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Bioprospecting of Gold Nanopartcicles Synthesizing bacteria from various sites of Bangalore

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Abstract

The science of nanotechnology and nanoparticles is the manipulation of matter on atomic sizes and molecular scales. Nanoparticle was subsequently established by the national nanotechnology initiative USA which defines recent science Nanotechnology is a new field for innovative idea and research. Nanotechnology deals with the Nanoparticles having a size of micron and one dimension used various fields of science and technology. This fields of nanotechnology and nanoparticles is defined by size is very broad and include the various fields. The main propose of study is too aimed at isolation and characterization of gold nanoparticles synthesizing bacteria from various underexplored mines places of Bangalore, Karnataka. Which include goldmines located at kolar gold fields and various rivers which intermediately connected to gold mines sector areas by synthesis and characterization of gold bionanoparticles. This science of bionanotechnology refers to the intersection of nanotechnology and biologically inspired nanotechnology uses biological systems as the inspiration for new technologies and the application, the use of microorganism is to help to synthesizing and to change the oxidation state of metal and their microbial. For isolation and characterization of gold synthesizing bacteria by using of various biochemical test and some kinds of serial dilution also needed for characterize the specific the bacteria. For better characterize the bacteria was done by DNA extraction and agarose gel electrophoresis, then the gel is kept under the UV spectroscopy for visible the DNA bands.

Keywords: Collection sample from KGF (kolar gold fields Bangalore), gold nanoparticles, biochemical characterization, Agarose gel Electrophoresis, nanotechnology, silver nanoparticles, antimicrobial activity

Introduction

Nanomaterials are cornerstones of nanotechnology and nanoscience, nanotechnology is broad and interdisciplinary area of research and development that has been grown worldwide. Particles which are basis of science of nanotechnology are ultra fine particles of matter which are measured on the basis use of nanoscale and less than 100 nm in size of particles. Nanoparticles are generally considered as particles with a size up to 100 nm, that have completely new or improved properties as compared to the bulk material that they are collected based on particular characteristics such as size, distribution and morphology. Currently developments in nanoscience and nanotechnology have brought potential building blocks for electronic, optoelectronics, medicines and solar cells. Nanoparticles of noble metals, such as gold, silver and platinum are broadly used in many fields and also directly come in contact with the human body, such as shampoos, soaps, detergents, shoes, cosmetic products, and tooth paste, besides medical and pharmaceutical applications.

The nanotechnology is very small objects which indeed this branch of science and technology which deals with materials having at least one micron dimensional in the sizes range of 1 to100 nm.

The structure is vary according to their dimension it's depend on the nano materials is classified, the micron or it may be called zero dimensional nano structure are nanoparticles. The one dimensional structure of nanomaterial's is structure is fibers or nano rods, some cases nano rods may be consider as one dimensional structure of nano materials.

Classification of nanoparticles

Nanoparticles consisting of different physical and chemical properties has been constructed in nanotechnology, classification of nanoparticles are changing and currently nanoparticles can be seaparated into several different, first there are nanoclusters that are semi crystalline with dimension within 1-10 nm and narrow size, second there are nanopowders that results aggregation of non-crystalline nanomaterials with dimension 10-100 nm, third are the nanocrystal that are single crystalline nanomaterials with dimensions 100-1000 nm.

Gold Nanoparticles

Gold nanoparticles are widely used in biotechnology and biomedical field because of their large surface area, and high electron conductivity. The modification of the nanometers is conducted to enhance the interaction of these nanoparticles with biological cells. Enhanced permeability and retention are the unique property of nanoparticles to accumulate and interact with the tumor cells. Drug delivery systems depend on nanoparticles, which is used in targeting malignant brain tumors where the conventional therapy is not as much effective. The gold nanoparticles proved to be the safest and much less toxic agents for drug delivery. Nanoparticles such as dendrites, quantum dots, polymer gels, and gold nanoparticles have more properties and widely used in some application such as drug delivery systems and imaging. Inorganic nanoparticles are widely used as a contrast agent in some application, especially molecular imaging such as computed tomography, positron emission tomography, magnetic resonance imaging, optical imaging, and ultrasound.

Gold nanoparticles synthesis

Nanoparticle synthesis is generally carried out by a variety of physical and chemical methods, such as laser ablation, pyrolysis, chemical or physical vapors deposition, lithography electro-deposition, solar gel etc., which are not eco friendly. Although the commercial methodologies have proven as efficient tools for synthesizing, but their continuous use may pose a great threat to human health and the environment because of the use of toxic and hazardous reagents and generation of toxic by-products in some instances. Green mediated synthesis and characterization of nanoparticles have emerged as a significant division of nanotechnology in the last decade, particularly for noble metals such as gold, silver, platinum and palladium, the chemical or physical approaches are usually synthesize metals nanoparticles because of their intrinsic advantage in producing well defined nanoparticles with quite controlled shape and sizes.

Metal nanoparticles have a high specific surface area and surface atoms, because of their outstanding physicochemical characteristics, including optical, catalytic, electronic, magnetic and antibacterial properties. Synthesis of metal nanoparticles is

enormous due to their potential applicability in different areas such as electronics, chemistry, energy, and medicine development. Metal nanoparticles, particularly noble metals, have been studied mainly because of their strong optical absorption in the visible region caused by the group excitation of the free electron gas. The silver nanoparticles have a large area of interest as they have a large number of applications: nonlinear optics, spectrally selective coating for solar energy absorption, biolabeling, and intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions, antibacterial materials, chemically stable materials and good electrical conductors.

Properties of nanomaterials

1. Optical Properties

Gold nano particles are most probably chemical inert and having greater biological compatibility; the optical properties such as Plasmon resonance are fluorescence and chemiluminescence is better explored by gold nanoparticles. Most of gold nanoparticles provide an particularly microscopic probes for the study of different types causative agent of cancer cells, that gold nanoparticles accumulate in the various cancer cells and then its shown the its particular effect of cytotoxic , apoptosis of the particularly specific cell and its specific part of receptors part of the cell.

2. Antibacterial properties

The antibacterial properties of gold nanoparticles is shown antibacterial potential for the various sources of bacteria gram negative bacteria like *E. coli* and gram positive bacteria like pseudomonas. In comparison of gold nanoparticles that silver nanoparticles improve the activity of penicillin, gentamicin in bacteria they may altering the membrane permeability and improving drug delivery. The gold nanoparticles may have that antimicrobial property to induce with addition of various types organic drugs.

3. Biochemical Sensors

Biochemical sensors of gold nanoparticles is most commonly used chemical and biological analytics, the main properties of gold nanoparticles for biochemical sensing is main function of sensing depends on the detection the various methodology and analysis the particularly subsections, these are include colorimetric, electro chemical sensing and fluorometric based on various methods of analysis. The most commonly surface Plasmon resonance and Raman based sensor is commonly used to analysis and detection of sensing the groups of elements.

4. Catalytic properties

The catalytic properties of gold nanoparticles of various sizes is prepared by using of two methods of reduction in reverse solution, gold nanoparticles are capable of that absorbing molecular hydrogen and its shown the catalytic activity in the such reaction, the main reaction is the sizes dependence of the catalytic activity of gold nanoparticles in the reaction of H_2- O_2 exchange of the methods of preparation was observed. Most of gold nanoparticles less than 3-5 nm in the diameter are nearly catalytic activity for several bio chemical reactions. The main focus that effect of chemical activity gold nanoparticles may change with particles shape and sizes.

Materials and Methods

i. Survey and selection of the sites

A survey was conducted for selection of various sites of gold mines and related water sources area. Goldmines kolar gold filed which is 45km far from Bangalore city that were survey and selected the sample.

ii. Sample collection

Four samples were collected from different station of gold field's soil station 1, station 2 and similarly different station of water sample also collected. The entire samples were kept at 4^0c in refrigerator in laboratory till further experimentation.

Figure: 1 sample collection

iii. Medium for isolation of bacteria

Two different culture media were taken for isolation of gold nanoparticles synthesizing bacteria by used Nutrient agar media and EMB agar media, medium producing maximum growth of bacterial colonies was selected for isolation of bacteria.

iv. Inoculation of sample on medium

One gram of soil and 1 ml of water sample collected was dissolved in 9ml of sterile water and by using serial dilution techniques, isolation of bacterial isolate was done. Each time 0.1ml of sample was taken from serial diluted test tubes and spread on the nutrient media and EMB media plates, these plates were incubated at 37^0c for 24-48 hours for bacterial growth. Individually colonies were restreaked repeatedly and the isolated colonies were stored at 4^0c in refrigerator.

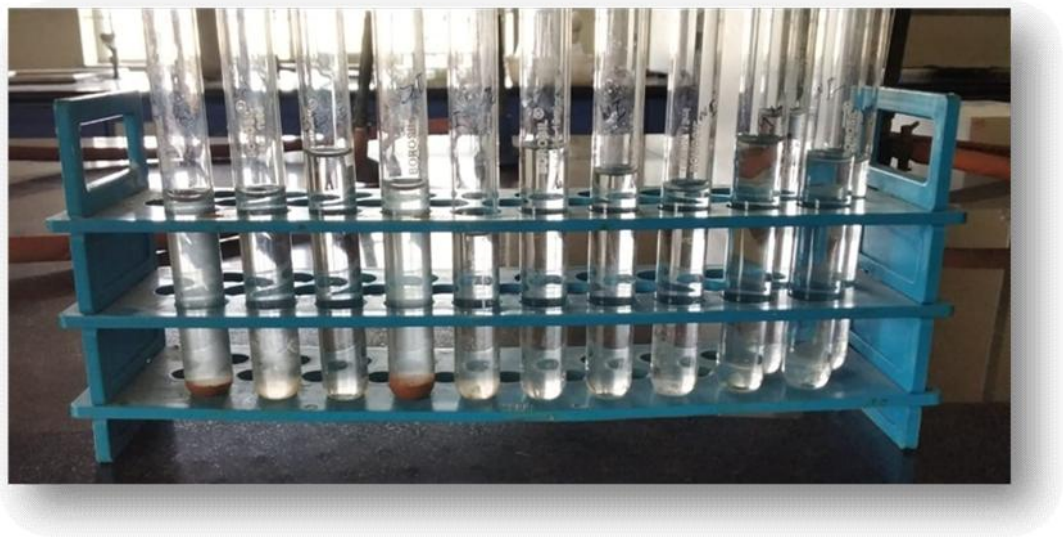

Figure: 2 serial dilutions

v. Microscopic characteristics of isolates bacteria

Gram staining

Take a clean slide make smear and heat fix it, flooded the fixed smear with crystal violets lets dry for 2min. pour off the strain and gently wash with tape water, flood with grams iodine and allow it for 2min then gently wash off with tape water shake off the excess water from the surface. Decolorize with 95% ethanol for 3 seconds until blue dye no longer flows from smear then finally add counter strain saffranin the observe under microscope.

vi. Biochemical characterization

Indole test

Peptone broth was prepared and sterilized at 121^0c for 15 min and inoculated with test organism, incubated the medium at 37^oc for 24 hours, Added 1 ml of kovac reagent to tubes including control. Shook and observed the tubes for presence of rings.

Methyl Red test

Prepared MR-VP broth in two flasks, inoculate the broth with the test organism and incubated for 24 hours at 37^0c, after 24 hours of incubation transferred 5 ml of broth into two test tubes. To the each broth culture added 5 drops MR indicator the tubes and shake them. Examine the colors of the each culture.

Voges – proskauer test

Prepared MR-VP broth in two flask, inoculate the broth with the test organism and incubated foe 24 hours, prepared BARRITT regent A and B. after 24 hours of incubation 0.5 ml of reagent A and 0.2 ml of reagent B was added to the broth and observe for color change.

Citrate utilization test

Prepared citrate agar slant and inoculated each of the test organism into appropriately labeled tubes by means of a loop, the slant was left UN inoculated that serve as control, incubated for 24 hours at 37^0c. After 24 hour all agar slant were examined for the presence of growth and coloration of the medium.

Catalase test

Transferred small quantity of culture from the plates on glass slide, add 1 drop of 3 % H_2O_2 observe bubbles formation.

Oxidase test

Taken oxidase disc in clean microscopic slide, pasted the culture on the oxidase disc and observed for color changes.

Nitrate test

Prepared nitrate broth and inoculated each of the test organisms into its appropriately labeled tubes means of a loop. The last slant was left un inoculated that serve as control, incubated all culture for 24 hours at 37^oc , after 24 hours add one dropper full of sulfanilic acid and one dropper full of α naphthylamine to each broth. Broth were examined for the change in coloration of the medium, a color change to red indicates a positive nitrate reduction test.

Starch test

Prepared starch agar and inoculated each of the test organism into its appropriately labeled tubes by means of a loop, the last plates was left un inoculated that serve as control, incubated all culture for 24 hours at 37^0 c. after 24 hours all agar slants were examined for the presence of growth and zone formation on the medium, add iodine solution to see the zone formed more vividly.

Gelatin test

Prepared gelatin slant and inoculated each of the test organism into its appropriately labeled tubes by means of a loop. The slant was left un inoculated that serve as control, incubated all culture at the bacterium optimal growth temperature for up to 1 week and checked every day for gelatin liquefaction. Gelatin normally liquefies at 28^0c and above, so to confirm that liquefaction was due to gelatinase activity. The tubes are immersed in an ice bath for 15 to 30 min, Afterwards tubes are tilted to observe if gelatin has been hydrolyzed, hydrolyzed gelatin will results in liquid medium even after exposure to cold temperature (ICEBATH), and while the UN inoculated control medium will remain solid.

vii. Antimicrobial susceptibility

The performance of antimicrobial susceptibility testing by clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents.

The disk diffusion susceptibility methods is simple and practical and has been well standardized, the test performed by applying bacterial inoculums of approximately 1-2 * 10^8 cfu/ml to the surface of large Mueller Hinton Agar plates, up to 12 commercially prepared fixed concentration paper antibiotics disk are placed on the inoculated agar surface. Plates are incubated for 16 – 24 hours at 37^0c prior to determination of results; the zones of growth inhibition around each of the antibiotics are measured to the nearest millimeter. The diameter of the zones is related to the susceptibility of the isolate and to the diffusion rate of the drug through agar medium.

viii. Agarose gel electrophoresis

Agarose gel electrophoresis is methods used to separate a mixed population of macromolecules such as DNA or protein in Matrix of agarose, it is easy to separate DNA of size range most often in laboratories.

- Casting the gel is prepared by dissolving the agarose powder in an buffer as TAE buffer to be used to electrophoresis,
- The agarose is dispersed in buffer before heating it near boiling point but avoid boiling.
- The melted agarose is allowed to cool sufficiently before pouring the solution into cast if the agarose solution is too hot.
- A comb is placed in the cast to create wells for loading sample and the gel should be completely set before used.
- Once gel is set the comb is removed, leaving wells where DNA sample can be loaded.
- Loading the buffer is mixed with DNA sample before the mixture is loaded into the wells
- The loading buffer contains a dense compound which May be glycerol, sucrose and ficoll that raise the density of the sample so that the DNA sample may sink to the bottom of the wells.
- DNA and RNA normally visualized by staining with ETBR, which intercalates into the major grooves of the DNA and fluoresce under UV light.

Figure: 3 Agarose gel electrophoresis

Results and Discussion

Table: 1 colony characterization

Sl.no	Isolate	Color of colony	Sizes mm	Shape	Optical properties	Elevation
1	Station1 soil	White	1.7	Circular	Opaque	Raised
2	Station 2 soil	White	1.6	Circular	Opaque	Raised
3	Station 1 water	Creamish white	1.9	Irregular	Opaque	Raised
4	Station 2 water	White	2.1	Circular	Opaque	Raised

Table: 2 Biochemical characterizations

Sample	Indole	Methyl red	Vp test	Citrate utilization	Catalase test	Oxidase test	Nitrate test	Strach test
Soil 1	+	--	+	+	-	+	-	+
Soil 2	--	+	+	--	+	+	+	--
Water 1	+	+	--	--	--	--	+	+
Water 2	--	+	+	--	+	+	--	+

Table: 3 Microscopic analysis

Sample	Soil station 1	Soil station 2	Water station 1	Water station 2
Gram +	+			+
Gram -		--	+	

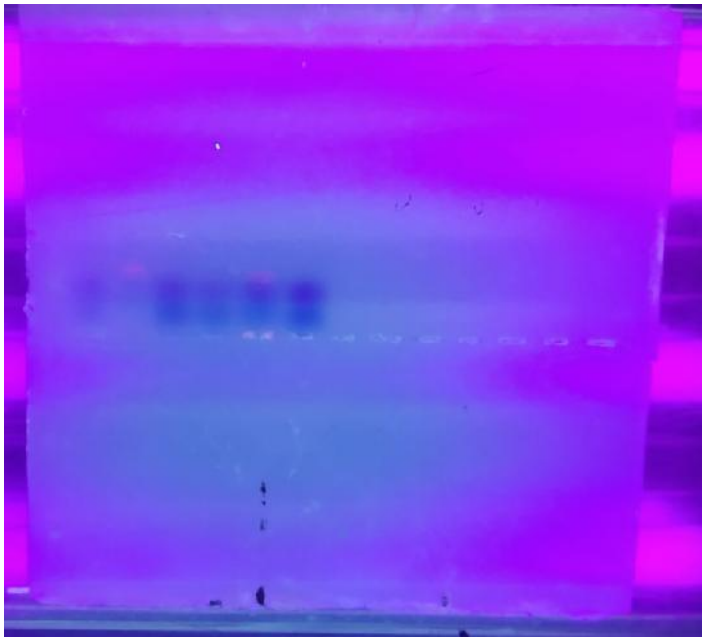

Figure: 4 Agarose gel electrophoresis sample1

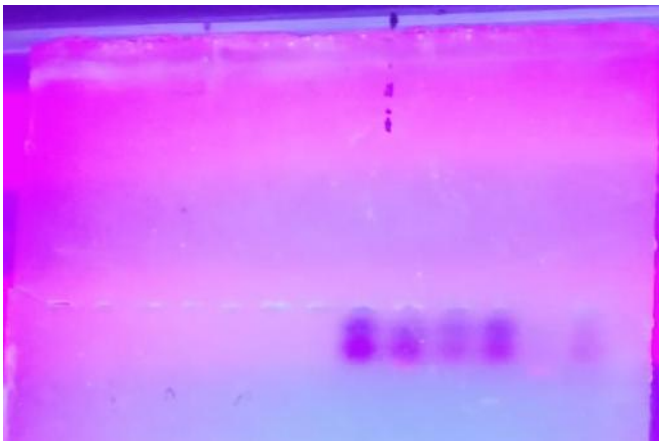

Figure: 5 Agarose gel electrophoresis sample 2

Figure: 6 formations of gold synthesizing bacteria

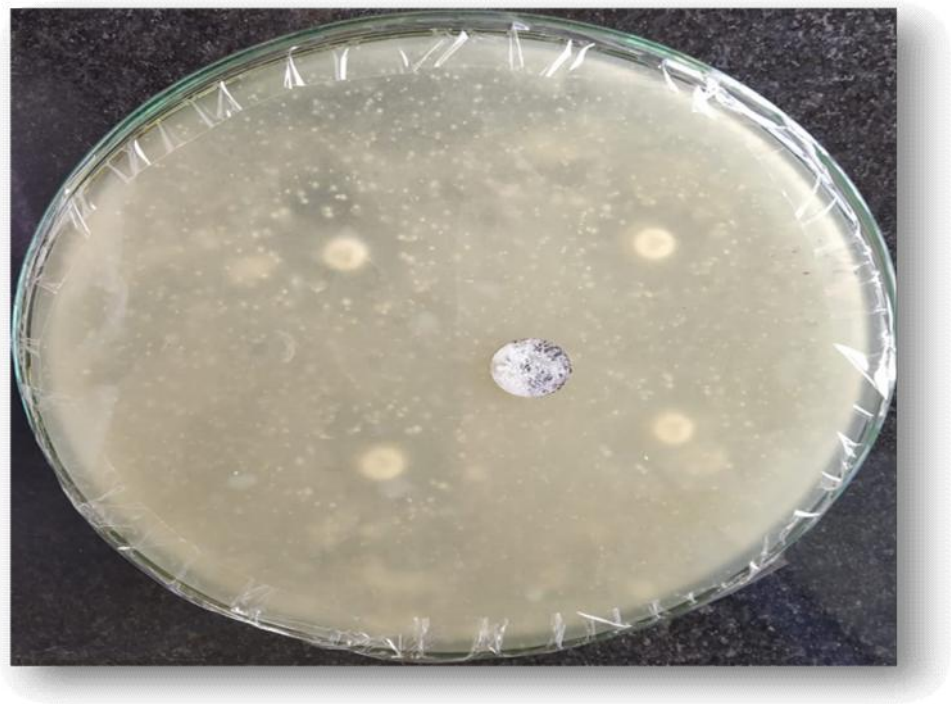

Figure: 7 Antimicrobial susceptibility tests

Conclusion

Kolar gold fields and its related area water were chosen 4 types of sample with different station of water and soil sample, including all sample were test all biochemical test and microscopic test we got all results according to that we concluded the particular gold synthesizing bacteria , for major sources of present of gold synthesizing bacteria if it is present then the media color will change from another color but here we found it the lees amount of gold particles is there so synthesizing bacteria did not change it color on media bottle, its change yellow to dark yellow color which indicate the bacteria is present with less concentration in amount.

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