proteins etc.

Nanoparticles are also used in environment, pharmaceutical, energy, cosmetics and biomedical field. Gold nanoparticles can be used in sterile cloths. They can be used in biomedical field. Have applications in optics, photonics and optoelectronics. Silver nanoparticles synthesized from fungus and bacteria have a number of applications in industries and in case of biolabelling. Selenium nanoparticles used as a new carrier for horseradish peroxidase to construct $H_2O_2$ biosensors.

A recent discovery of tellurium nanoparicles also has many applications like in electronics, mining, industries, textiles and in optics. Tellurium has many applications in medical field. The field of nanotechnology has an impressive development in the synthesis of tellurium nanoparticles.

Tellurium is a natural occurring element. The word tellurium comes from the latin word "tellus", which mean "earth". It was discovered by F.J. Mueller von Reichenstein in 1782 in a mineral containing tellurium and gold. Tellurium belongs to the group of chalcogens, which also includes the oxygen, sulphur, selenium and polonium. The rare element, Tellurium, has been regarded as a toxic element with symbol Te and atomic number 52. The compounds of Te are highly toxic. The gold telluride minerals are the most notable natural gold compounds. However, they are not a commercially significant source of Te itself, Tellurium is found most commonly as a byproduct from the electrolytic refining of copper.

The elemental and non-toxic form of tellurium is very rare. It is mainy found in its toxic form. It exists in nature in tellurite and tellurate form. These Tellurium salts are considered highly toxic towards many micro-organisms at concentration as low as 1 μg/ml. Aerobic reduction of Te compound allows some species to resist $K_2TeO_3$ at very high concentrations. However, tellurite resistant bacteria do exist in nature and they are often capable of reducing tellurite (IV) to its less toxic elemental form Te (0). By using micro-organisms, toxic metal ions, including tellurite and tellurate are usually transformed into inert metal form. It is poorly understood as to why bacteria are resistant to tellurite but is thought to be associated with tellurite reduction and precipitation of metallic tellurium.

Tellurium is used in many fields. The main use of Tellurium compounds is in steel industries to improve the properties of steel. Also use in solar panels and in vulcanization of rubber and in case of alloys of copper, steel and lead, in new rechargeable batteries, electronics, textiles, mining and also in chemical industries. Compounds of Tellurium are also used to color the glasses. Tellurium has also featured in the development of fluorescent detection probes as CdTe quantum dots with high quantum yield . Historically, Tellurium has found as an antimicrobial agent to treat many microbial infection prior to the discovery of antibiotics. In 1932, the antibacterial properties of Penicillin a Tellurite are compared by Sir Alexander

Fleming, and in nearly all cases, tellurite-insensitive bacteria was Penicillin-sensitive and vice-versa.

The low toxicity of tellurium nanoparticles makes them an excellent material and have many biological application. For e.g. in medicine, in the treatment of tuberculosis, dermatitis, cystitis and eye infection.

Keeping this in view, the present study attempted to elucidate the microbial reduction of sodium tellurite to Te(0) and characteristics of reduced Te(0) present in extra and intracellular matrices.

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# Chapter 2

# Literature Review

# 2.0 Literature review

The field of nanotechnology spans the synthesis of nanoscale matter and understanding their physiochemical and optoelectronic properties. As far as the field of nanotechnology growing, there is a need to develop non-toxic and clean synthetic procedures. In the field of nanoparticle preparation, the researchers have been looking at biological systems for inspiration. Many organisms are known to synthesize nanoparticles.

The nanomaterial level is the most advanced at present, both in scientific knowledge and commercial applications. Nanoparticles are considered to be the building blocks for nanotechnology and are referred to particles with at least one dimension <100 nm (Biswas and Wu, 2005). The synthesis of nanoparticles of different chemical compositions, sizes and controlled monodispersity is an important area of research in nanotechnology. Both chemical and biological methods are using for the synthesis of nanoparticles. In chemical synthesis of nanoparticles, a variety of preparation routes have been reported. Notable examples include, reverse micelles process, salt reduction, microwave dielectric heating reduction, ultrasonic irradiation, radiolysis, solvothermal synthesis, electrochemical synthesis, etc (Guzman *et al*., 2009).

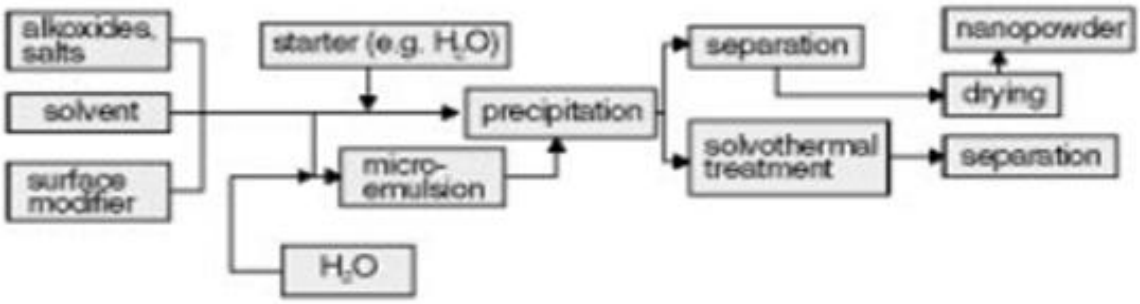



**Fig 1: General scheme of chemical route to nanoparticles (Helmut Scmidt, 2001)**

The chemical methods are fraught with many problems including use of toxic solvents, generation of hazardous by-products, and high energy consumption. A promising approach to achieve this objective is to exploit the array of biological resources in nature. Indeed, over the

past several years, plants, algae, fungi, bacteria, and viruses have been used for production of low-cost, energy-efficient, and nontoxic metallic nanoparticles (Thakkar *et al*., 2009). Biotransformation is a route for growth of nanoscale structures (Mukherjee *et al*., 2002). Now a day, biological synthesis plays a very important role in the generation of nanoparticles.

## 2.1 Biosynthesis of nanoparticles

Diverse variety of organisms plays a role in the synthesis of nanoparticles (bacteria, fungi, yeast, plants etc). Most of these use the synthetic process as a means to detoxify toxic elements (**Table 1**).

### *2.1.1 Bacteria*

A number of bacterial sp. are known to reduces toxic form of some elements into its less toxic form (biotransformation). *Rhizobium sp*. reduces the soluble and toxic selenite anion to insoluble elemental red selenium ($Se^0$) under aerobic and denitrifying conditions. (Hunter and Kuykendall, 2007). *Sulfurospirillum barnesii, Bacillus selenitireducens,* and *Seleni-halanaerobacter shriftii* are three physiologically and phylogenetically diverse species of selenate- and selenite-respiring bacteria, which are used for the production of Selenium nanospheres. (Oremland *et al*., 2003). *Klebsiella pneumoniae* is able to transform cadmium ions into nanometer-sized cadmium sulphide particles which are deposited onto the cell surface. Such a biosynthetic mechanism reduces the toxic effect of any cadmium species, which may be present in the bacterium's local environment (Smith *et al*., 1999).

*Lactobacilli* which are common in buttermilk assist the growth of gold, silver, and gold-silver alloy crystals of submicron dimensions upon exposure to the precursor ions (Nair and pradeep, 2002). *Pseudomonas aeruginosa* was used for the reduction of gold ions resulting in extracellular biosynthesis of gold nanoparticles (Husseiny *et al*., 2007). *Pseudomonas stutzeri* AG259 isolated from silver mine when placed in a concentrated aqueous solution of $AgNO_3$ resulted in the reduction of the $Ag^+$ ions and formation of silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria (Absar *et al*., 2011). Morphological control over the shape of gold nanoparticles has been achieved by using *Plectonema boryanum* UTEX 485, filamentous cyanobacteria. The mechanism of gold bioaccumulation by cyanobacteria from (III)-chloride solutions have documented that interaction of cyanobacteria with aqueous gold (III)-chloride initially promoted the precipitation of

nanoparticles of amorphous gold(I)-sulfide at the cell walls, and finally deposited metallic gold in the form of octahedral (II) platelets near cell surfaces and in solutions (Lengke *et al*., 2006b). *Bacillus sp*. isolated from the Caspian Sea of northern Iran was used for the synthesis of Tellurium nanoparticles. This bacterial strain was used for intracellular biosynthesis of nanoparticles. It synthesized the nanoparticles with dimension of about 20nm × 180nm (Zare *et al*., 2012).

**2.1.2. *Yeast***

It has long been recognized that among the eukaryotes, yeasts are explored mostly in the biosynthesis of the semiconductor nanoparticles. In the biosynthesis of cadmium sulphide (CdS) nanocrystals, *Candida glabrata* and *Schizosaccharomyces pombe* were used for the first time. These nanocrystals were produced using cadmium salts. In addition, they are used in quantum semiconductor crystallites (Dameron *et al*., 1989). Exposure of *Candida glabrata* (Reese and Winge, *1988*; Dameron *et al*., *1989*) to $Cd^{2+}$ ions leads to the intracellular formation of CdS quantum dots. Chemically synthesized nanoparticles have been used for device applications, but biogenic nanoparticles are yet to be explored for the same applications. Cadmium sulphide quantum dots synthesized intracellularly in *Schizosaccharomyces pombe* yeast cells exhibit ideal diode characteristics (*Kowshik et al., 2002a, b*).

Silver nanoparticles in the size range of 2–5 nm were synthesized extracellularly by a silver-tolerant yeast strain MKY3, when challenged with 1mM soluble silver in the log phase of growth. (Kowshik *et al.,* 2003).

**2.1.3. *Fungi***

The use of fungi is potentially exciting since they secrete large amounts of enzymes and are simpler to deal with in the laboratory. *Fusarium oxysporum* reduces aqueous silver ions leading to the formation of extremely stable silver hydrosol. The silver nanoparticles are in the range of 5-15 nm in dimensions and are stabilized in solution by proteins secreted by the fungus. It is believed that the reduction of the metal ions occurs by an enzymatic process, thus creating the possibility of developing a rational, fungal-based method for the synthesis of nanomaterials over a range of chemical compositions (Ahmad *et al*., 2002). *Spergillus fumigates is* also being using for the extracellular biosynthesis of nanoparticles. In this case, the synthesis process was quite fast and silver nanoparticles were formed within minutes of silver ion coming in contact with the cell filtrate (Bhainsa and Dsouza, 2006 *). Alternaria alternata* is also used for the

extracellular biosynthesis of silver nanoparticles. In addition, *Verticillium sp.,* when treated with an aqueous solution of $AuCl_4$ or Ag ions, resulted in the in situ reduction and consequent intracellular formation of gold and silver nanoparticles of good monodispersity.

### *2.1.4. Actinomycetes*

*Thermomonospora sp*. when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles with a much improved polydispersity. The enzymatic process plays a role in reduction of metal ions and stabilization of Gold nanoparticles (Ahmad *et al*., 2003a, b). They concluded that the monodisperse gold nanoparticles synthesis could be due to extreme biological conditions such as alkaline and slightly elevated temperature conditions used for the synthesis of nanoparticles. *Rhodococcus sp*. has been used for intracellular synthesis of gold nanoparticles. Concentration of nanoparticles was more on the cytoplasmic membrane than on the cell wall. This is due to reduction of the metal ions by enzymes present on cell wall. These metal ions were not toxic to cell and were continued to multiply even after the biosynthesis of gold nanoparticles (Ahmed *et al*., 2003a).

**Table 1. Some biological entities used in the synthesis of nanoparticles (**Mandal *et al*., 2006)

| **Biological activity** | **Nanoparticles produced** | **Refrence** |
|---|---|---|
| *Bacillus sp BZ* | Tellurium | Zare *et al*., 2012 |
| *Pseudomonas aeruginosa* | Gold | Husseiny *et al.,* 2007 |
| *Fusarium oxysporum* | Zirconia | Bansal *et al*., 2007 |
| *Fusarium oxysporum* | Barium titanate | Bansal *et al*., 2006 |
| *Verticillium sp.* | Magnetite | Bharde *et al*., 2006 |
| *Aspergillus fumigatus* | Silver | Bhainsa and D'Souza, 2006 |
| *Aloe vera* | Gold and silver | Ahmad *et al*., 2006 |
| *Trichothecium sp.* | Gold | Ahmad *et al*., 2005 |
| *Fusarium oxysporum* | Silica and titanium | Bansal *et al*., 2005 |
| *Avena sativa* | Gold | Armendariz *et al*., 2004 |
| *Azadirachta indica* | Silver and gold bimetal | Shankar *et al*., 2004 |
| *Fusarium oxysporum* | Zirconia | Bansal *et al*., 2004 |

**Table 1. Some biological entities used in the synthesis of nanoparticles (**Mandal *et al*., 2006)

| **Biological activity** | **Nanoparticles produced** | **Refrence** |
|---|---|---|
| *Verticillium* | Silver | Senapati *et al*., 2004 |
| *Thermonospora sp* | Gold | Sastry *et al*., 2003 |
| *Pelargonium graveolens* | Silver | Shankar *et al*., 2003 |
| *Rhodococcus* | Gold | Ahmad *et al*., 2003 |
| *MKY3* | Silver | Kowshik *et al*., 2003 |
| *Colletotrichum sp.* | Gold | Shankar *et al*., 2003a |
| *Schizosaccharomyces pombe* | Cadmium sulfide | Kowshik et al., 2002 |
| *Magnetotactic bacteria* | Magnetite, greigite | Roh *et al*., 2001 |

### 2.1.5 *Plants*

Plants have also been observed as a good source for the synthesis of quantum dots which has huge application in nanotechnology. One of the interesting investigations with *Alfalfa* documents the feasibility for the synthesis of quantum dots by living plants. *Alfalfa* roots have capability for absorbing Ag (0) from agar medium and transferring them to shoot of the plant in the same oxidation state. By joining themselves to form larger arrangements, in shoot these Ag atoms arranged themselves to form nanoparticles. The accumulated Ag atoms inside the plant tissue underwent nucleation shown by transmission electron microscopy. This resulted in the formation of nanoparticles (Gardea-Torresday *et al*., 2003). Use of Geranium (*Pelargonium graveolens*) leaf extract is used for extracellular synthesis of silver nanoparticles. On treating aqueous silver nitrate solution with geranium leaf extract, rapid reduction of the silver ions is observed leading to the formation of highly stable, crystalline silver nanoparticles in solution (Shankar *et al*., 2003). A protein with a molecular weight of 30 kDa extracted from *Capsicum annuum L* not only reduces the $SeO_2^{-3}$ ions to $Se^0$, but also controls the nucleation and growth of $S^0$ (Li *et al*., 2007). Armendariz *et al*., 2004 have reported the pH-dependent binding trend of Au (III) ions to *Avena sativa* (Oat) biomass and subsequent formation of Au nanoparticles of variable sizes. Rod-shaped nanoparticles were common at all pH values used during the study. Small nanoparticles (5-20nm) with large quantities were formed at pH 3 and 4, while large nanoparticles (25-85nm) with small quantities were formed at pH 2.

## 2.2 Applications of nanoparticles

Nanotechnology has become a popular term representing the main efforts of the current science and technology. Nanotechnology is unique in that it represents not just one specific area, but a vast variety of disciplines ranging from basic material science to personal care applications. The synthesis of nanoparticles is a cornerstone of nanotechnology. As far as the synthesis of nanoparticles is concerned, there is an ever-growing need to develop clean, non-toxic, and environmentally friendly ("green chemistry") synthetic procedures (Mukherjee *et al*., 2001). There are some applications of nanotechnology which would help in understanding the use of diverse living organisms in nanodevices production and the use of nanoparticles in various products.

Nanoscale materials are used in different areas such as electronic, magnetic, optoelectronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytic, and materials applications. Nanotechnology plays a role in improving air, water, and soil quality in the environment. It can also improve detection and sensing of pollutants and help in the development of new technologies for remediation (Biswas and Wu, 2012). Bioremediation of radioactive waste resulted from the nuclear power plants and nuclear weapon production such as uranium have been achieved by using nanoparticles. Cells and S-layer proteins of *Bacillus sphaericus* JG-A12 have special capabilities for clean-up of uranium contaminated waste waters (Duran *et al*., 2007).

Nanoparticles have found applications in antibacterial effects. *Fusarium oxysporum*, extracellularly produced silver or gold nanoparticles can be incorporated in sterile cloths that can be useful in hospitals to prevent or to minimize the infection with pathogenic bacteria such as *Staphylococcus aureus* (Dura'n *et al*., 2007). Either intracellularly or extracellularly produced nanoparticles by using living organisms have great value. Silver nanoparticles have a number of applications such as in non-linear optics, spectrally selective coating for solar energy adsorption, biolabelling (Klaus *et al*., 2001). Other applications like catalyst in chemical reaction, intercalation material for electrical batteries, as optical receptors and as antibacterial capacities (Dura'n *et al*., 2005).

From the last 400 years, gold nanoparticles have been used for the treatment of certain illness, and staining of glass and enamels (Tanaka, 1999). The size dependent chemical and electrochemical reactivities for gold nanoparticles have been well documented. The gold

nanoparticles have unique and highly anisotropic planes shapes and find applications in photonics, optical sensing and optoelectronics. Further, the effect of different organic solvent vapors like methanol, benzene and acetone on the conductivity of tamarind leaf extract reduced gold nanoptriangles suggests the application of gold nanotriangles to future chemical sensors (Ankamwar *et al*., 2005).

Selenium is well reported element for anti-oxidant properties in humans and animals. Its colloidal form has been utilized for nutritional suppliments and in medical diagnostic. Selenium nanoparticles were used as a new carrier for horseradish peroxidase to construct $H_2O_2$ biosensors with good performances. Selenium is well known for its photoelectrical, semiconductor and biochemical properties. Selenium has shown its importance as a photo conductive material in applications such as solar cells, rectifiers, photographic exposure meters and xerography (Yang *et al*., 1990). In recent years, increasing attention has also been attracted toward the biosynthesis of other important metal or mineral nanoparticles such as tellurium (Te) nanoparticles. The low toxicity of Te nanoparticles makes them excellent material for application in medicine. Used as an antimicrobial agent in the treatment of leprosy, tuberculosis, dermatitis and sever eye infections. It is used to improve the properties of steel, also used in solar panels, glasses, new rechargeable batteries. In addition, have applications in metallurgy, textile, electronics and mining and in chemical industries (Zare *et al*., 2012). Diverse variety of applications of nanoparticles sourced from various elements are outlined in Table 2.

**Table 2. Metal nanoparticles and their applications**

| Nanoparticle | Applications | References |
|---|---|---|
| Gold | In drug delivery. | Yang *et al*., 2006 |
| | Treatment of certain illness and staining of glass. | Duran *et al*., 2007 |
| | In sterile cloths to prevent the infection. | Ankamwar *et al*., 2005 |
| | In optoelectronics, photonics and as future chemical sensors. | Huang *et al*., 2007 |
| | Imaging and biomedicine. | Jain *et al*., 2006 |
| Silver | In non-linear optics, selective coating for solar energy adsorption and in biolabelling. | Klaus *et al*., 2001 |
| | As an anti-bacterial. | Sharma *et al*., 2009, Mohan *et al*., 2007 and Rai *et al*., 2009 |

| Nanoparticle | Applications | References |
|---|---|---|
| Selenium | Show anti-oxidant property in humans and animals.<br>Have photoelectrical and biochemical properties.<br>Also have applications in photographic exposure meters and xerography.<br>Have optical properties. | Yang *et al*., 1990;<br>Wang *et al*., 2005<br>Gao *et al*., 2002<br>Ingole *et al*., 2010<br>Rajalakshmi *et al*., 1999 |
| Tellurium | Applications in medicine.<br>As an antimicrobial agent.<br>Used in fluorescent detective probes. | Zare *et al*., 2012 |

## 2.3 Biosynthesis and recovery of Te nanoparticles

In the synthesis of organic and inorganic nanoparticles, nanotechnology field has seen impressive development. The different nanosized materials exhibit unique optical, chemical, biological, photoelectrochemical and electronic properties. This is because of their high surface area and large surface/volume. Tellurium nanoparticles have different applications in different fields.

In a study, a Te-transforming *Bacillus* sp. BZ was isolated from the Caspain Sea in northern Iran. Various tests and rDNA analysis were used to identify the isolates. Then it was used to prepare Te nanoparticles. The isolate was subsequently used for the intracellular biosynthesis of elemental Te nanoparticles. Liquid nitrogen was used to release the biogenic nanoparticles and purified by an n-octyl alcohol water extraction system. The size, shape and composition of synthesized nanoparticles were characterized by various methods.

The rod shaped nanoparticles with dimensions of about 20nm×180nm were shown by Transmission electron micrography. The energy dispersive X-ray and X-ray diffraction spectra demonstrated that the synthesized nanoparticles consisted of only Te in elemental form and have a hexagonal structure.

It has applications in different fields including improving the properties of steel, also use in solar panels, glasses, new rechargeable batteries. Als in electronics, textiles, mining and chemical industries. Tellurium compound have tradinally been used as an antimicrobial agent for the treatment of leprosy, tuberculosis, dermatitis, cystitis and sever eye infections (Zare *et al*., 2012). The various physical, chemical and in particular spectroscopic properties of tellurium have raised the possibility that tellurium- containing substances may serve as effective biological markers. Tellurium based antibiotics were used in earlier centuries. (Ba *et al*., 2010).

### *2.3.1. Tellurium chemistry*

Tellurium is a natural occurring element with atomic number 52 and symbol Te. It was discovered by F.J. Muller von Reichenstein in 1782 in a mineral containing tellurium and gold. The word Tellurium comes from the Latin word "Tellus", which means "earth". Tellurium belongs to the group chalcogens, which also includes the oxygen, sulphur, selenium and polonium. These close relatives of Te, occupy pivotal role in biochemistry.

The Te compounds can be divided into three groups: tellurium-containing complex-like structures, inorganic tellurides, and organotellurides. This distinction is not always applicable; it allows to rationalize and to understand some basic features of tellurium compounds, like the stability and reactivity.

Like sulfur and selenium, Te has numerous oxidation states ranging from -2 (H 2Te) to +6 ($TeO_4$2). Various forms of Te are found in nature such as $TeO_2$ (+4) and $TeO_3$ (+6). Tellurites $TeO_3$2- and tellurates $TeO_4$2- are the corresponding forms of Te. Tellurates are generally more stable than tellurites. Some 0f the common Te compounds known in chemical synthesis are as follows (Cunha *et al*., 2009):

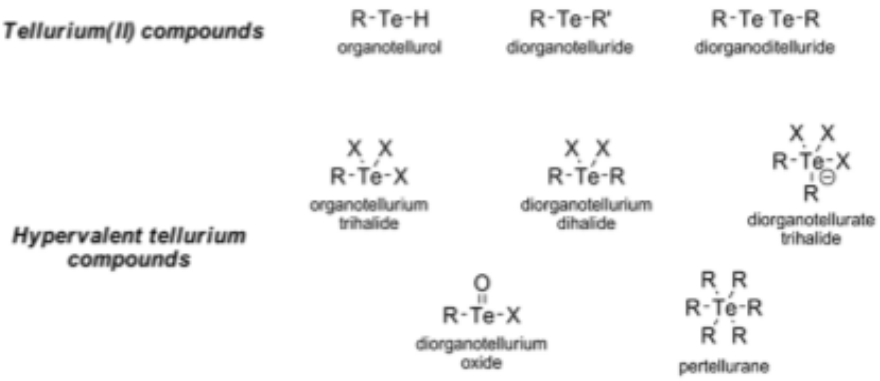



**Fig 2: Classifications of Te compounds**

The compounds of Te are highly toxic. In the field of biology, Tellurium is a truly alien element, with no apparent role in either pro- or eukaryotic organisms. Selenium which is discovered by Jones Jacob Berzelius in 1817 is relative to Te. Unlike selenium, tellurium is not an essential biological trace element. Tellurides, tellurites and tellurates are the inorganic salts of

tellurium. The chemistries of elements belong to same group as that of Te is intertwined *in vitro* and *in vivo*. Many Te compounds kill various microorganisms, including bacteria and plasmodia. Some Te compounds also induce apoptosis in certain cancer cells.

Tellurium also exhibits interesting spectroscopic properties, which are useful for the characterisation of organotellurium agents. Naturally occurring tellurium is a mixture of several isotopes, namely 120Te (natural abundance 0.09%), 122Te (2.55%), 123Te (0.89%), 124Te (4.74%), 125Te (7.07%), 126Te (18.84%), 128Te (31.74%) and 130Te (34.08%).

One of sulfur and selenium resembles the 'inorganic' chemistry of tellurium but there are also notable differences. The +4 and +6 oxidation states have comparable stability in case of tellurium, in contrast to sulfite, tellurite is not easily oxidized to tellurate, as reflected in the redox potentials (E0(SO32-/SO42-) =-0.936 V and E0(TeO32-/TeO42-) = +0.07 V in alkaline solution) (Ba *et al*., 2010).

In 1996, the biochemistry of Te in the context of animal and human toxicology was reviewed by Taylor. A nutritional role has never been identified for Te despite many chemical homologies between selenium and tellurium. At low concentrations, Te induces both acute and chronic toxicity in a number of organisms. According to some studies the trace amount of Te is also present in body fluids such as in blood and urine. Te in form of Tellurocysteine and telluromethionine can be found in bacteria, fungi and yeast.

Because of less frequent contact of man and animal with Te, the toxicology of tellurium has been received less attention than that of selenium. Tellurium is less soluble than selenium in physiological pH. $H_2Te$ is reduced form of tellurium which is more readily decomposes, by light or air, than $H_2Se$; for this reason, some authors attribute to tellurium a lower potential toxicity than selenium.

In the cholesterol biosynthesis pathway, both Te and its oxyanion forms act on the enzyme squalene monooxygenase which is the second enzyme of this pathway. From the blockage of cholesterol synthesis, a transient demyelination of peripheral nerves occur and the same effect has been observed with selenite and other methylselenium compounds. Squalene monooxygenase is sensitive to Tellurium and Selenium compounds because of their binding to vicinal cysteins; the methylation of tellurium in vivo may enhance the toxicity of tellurium for this enzyme. Cysteine proteases also deactivate by most of the Te (IV) derivatives. This seems to be related to the ability of Te (IV) compounds to react with the thiolcatalytic site of cysteine

proteases. The compounds of Te (IV) donot exhibit any such inhibitory activity as they are inert towards thiolmoieties. Te compounds interact with biological systems by specific chemical linteraction with endogenousthiols.

The biological interactions of Te and its inorganic compounds are in a specific way. Some bacteria and fungi strains are able to metabolize Te salts in the reductive metabolism, leading to biologically inert $Te^0$ or tellurolate ($Te^{2-}$), which is methylated to volatile, garlic smelling. The reduction and methylation of Te also occur in mammals including humans. If the solution of salts injected into the animals, it may lead to the formation of $Te^0$ and $(H_3C)_2$ Te, which are eliminated by breath, urine and sweat. Tellurate is about 2 to10-times less toxic than tellurite in most organisms studied. From last 90 years, tellurite use as an additive in medium for the identification of tellurite resistant microorganisms e.g. in selective media to isolate a wide range of pathogens like *Corynebacterium diphtheriae*, *Vibriocholerae*, *Shigella* spp. Tellurite and tellurate have also been proposed for use in selective media for identification of fecal Streptococci (Cunha *et al*, 2009). In some cases, in the presence of sodium tellurite when fungi are grown without a sulfur source, tellurium-containing amino and even tellurium-containing proteins are formed. Instead of sulfur this more or less random incorporation of heavier chalcogen analogues is well known for selenium, which substitutes for sulfur in the amino acid methionine, for instance in yeast and the similar processes also occur in the case of tellurium, at least in fungi. Such role of Tellurium in plants and higher organisms is not clear (Ba *et al*., 2010). Keeping in view, the growing importance of Te in terms of its applications as nano-materials for industrial and biomedical applications, the present study examined the potential nature of two known selenium tolerant bacteria $C_3$a and $C_3$b to assimilate and reduce tellurium.

# Chapter 3

## Objective and Work Elements

# 3.0 Objective & Work Elements

## 3.1 Objective

Studies on tolerance and biotransformation of Tellurium by soil bacteria

## 3.2 Work elements

1) Determination of bacterial tolerance to Te.
2) Generation and characterization of Te(0) nanoparticles.
3) Examine the antimicrobial activity of Te(0) nanoparticles

# Chapter 4
# Materials and Methods

# 4.0 Materials and Methods

## 4.1 Microbial strains under study

Two strains (C3a and C3b) isolated from rhizophere soils of seleniferous fields were taken for the study. The strains were earlier observed to be phylogenetically similar (≅ 98%) to *Bacillus subtilis* (C3a) and *Cellulomonas denverensis* (C3b). These strains were also observed to be tolerant to significantly high concentrations of selenium oxyanions. Three pathogenic bacterial strains i.e. *S.epidermidis*, *S.aureus* and *E.coli* MTCC 1302 were used to evaluate the antimicrobial activity of tellurium nanoparticles.

C3a and C3b were sub-cultured in tryptone soya broth (TSB – Himedia, India) comprising (g/l) dipotassium hydrogen phosphate - 2.5; casein peptone -17.0; soy peptone - 3.5; sodium chloride - 5.0; Glucose - 2.5. The final medium was set at pH - 7.3. The culture was grown at 37$^{o}$C for 24h. The stock of the active strains were maintained in tryptone soya agar (TSA – Himedia, India) containing (g/l) Casein peptone - 17.0, Soya peptone - 3.0, Sodium chloride - 5.0, Dipotassium hydrogen phosphate - 2.5, Glucose - 2.5 and the pH was 7.3. Muller-Hinton Agar (MSA- Himedia, India) and Muller-Hinton Broth (MHB – Himedia, India) was used for antimicrobial tests.

## 4.2 Determination of tolerance to tellurium (Te)

Sodium tellurite ($Na_2TeO_3$) was procured from Loba Chemie, Mumbai (India). Stock solution of 7800µM tellurite was prepared by dissolving 0.1735 gm of $Na_2TeO_3$ in 100ml of double distilled water. Te spiked TSA plates were prepared by addition of 3.8ml, 6.4ml and 8.9ml of tellurite, through sterile 0.22µ syringe filter, to 100 ml of agar solution of autoclaved and cooled TSA, leading to final concentrations of 300, 500 and 700µM respectively. TSA plates without Te were prepared for control studies.

The strains were cultivated by streaking them on Te supplemented TSA plates or inoculating them in Te supplemented TSB, of varied concentrations. The strains in broth or agar were

incubated at 37$^{o}$C for 8-12h. Appearance of black colour colonies indicated the growth and tolerance of the strains to a particular concentration.

### 4.3 Generation of Te particles

Based on the tolerance profile of the strains, actively growing cultures were exposed to 500 µM Te in TSB for 12 h. The biomass were then centrifuged at 8000 g and separated. The biomass was air dried at 40$^{o}$C and stored. The cell free supernatant (CFS) was filtered through 0.22 m□ to remove biomass.

### 4.4 Characterization of Te particles

The cultures grown on Te supplemented medium were centrifuged at 8000 g for 5min and the resultant supernatant was used for size and charge analysis by dynamic light scattering (DLS) method and zeta potential respectively. The *Zeta PALS* (Brookhaven Instrumetns Corp., USA) was used for determining the above parameters. PALS stands for phase analysis light scattering. PALS is based on measurement of the velocity of moving particles that scatter laser light and ca measure velocities that are as little as 1000 times smaller. The velocity that is measured is the product of the electrophoretic mobility and the electrical field. Zeta potential is represented in millivolts (mV).

For determining the size of nanoparticles generated by bacteria, DLS technique was used which is one of most popular light scattering technique for nanoparticles, proteins, polymers, emulsions etc. DLS which is also known as **Photon Correlation Spectroscopy** or **Quasi-Elastic Light Scattering** DLS measures variation in scattered intensity with time at a fixed scattering angle (typically 90$^{o}$C). The sample is illuminated by a laser beam and the fluctuations of the scattered light are detected at a known scattering angle θ by a fast photon detector. Simple DLS instruments that measure at a fixed angle can determine the mean particle size in a limited size range. More elaborated multi-angle instruments can determine the full **particle size distribution**.

### 4.5 X-Ray Diffraction (XRD) analysis

The cultures grown on Te supplemented medium (i.e. 300µM, 500µM and 700µM) were centrifuged at 10000 g for 10min. After centrifugation, supernatant was discarded and pellet

containing Te nanoparticles was washed, air dried in hot air oven (at 65˚C). After drying, the pellet was powdered and the crystalline nature of the particles was studied by XRD analysis. The crystallography was carried out using X-ray diffractometer (PANanalytical X'Pert Pro, USA) facilitated by Sophisticated Analytical Instrumentation Facility, Panjab University, Chandigarh. XRD was set at 40 Kv and 30 mA with Cu-Kα radiation.

### 4.6 Evaluation of antimicrobial effect of Te nanoparticles

Microbial susceptibility tests were carried out by agar well diffusion method, disc assay and by inoculating bacteria in Muller-Hinton broth with different concentration of tellurium nanoparticles.

Three different bacteria strains (*S. epidermidis*, *S. aureus* and *E. coli* MTCC 1302) were used to check the antimicrobial activity of Te nanoparticles. These three organisms were inoculated into 3 different test tubes containing 10 ml of Muller-Hinton broth and then incubated at 37˚C for 24 h.

For the preparation of the Te particles, Te tolerant bacteria were grown in Te supplemented media (500µM) were centrifuged at 15000rpm for 10min. After the centrifugation step, the supernatant was discarded and pallet was re-suspended into the water. The sample was centrifuged twice to wash the particles. Following the washing, the sample was this at 5000 rpm for 5min to settle down the large-sized particles. The supernatant containing Te nanoparticles was taken to evaluate the antimicrobial activity.

For carrying out anti-microbial assay, Muller-Hinton agar (MHA) plates were prepared by adding 22.5ml of MHA into the sterile petriplates. In well diffusion method, wells were made on the solidified plates with the help of cork borer. 20µl of extract of Te nanoparticles (3mg $ml^{-1}$) were added into the wells and then sealed with molten agar. 0.5 McFarland adjusted different clinical strains (*S. epidermidis*, *S. aureus* and *E.coli* MTCC 1302) were inoculated on different plates with the help of cotton swabs. Plates were incubated at 37˚C for 18h. In disc assay, organisms were inoculated on the plates with cotton swabs then discs were placed on the plates with sterile solid medium. 20µl of extract of Te nanoparticles (3mg $ml^{-1}$) were added on the discs and then incubated at 37˚C for 18h.

To observe the visible inhibitory activity of Te nanoparticles, the test was carried out on microtitre plate. 50µl of Muller-Hinton broth and 25µl of extract (3mg $ml^{-1}$) were added into the wells on microtitre plate. Then, test organism was added in different wells. The microtitre plate was incubated at 37˚C for 18 h. After 18 h, TTC solution (2, 3, 5-triphenyl-2H-tetrazolium solution) was added into each well to visualize the inhibitory action of Te nanoparticles.

Minimum inhibitory concentration (MIC) test was done to check the lowest concentration of Te nanoparticles to inhibit the growth of different bacterial strains. Muller-Hinton broth (MHB) supplemented with different concentration of Te nanoparticles ranging from 0.6 to 4 mg $ml^{-1}$ was prepared in different test tubes by dilution method and was inoculated with 0.5 McFarland adjusted different cultures. The test tubes were incubated at 37˚C for 18 h. After incubation period, 200µl from each test tube was added into wells of microtitre plate and then absorbance was detected by using microplate reader at 600nm.

# Chapter 5
# Results and Discussion

# 5.0 Results and discussion

Nanotechnology is a rapidly progressing field and has a tremendous impact on fields such as materials, electronics and medicine (Mnyusiwalla *et al*., 2003). The synthesis of nanoparticles of different chemical compositions, sizes and controlled monodispersity is an important area of research in nanotechnology. There are various chemical (Kalele *et al*., 2006; Murray *et al*,. 2000) and physical (Ayyub *et al*., 2001) methods to generate nanoparticles. The chemical methods are fraught with many problems including use of toxic solvents, generation of hazardous by-products, and high energy consumption. A promising approach to achieve this objective is to exploit the array of biological resources in nature. The present study focused to understand such nanostructural formations by bacteria.

On subjecting the two test bacteria i.e. $C_3$a and $C_3$b to different concentrations (i.e, 300, 500 and 700µM) of sodium tellurite, visual observations of the biotransformation indicated the reduction of tellurium oxyanions to elemental tellurium with growth medium or biomass turning black in colour. This colour was very distinct as compared to control. On examing the viability of the organisms exposed to different concentrations, it was observed that the test organisms were tolerant up to 700µM sodium tellurite. The **Figure 3** and **Figure 4** show the transformation of sodium tellurite into tellurium by soil bacteria in growth medium (tryptone soya broth) and on TSA plates. The observation on tellurium was similar to earlier studies by Zare and co-workers (2012) in which nanoparticles were generated by *Bacillus* sp. BZ isolated from the Caspian Sea in northern Iran.

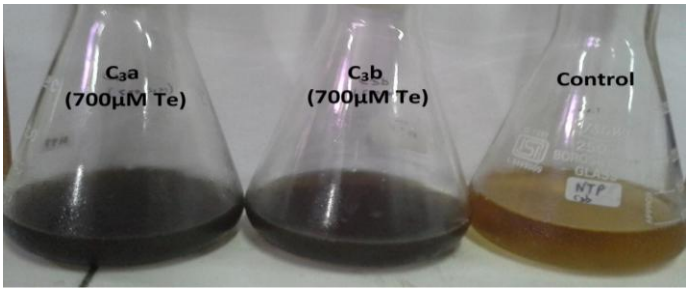



**Figure 3. Reduction of tellurite by soil bacteria as indicated by formation of elemental Te in growth medium**

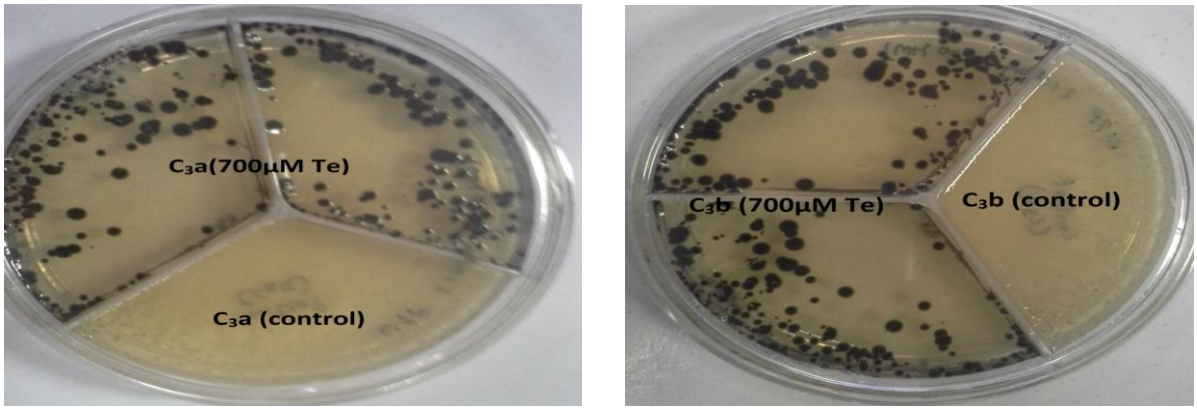


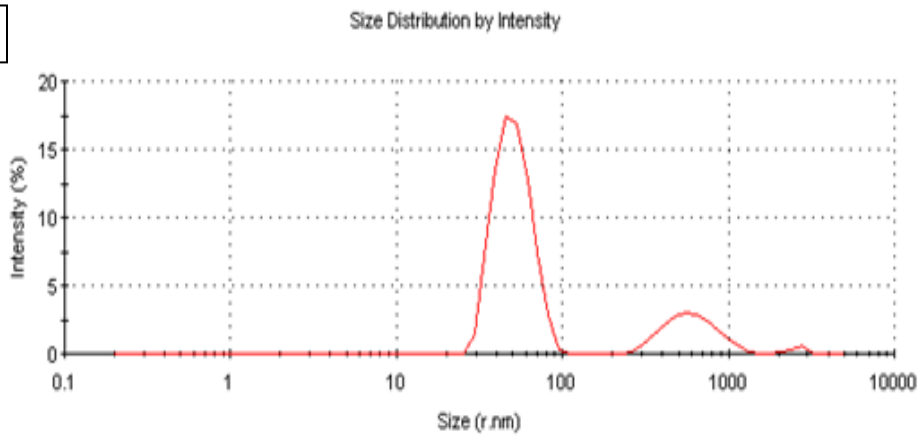

**Figure 4. Reduction of tellurite to elemental Te as depicted by formation of black colonies**

The **Figures 3** and **4** also show the tolerance of tellurium by soil bacteria at concentration 700µM. It gives black coloured colonies in case of tellurite containing plates and black coloured solution in case of broth. Element selenium belongs to same group as that of tellurium but in case of selenium red coloured colonies on TSA plates and red coloured solution in TSB formed after incubation period. In case of Te, change in colour starts after 6 hours in TSB and in case of selenium it starts after 2 hours in TSB. Many reports are present on the reduction of selenium nanoparticles. In June 2010, Fesarakhi and co-workers reported that *Klebsiella pneumoniae* reduced selenite concentration up to 0.2mg $ml^{-1}$ but in our study, C3a and C3b strains reduced tellurite upto 1.73mg $ml^{-1}$ which is much higher than that of selenium. According to Dhanjal and Cameotra in 2010, *Bacillus cereus* was able to tolerate high concentration of selenite i.e. up to 5mM but in this case generated nanoparticles was sized between 100-200nm. At low concentration (700µM) than that of selenite, $C_3$a and $C_3$b strains were able to generate >100nm sized tellurium nanoparticles by reduction process.

### 5.1 Characterization of reduced Te

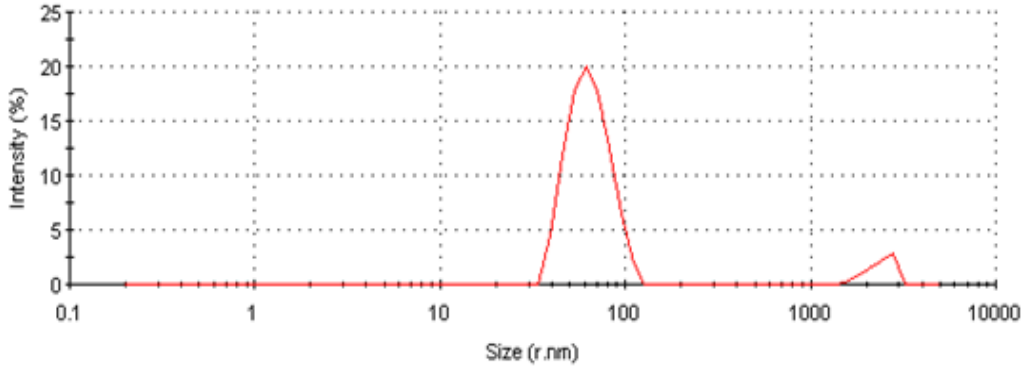
Two different approaches were followed to understand the characteristics and nature of the reduced tellurium in bacterial cells. **Dynamic light scattering** was carried out to determine the size distribution of Te (0). The average size of Te (0) nanostructures formed in bacteria was 60nm in case of $C_3$a (**Figure 5a**) and 69nm in $C_3$b (**Figure 5b**).

Fig. 5a




| | | Size (r.nm): | % Intensity | Width (r.nm): |
|---|---|---|---|---|
| Z-Average (r.nm): 60.43 | Peak 1: | 51.17 | 79.9 | 12.65 |
| PdI: 0.343 | Peak 2: | 613.2 | 19.1 | 213.8 |
| Intercept: 0.890 | Peak 3: | 2592 | 1.0 | 242.0 |

Fig. 5b




| | | Size (r.nm): | % Intensity | Width (r.nm): |
|---|---|---|---|---|
| Z-Average (r.nm): 69.34 | Peak 1: | 64.56 | 92.8 | 16.14 |
| PdI: 0.300 | Peak 2: | 2411 | 7.2 | 356.3 |
| Intercept: 0.839 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Figure 5. Average size, relative density and size distribution of the Te(0) nanostructures generated by reduction of tellurite by C3a and C3b strains**

Poly dispersity Index (PDI) tells about the mean size of nanoparticles. The PDI values ranges from 0 to 1; a higher value indicates a less homogenous nanoparticle size distribution. All nanometric systems presented significant differences for the evaluated variables ($P<0.1$). The highest PDI indicates that nanoparticles generated by this system had the lowest homogeneity in size distribution. The lowest value for evaluated variables were obtained in $C_3$b, it showed a mean particle size of 69.34 nm and a PDI of 0.300 that is directly related to best particle size. In case of $C_3$a, mean particle size was better than $C_3$b but have high PDI i.e. 0.343 which means $C_3$a is less homogenous particle as compare to $C_3$b.

The size-distribution profile demonstrated that the biosynthetic method yielded Te (0) nanomaterials, of a narrow size range, stable in water. Most of studies have been done on selenium nanoparticles because of their applications in medicine and other fields but according to Ba *et al* tellurium also have a number of applications in medicine. These applications mainly depend upon the size of nanoparticles. In case of small particles the larger surface area available for interaction will give more bactericidal effect than the larger particles (Govindaraju *et al*., 2010). In a very recent research, selenium nanospheres were synthesized from bacterial strain JS-11 and according to DLS analysis the aggregate size of generated nanospheres was 264nm in deionized ultrapure water. In an another study, 100-200nm selenium nanoparticles were produced by *Bacillus cereus* (Dhanjal and Cameotra, 2010). In our study, C3a and C3b strains was able to generate tellurium nanoparticles with 10-80nm size. Study was also carried out on extracellular biogenesis of gold nanoparticles using *Thermonospora* sp. by Sastry and co-workers (2003) resulted in generation of gold nanoparticles sized 7-12nm and were spherical in nature. The uniqueness of this observation by the researchers was the formation of gold particles at extreme conditions of pH 9-11 and temperature above 50˚C.

Further, to characterize and understand the nature of the Te particles formed by the test strains, the biomass which was centrifuged, dried and analyzed using **X-ray diffractometer** indicated the presence of crystallite nanostructures (**Figure 6**).

Fig 6a

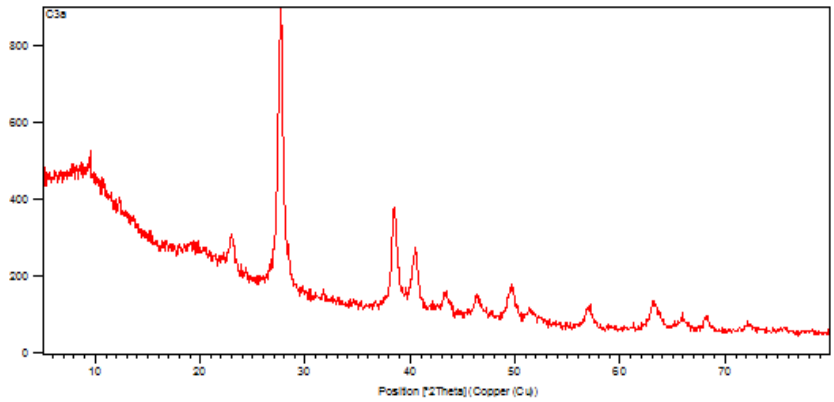



Fig 6b

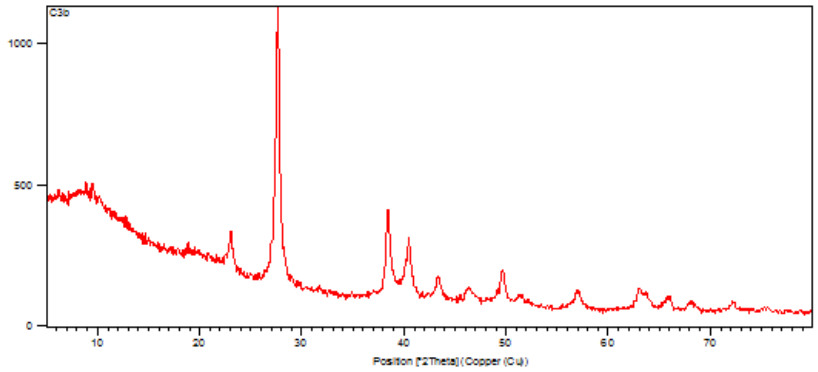



**Figure 6. X-ray diffraction of dried biomass (C3a (a) and C3b (b)) indicating formation of Te(0) nanocrystallites**

The results of X-ray diffraction indicate the pure crystalline form of tellurium nanoparticles. All the reflections can be indexed on hexagonal phase of tellurium crystal. The nanoparticles are well-crystalline, with limited peaks of other phases are observed, indicating the relatively pure crystals. The peak broading of XRD patterns indicates the significantly small size of the resulting crystallites.

The crystallite characteristics matched with powder diffraction file (ICDD-PDF 36-1452) of pure Te(0) particles. The size determination using Debye-Scherrer equation (Eq.1) is represented in **Table 3**.

**Debye-Scherrer equation = (K x λ) / (w x Cosθ)** *....Eq.1*

*Where K is constant = 0.91*

*λ = Wavelength of CuKα radiation*

*W = width at half peak height*

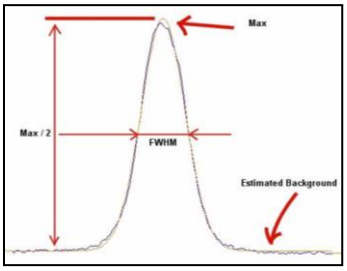
*θ = Bragg angle*

**Table 3 – XRD spectral data and size distribution as calculated by Debye-Scherrer Eq.**

| 2θ | d | FWHM* at 2θ | Relative intensity ($I/I_0$) | Size (nm) | Lattice orientation** | | |
|---|---|---|---|---|---|---|---|
| | | | | | h | k | l |
| **C3a** | | | | | | | |
| 27.6679 | 0.1673 | 3.22421 | 100.0 | 48.93 | 1 | 0 | 1 |
| 38.4088 | 0.1673 | 2.34371 | 35.70 | 50.30 | 1 | 0 | 2 |
| 40.5519 | 0.4015 | 2.22466 | 20.97 | 21.10 | 1 | 1 | 0 |
| 49.7824 | 0.5353 | 1.83165 | 11.67 | 16.36 | 1 | 1 | 1 |
| 22.9834 | 0.401 | 3.86966 | 11.59 | 20.20 | 2 | 0 | 1 |
| **C3b** | | | | | | | |
| 27.6201 | 0.1673 | 3.22968 | 100.0 | 48.92 | 1 | 0 | 1 |
| 38.3867 | 0.1840 | 2.34501 | 30.41 | 45.73 | 1 | 0 | 2 |
| 40.4547 | 0.1673 | 2.22978 | 21.23 | 50.63 | 1 | 1 | 0 |
| 23.0427 | 0.1673 | 3.85984 | 13.40 | 48.48 | 1 | 1 | 1 |
| 49.6725 | 0.4015 | 1.83545 | 11.20 | 21.81 | 2 | 0 | 1 |

* **Full width at half maximum of the XRD peak**

** **Lattice orientation based on ICDD-PDF Te(0) – 36.1454**




An estimated mean size of the crystallites obtained in the present study as calculated using the Debye-Scherrer equation indicated a mean size of 33.33 nm in case of C3a and 36.22 nm in case of C3b (**Table 3**). The observations, thus, confirm that the crystallites formed through reduction of tellurite to Te(0) were nano size in nature. Similarly, $TiO_2$ nanoparticles were also produced by *Lactobacillus* sp. and *Sachharomyces cerevisae* by Jha and co-workers (2009) wherein X-ray diffraction method was performed to ascertain the formation of $TiO_2$ nanoparticles. The aggregate size of generated nanoparticles in this case was 8–35 nm.

Concentric Scherrer rings in the selected area electron diffraction pattern indicated that the nanoparticles are having all possible orientations. According to XRD analysis the size of $TiO_2$ nanoparticles by *Lactobacillus* sp. and *Sachharomyces cerevisae* in this was same as that in case of Te nanoparticles by bacterial strains $C_3$a and $C_3$b.

Studies carried out on biosynthesis of gold nanoparticles by *Rhodopseudomonas capsulate* shows the X-ray diffraction analysis of synthesized nanostructures. In this analysis, no spurious diffraction due to crystallographic impurities was found. A strong diffraction peak located at 38.09˚ is ascribed to (1 1 1) peaks was much lower than the standard value (0.068 versus 0.53). The ratio between the (2 2 0) and (1 1 1) peaks was also much lower than the standard value (0.039 versus 0.33). According to these observations the gold nanoparticles formed by the reduction of Au (III) by bacteria *Rhodoseudomonas capsulate are* dominate by (1 1 1) facts, and most of the (1 1 1) planes parallel to the surface of the supporting substrate were sampled (He *et al*., 2007). Gurunathan and co-workers (2009) reported the synthesis of silver nanoparticles by *E.coli*. This study reports the optimal conditions for maximum synthesis of silver nanoparticles through reduction of $Ag^+$ ions by the culture supernatant of *Escherichia coli*. XRD pattern of silver nanoparticles formed after reaction of culture supernatant with silver nitrate ($1 \times 10^{-3}$ M) for 24 h. The XRD pattern shows four intense peaks in the whole spectrum of $2\theta$ values ranging from 20 to 80. Note $2\theta$ peak values of 39.01°, 46.48°, 64.69° and 77.62°, corresponding to 1 1 1, 2 0 0, 2 2 0, and 3 1 1 planes, respectively, for silver. XRD analysis demonstrated that the aggregate size of synthesized nanoparticles was 50nm. According to above three XRD analyses, the size of nanoparticles in all these cases were almost same i.e. 10-50 nm.

**5.2 Anti-microbial activity of Te (0) nanocrystals**

Tellurium ions have long been known to have strong inhibitory effects as well as a broad spectrum of antimicrobial activities. Antimicrobial tests were used to check the inhibitory action of Te nanoaprticles against different test organisms (*S.epidermidis*, *S.aureus* and *E.coli* MTCC 1302). The tests were carried out following two different protocols viz., agar well diffusion assay and disc assay.

In agar well diffusion method, after 24 h of incubation period, a clear zone was observed against *E.coli* MTCC 1302 (in C3a) and *S. epidermidis* (in C3b) (**Figure 7**). The inhibitory effect

of nanomaterials was similar in both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria.

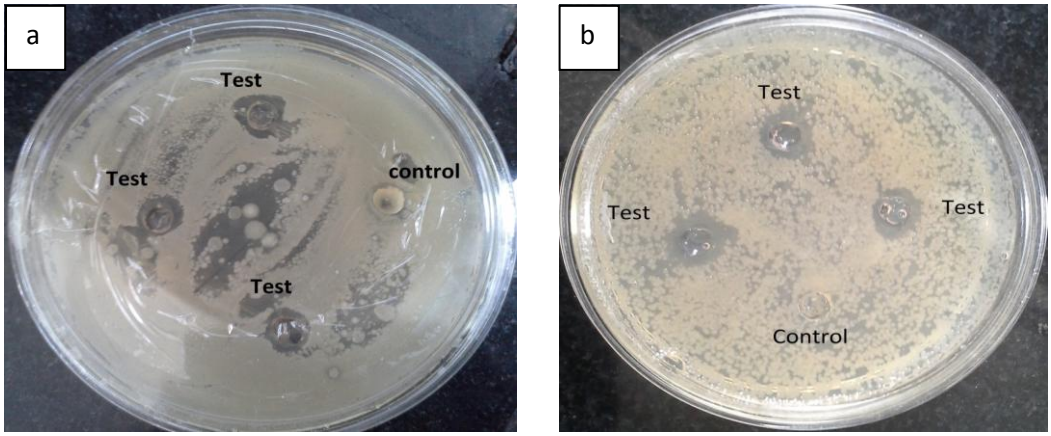



**Figure 7. Anti-microbial activity of Te nanoparticles generated by C3a and C3b on (a) *Ecoli* MTCC 1302 and (b) *S.epidermidis* respectively**

In well diffusion method, although the extent of diffusion is limited due to particulate nature of the substance, the anti-microbial activity was still significant.

In disc assay, after 24 h, a clear zone of inhibition was observed around the discs against *E.coli* MTCC 1302 in both the cases i.e $C_3$a and $C_3$b (**Figure 8)**. The clear zone shows the inhibitory action of Te nanoparticles against *E.coli* MTCC 1302.

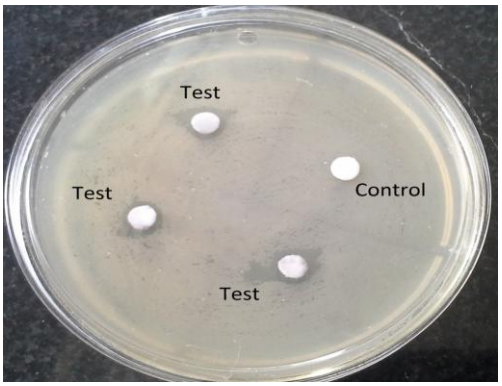



**Figure 8. Anti-microbial activity of Te nanoparticles generated by C3a on *Ecoli* MTCC 1302 as depicted by disc assay**

Disc tests are basically qualitative, although it is possible to get some information on the degree of activity depending on zone size. Although, the zone size was not significantto measure, it was clearly evident that the bacteria (*E.coli* MTCC 1302) was susceptible to inhibitory effect of Te nanoparticles.

Susceptibility test was also carried out by inoculating test organisms (*S.aureus* - well 1 and 2; *S.epidermidis* - well 4 and 5; and *E.coli* MTCC 1302 - well 7 and 8) from different MHB test tubes containing Te nanoparticles (3mg $ml^{-1}$). After incubation period, the TTC was added in each well of microtitre plate and again incubated for 30mins at 37˚C. After half an hour, the antimicrobial activity of Te(0) was observed by the colour change (**Figure 9**). In case of living cells, the colour changed to pink or purple and in case if the microbial growth was inhibited by the reagent then the colour remained indicating the inhibition of microbial growth.

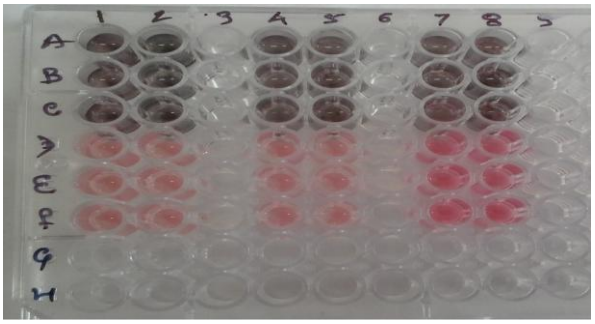


**Figure 9. Anti-microbial activity of Te nanoparticles generated by C3a as depicted by TTC assay**

In **Figure 9,** wells A, B and C contains test organisms i.e *S.aureus* (well 1 and 2), *S.epidermidis* (well 4 and 5) and *E.coli* MTCC 1302 (well 7 and 8) with Te nanoparticles (3mg $ml^{-1}$) and well D, E and F are control i.e. without Te nanoparticles. On addition of TTC solution, no change was observed in colour in wells containing Te nanoparticles indicating total inhibition of growth of cells. But in case of control, the colour changed to pink due to metabolic activity of cells. TTC solution binds with living cells and gives pink colour. TTC is a redox indicator, which turns pinkish-red when reduced. A living bacterium will move throughout the medium, oxidizing the carbon compounds and reducing the indicator to produce a pink color wherever it goes. A non-motile (non-living) organism will be unable to move so that there is no any change

in color. The results are further outlined in **Table 4** where in NG represents 'no growth' and G represents growth of organisms.

**Table 4. Antimicrobial effect of Te nanoparticles against test organisms in comparison to control**
***NG - 'no growth' and G – 'growth'***

| | | *S.aureus* | | | *S .epidermidis* | | | *E.coli* MTCC 1302 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_3a$ | $C_3b$ | | $C_3a$ | $C_3b$ | | $C_3a$ | $C_3b$ | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Test | A | NG | NG | | NG | NG | | NG | NG | | | | |
| | B | NG | NG | | NG | NG | | NG | NG | | | | |
| | C | NG | NG | | NG | NG | | NG | NG | | | | |
| Control | D | G | G | | G | G | | G | G | | | | |
| | E | G | G | | G | G | | G | G | | | | |
| | F | G | G | | G | G | | G | G | | | | |
| | G | | | | | | | | | | | | |
| | H | | | | | | | | | | | | |

Minimum inhibitory concentration (MIC assay) was carried out to determine the lowest concentration of Te nanoparticles that inhibits the activity of pathogenic bacterial strains. After incubation of test strains in MHB for 18h with varying percent supplementation of Te nanoparticles, the observation was taken at 600 nm in microplate reader.

**Table 5: Growth profile of *E.coli* MTCC 1302 on exposure to Te nanoparticles as depicted by absorbance at 600nm**

| Conc. (Te nanoparticles) | $C_3a$(OD at 600nm) | $C_3b$(OD at 600nm) |
|---|---|---|
| 0% | 0.62 | 0.63 |
| 10% | 0.56 | 0.53 |
| 50% | 0.52 | 0.50 |
| 100% | 0.45 | 0.47 |

Synthesis of nanosized particles with antibacterial properties (nanobiotics), is of great interest in the development of new pharmaceutical products. The use of Te nanoparticles as an antimicrobial agent is relatively new and has attracted significant attention in recent years. In this study, the antimicrobial activity of Te nanoparticles synthesized against different species of highly pathogenic bacteria and Te nanoparticles has been used as an antimicrobial agent. The Te

nanoparticles synthesized were proved to have antimicrobial activity against all the tested microorganisms. The mechanism of the bactericidal effect of Te nanoparticles against bacteria is not very well known. In the case of silver nanoparticles, the particles attach to the surface of the cell membrane and alter the permeability and respiratory activity. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles (Govindaraju *et al*., 2010). According to this, it can be presumed that the used Te nanoparticles have good antimicrobial activity with smaller size and larger surface area. The size of metallic nanoparticles ensures that a significantly large surface area of the particles is in contact with the bacterial effluent. Considering a hypothetical case with particles of uniform size, a reduction in the particle size from ~10 μm to 10 nm will increase the contact surface area by $10^9$. Such a large contact surface is expected to enhance the extent of bacterial elimination (Pal *et al*., 2007). Our result demonstrated the ability of C3a and C3b strains on synthesizing Te nanoparticles of small size and their antimicrobial activity represent a significant advancement in the nanomaterial with realistic implications.

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