Phycosynthesis of silver nanoparticles using *Spirulina platensis* as a potential source of antibacterial agent

INTRODUCTION

Nanoscience is currently a fast growing niche and nano-technology is at the cutting edge of this rapidly evolving area (Mandal *et al.*, 2006). Nano-technology collectively describes technology and science involving nano-scale particles (nanoparticles) that increases the scope of investigating and regulating the interplay at cell level between synthetic materials and biological systems (Du *et al.*, 2007). It can be employed as an efficient tool to explore the finest process in biological processes (Sondi & Salopak, Sondi, 2004) and biomedical sciences (Hutten *et al.*, 2004).

The word “nano” is used to indicate one billionth of a meter or 10^-9. The term nano technology was coined by Norio Taniguchi a, researcher at the University of Tokyo, Japan. Nanotechnology is field that is burgeoning day by day making an impact in all spheres of human-life (Vaidyanathanh *et al.*, 2009). Besides this, NPs play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering (Marones *et al.*, 2004). In the current context, importance is being given to the fabrication of a wide range of nano-materials for developing environmentally benign nanotechnologies in material synthesis (Bhattacharya *et al.*, 2005).

One of the major developments in nanotechnology is the production and application of nanoparticles in biology. New methods to produce nanoparticles are constantly being studied and developed. The enormous interest in the bio-synthesis of NPs is due to their unusual optical (Krotiknowska *et al.*, 2003), chemical (Kumar *et al.*, 2003), Photochemical (Chandrasekharan *et al.*, 2000), electronic (Peto *et al.*, 2002) and magnetic (Watson *et al.*, 1999), properties, NPS are either newly created via nanotechnology or are present naturally over the earths crust or in the environment caused by weathering of Au deposits. The metal formed by evaporation is coupled with minerals and been deposited rapidly from saline ground water. Nanotechnology has been defined as a technology that mainly consist of the process of separation, consolidation and deformation of materials by one atom or molecule (Taniguchi, 1974).

The micro-organisms, such as bacteria, yeast and fungi play an important role in remediation of toxic metals through reduction of the metal ions; this was considered interesting as nanofactory, using the dissimilatory properties of fungi and hence these biological systems have been extensively used for the rapid and eco-friendly biosynthesis of metal nanoparticles.
Uni and multicellular organisms have shown immense potential for the synthesis of nanoparticles, as they can synthesize inorganic materials either intracellularly or extracellularly. More recent and detailed investigations into the use of microbes in the synthesis of different metal nanoparticles include bacteria for gold (Beveridge and Murray, 1980; Southam and Beveridge, 1996; Fortin and Beveridge, 2000), silver (Joerger et al., 2000; Klaus et al., 1998), ZnS (Labrenz et al., 2000), magnetite (Lovley et al., 1987, 1999, 2001; Nair and Pradeep, 2002), CdS (Smith et al., 1998;Philipse and Maas, 2002), iron sulfide (Watson et al., 1999, 2000), yeast for PbS (Kowshik et al., 2002a), CdS (Kowshik et al., 2002b), and silver (Kowshik et al., 2003), and algae for gold (Robinson et al., 1997).

Recently the development of resistant or even multi resistant pathogens has become a major problem for instance *Staphylococcus aureus* resistance to methicillin and *Candida albicans* resistance to fluconazole have to be mentioned (Schaller et al., 2004) on the other hand, the introduction of newly devised wound dressing has been a major breakthrough in the management of wound or infections. In order to prevent or reduce infection a new generation of dressing incorporating antimicrobial agents like silver was developed (Yin et al., 1999).

It is well known that silver ions and silver based compounds are highly toxic to microorganisms. Thus silver ions have been used in many kinds of formations (Sondi et al., 2004) and recently it was shown that hybrids of silver nanoparticles with amphiphilic hyper branched macro molecules exhibit effective antimicrobial surface coating (Aymonier et al., 2002). Nanometer sized silver particles synthesized by inert gas condensation or co-condensation techniques showed anti bacterial activity against *E.coli* at low concentrations. In addition it was observed a relationship between the antibacterial properties and the total surface area of the nanoparticles. Smaller particles with a larger surface area were more efficient in the antibacterial activity tests (Baker et al., 2005).

Silver is a non toxic safe inorganic antibacterial agent used for centuries and is capable of killing about 650 types of diseases causing micro-organisms (Jeorg et al., 2005). Silver has been described or being “oligodynamic” because of its ability to exert a bactericidal effect at minute concentrations (Percivala et al., 2005). It has a significant potential for a wide range of biological applications such as antifungal agent, antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti inflammatory (Taylor et al., 2005). Silver ions and its compounds are highly toxic to micro-organisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells. Therefore silver ions, being antibacterial component, are employed information of dental resin
composites, bone cement, ion exchange fibers and coatings for medical devices (Panacek et al., 2006: Alt et al., 2004). Compounds of silver such as silver, nitrate and silver sulfadiazine are used to prevent bacterial growth in drinking water, sterilization and burn care. It is economical to consolidate silver in polymers, composites, fabrics and catheters for anti-bacterial functionality (Oloffs et al., 1944, Li et al., 2005, Zhang et al., 2004, Baker et al., 2005).

Your running shoes, socks and even computer keyboard may be impregnated with silver nanoparticles that can kill some bacteria keep you swelling sweet and preventing the spread of infection among computer users. Researchers in India point out that silver nanoparticles are not only antibacterial against so called Gram Positive bacteria, such as resistant strains Staphylococcus aureus and Streptococcus pneumoniae but also against Gram Negative Escherichia coli and Pseudomonas aeruginosa (Pattabi, 2010).

Scientists are reporting development and successful lab tests of “killer paper” a material intended for use as a new food packaging material that helps preserve foods by fighting that bacteria that cause spoilage. The silver coated paper showed potent antibacterial activity against E.coli and S.aureus, two causes of bacterial food poisoning killing all of the bacteria in just three hours. (Langmuir, 2011).

The use of nanoparticles derived from noble metals has spread too many areas including jewelry, medical fields, electronics, water treatment and sport utilities thus improving the longevity and comfort in human life. The application of nanoparticles as delivery vehicles for bactericidal agents represents a new paradigm in the design of antibacterial therapeutics. (Vijayaraghavan and Nalini, 2010). Major consumer goods manufactures like LG and Samsung already produce household items that silver nanoparticles. These products include nano-silver lined refrigerators air conditioners and washing machines (www.azom.com, 2011).

Other current applications for silver nanoparticles impregnated materials include:
* Toy
* Baby pacifiers
* Clothing
* Food Storage Containers
* Face Masks
* HEPA Filters
* Laundry Detergent

Medical application
Other potential applications for silver nanoparticles include:
* Diagnostic biomedical optical imaging
* Dressing and Bandages
* Biological implants
  (Like heart valves)

**BACTERIAL INFECTION**
The recent years infections caused by bacteria resistant to multiple antibiotics have been a significant problem. *Escherichia coli* is the head of the large bacterial family, Enterobactericidal, the enteric bacteria, which are facultatively anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease (Todar, 2011). It is an extremely reversible opportunistic pathogen (Cheesbrough, 2000) causes septic-mias and can infect the gall bladder, meningers, surgical wound skin lesions and the lungs especially in debilitate and immuno-deficit patients (Black, 1996).

*Staphylococcus aureus* is a well armed virulent pathogen that is currently the most common cause of infections in hospitalized patients (Archer, 1998). *S. aureus* is a facultatively anaerobic gram positive, which causes food poisons and usually grows on the usual membranes and skin. It is also found in the gastrointestinal and urinary tracts of warm-blooded animals. Also cause boils, abscesses, wound infection, pneumonia, toxic shock syndrome and other diseases (Cheesbrough, 2000).

*Bacillus subtilis* is not a human pathogen. It may contaminate food but rarely causes food poisoning. It produces the proteolytic enzyme subtilisin and spores can survive the extreme heat during cooking. *B. subtilis* is responsible for causing ropiness, a sticky, stringy, consistency caused by bacterial production of long chain polysaccharides in spoiled bread dough (Ryan and Ray, 2004).

*Bacillus fusiformis* was described originally as occurring in cases of hospital gangrene. It is more common at present in the form of Vincent’s angina, an antiflamatory condition of the throat (McConnell, 2010). The organism has been observed in ulcero-membranous angina, hospital gangrene, noma, appendicitis, diphtheria, forted bronchitis, gangrenous laryngitis, pyorrhea, alveolaris, brain abscess and in the healthy mouth (Peters, 1911).

The pathogenicity of 13 strains of *Bacillus licheniformis* was studied in immunodepressed mice. (Agerholm et al., 1997) There are several reports in the literature of human infections with *Bacillus licheniformis* (Farar, 1963; Banerjee et al 1988; Logan 1988) however these occurred in immunosuppresed individuals or following trauma (Tabbara and Tarabay, 1979).
Due to its ubiquitous presence as spores in soil and dust, *B. licheniformis* is widely known as a contaminant of food (Norris *et al.*, 1990).

Several studies revealed *S. platensis* or its extract could show physiological benefits as antioxidants, anti-microbial, anti-inflammatory, antiviral or anti-fungal (Carreri *et al.*, 2001, Mendes *et al.*, 2003, Pinero, 2001, Subhashim *et al.*, 2004). The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The majority of clinically used anti-microbial drugs have various draw back in terms of toxicity, efficacy, cost and their frequent use has led to the emergence of resistant strains.

Thus there is an urgent need to develop alternative biodegradable agents, which could be free from side effects. This search prompted the exploration of natural Algal product that could be exploited as biodegradable, more systematic and non-toxic anti-microbial agent with microbial toxic properties. There are few reports present regarding the anti-microbial activity of *Spirulina* against some pathogenic bacteria and fungi (Boussiba and Richmond 1978, Ozdemir *et al.*, 2004, Mendiola *et al.*, 2007, Santyo *et al.*, 2006) and anti-bacterial effect of silver nanoparticles (Morones *et al.*, 2005, Feng *et al.*, 2000, Sharma *et al.*, 2009, Kim *et al.*, 2007, Duran *et al.*, 2007). Thus anti microbial effects of silver (Ag) ion or salts are well known, but the effects of Ag nanoparticles on micro-organisms and anti-microbial mechanism have not been revealed clearly. But no report is available regarding effectiveness of antimicrobial activity of silver nanoparticles produced by *Spirulina platensis*. Therefore, the present work entitled, “Phycosynthesis of silver nanoparticles using *Spirulina platensis* as a potential source of antibacterial agent.” is chosen for the present study.

**PRELIMINARY WORK ON SPIRULINA PLATENSIS AGAINST PATHOGENIC BACTERIA**

Preliminary work of silver nanoparticles synthesized using living and non-living stains of *Spirulina platensis* and its antibacterial activity against some pathogenic bacteria such as *E. coli*, *B. fusiformis* etc have already done. Among non-living strains of *S. platensis* of Jalmahal Lake, Jaipur was found to show synthesis of AgNPs and its antibacterial activity against *E.coli* as compare to other non-living strains (Ramgarh and Rajkot strain). Living culture of *Spirulina platensis* also shows synthesis of silver nanoparticles and its antibacterial activity. Silver nanoparticles synthesized by *Spirulina platensis* were monitored and characterized by UV-vis spectroscopy and FTIR.
REVIEW OF LITERATURE
CHARACTERISATION OF SYNTHESIZED SILVER NANO PARTICLES USING SPIRULINA & OTHER ALGAE

Alga is diverse group in plant kingdom that is being explored for application in nanotechnology. Besides the production of NPs algae are also being explored for determining its mutational value, efficacy in bio-diesel improvement as well as its vast potential for therapeutic application (Sinha et al., 2009).

Interaction of single cell protein of Spirulina platensis with aqueous silver nitrate (AgNO₃) and chloroauric acid (HAuCl₄) was investigated for the synthesis of Ag, Au and Ag core Au shell nanoparticles (Govindaraju et al., 2008). High resolution transmission electron microscopy showed formation of nanoparticles in the range of 7-16 (Silver), 6-10 (Gold) and 17 – 25 nm (bimetallic 50:50 ratio). XRD analysis of the silver and gold particles confirmed the formation of metallic silver and gold. Fourier transform infrared spectroscopic (FTIR) measurements revealed the fact that the protein is the possible bimolecular responsible for the reduction and capping of the biosynthesized nanoparticles. In another attempt biological synthesis of silver, gold and Ag shell-Au core nanoparticles using single cell protein Spirulina platensis and seaweed Sargassum wightii have been achieved. (Singaravelu et al., 2007). Tsibakhashvili et al (2010), reported synthesis silver nanoparticles using blue green alga S. platensis and actinobacteria-Streptomyces spp. The samples have been characterized by UV-Visible spectrophotometry and XRD.

The formation of extracellular silver nanoparticles by photoautotrophic cyanobacterium Plectonema boryanum had been described (Langke et al., 2007). The reaction products were analyzed using transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS). Ali et al (2011), reports the extracellular biosynthesis of silver nanoparticles using marine cyanobacterium, Oscillatoria willei NTDM01 which reduces silver ions and stabilizes the silver nanoparticles by a secreted protein. The silver nitrate solution incubated with washed marine cyanobacteria changed to a yellow color from 72h onwards, indicating the formation of silver nanoparticles. The characteristics of the protein shell at 265nm were observed in Ultra violet spectrum for the silver nanoparticles in solution. While Fourier Transform Infra Red (FTIR) confirmed the presence of a protein shell which are responsible for the nanoparticles biosynthesis. Scanning Electron Microscopy (SEM) studies showed that the formation of agglomerated silver nanoparticles due to the capping agent in the range of 100 - 200nm. EDS spectrum of the silver nanoparticles was confirmed
the presence of elemental silver signal in high percentage. Apart from Eco-friendliness and easy availability and low cost cyanobacterial biomass production will be more advantageous when compared to other classes of micro organism. Extracellular synthesis of silver nanoparticles by brown seaweed, Sargassum wightti was characterized by means of UV-Vis spectroscopy, FTIR, XRD and HR-TEM. Antibacterial studies were carried out using the bacterial isolated from the infected silkworm. The recorded antibacterial effect of silver nanoparticles was found more potent when compared to the chemically synthesized silver nanoparticles and it is expected to be bio compatible. (Govindaraju et al, 2009). Bunghez et al (2010) obtained silver nanoparticles from AgNO₃ using red algae (Porphyridium purpureum). The red algae contain the red pigment-phycobilins, responsible for red color and for the strong absorption in visible spectrum. The properties and structure of silver nanoparticles have been put into evidence by means of: Fourier transform infrared spectroscopy-FTIR, optical microscopy, X-ray fluorescence spectrometry-EDXRF. Venkatpurwar and Pokharkar (2011) synthesize silver nanoparticles through green route using sulfated polysaccharide isolated from marine red algae, Porphyra vietnamensis. FTIR spectra revealed the involvement polysaccharide for reduction of silver nitrate. The dose dependent effect of synthesized silver nanoparticles revealed strong anti bacterial activity against gram negative bacteria as compared to gram positive bacteria.

ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES AGAINST BACTERIA
The antibacterial property of silver has been known for thousands of years with the ancient Greeks cooking from silver pots and the old adage’ born with a silver spoon in his mouth’ referring to more than just wealth. Eating with a silver spoon was known to be more hygienic. Manufacturing entire objects from pure silver is prohibitively expensive for consumer items but research has found that impregnating other materials with silver nanoparticles is a practical way to exploit the germ fighting properties of silver (www.azom.com, 2011). Application of silver nanoparticles can prevent accumulation of bacterial contaminants from accumulating in the further protecting the wearer from other infections. The success of silver nanoparticles against bacterial growth is due to damage of plasma membrane or bacterial enzymes. This results in morphological distortion of the bacterial cells, leading to impairment of bacterial metabolism and escape of cytoplasmic substance to the surroundings. The coating of medical instruments is one recent silver nanoparticles medical application. The treated instruments were observed to have a powerful bacterial action against Klebsiella pneumoniae, Bacillus anthracis sterne and Bacillus subtilis for 1 hour and 30 minutes. Staphylococcus
*Staphylococcus aureus* and *Acinetobacter baylyi* were contained for 5 hours. (www.silvernanoparticles.info, 2010).

The antibacterial activity of silver nanoparticles synthesized by *Streptomyces* species is proving against the multi drug resistant bacteria of Gram positive (*Staphylococcus aureus*, *S. epidermidis* and Gram negative strains (*E. coli*, *S. typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*) (Shirley *et al.*, 2010). Silver nanoparticles synthesized by *Fusarium oxysporum* strain incorporated in cotton cloth exhibit antibacterial activity against *Staphylococcus aureus* to become them sterile. (Marcato *et al.*, 2010). The AgNPs were evaluated for their anti-microbial activities against different pathogenic organisms. The most sensitive antimicrobial activity has been observed against methicillin resistant *S. aureus* followed by methicillin resistant *S. epidermidis* and *Streptococcus pyogenes* whereas only moderate anti-microbial activity was seen against *Salmonella typhi* and *S. pneumoniae* (Nanda and Saravanan, 2009).

Inhalation of silver nanoparticles results in “Miraculous” protection against pneumonia caused by *Pseudomonas aeruginosa*, according to a study conducted by researchers from the Washington University School of Medicine and the University of Akron, Ohio and presented at the 105th national Conference of the American Thoracic Society (Gutierrez, 2002).

Agile silver nanoparticle is a pure liquid silver suspension product which can achieve over 99.9% effect in inhibiting growth of numerous kinds of bacteria, including multiple drug resistance bacteria and fungi (Lok *et al.*, 2007). Agile silver nanoparticles can inhibit the growth of *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumanii* which are silver ion resistant and multiple-drug resistance.

**ANTIBACTERIAL ACTIVITY OF SPIRULINA PLATENSIS & OTHER ALGAE**

Algal organisms are rich source of structurally novel and biologically active metabolites. These induce antibiotics which in laboratory tests inhibited bacteria and fungi that incite diseases of humans (Kulik, 1995). Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activites such as antialgal, antibacterial, antifungal and antiviral activity (Noaman *et al.*, 2004). The crude extracts of *Spirulina platensis* were tested for their antimicrobial activities against different species of human pathogenic Bacteria [(*Klebsiella pneumoniae* (NCIM 2063), *Proteus vulgaris* (NCIM 2027), *Pseudomonas aeruginosa* (NCIM 2076) and *Escherichia coli* (NCIM 2065), *Salmonella typhi* (NCIM 2080),] by the agar-solid diffusion method (Mala *et al.*, 2009). Ozedmir *et al* (2004) tested *S. platensis* in vitro for their antimicrobial activity against four
Gram positive, six Gram negative bacteria and *Candida albicans* ATCC 1023. In another attempt, 3 cyanobacteria (*Anabaena oryzae*, *Tolypothrix ceytonica* and *Spirulina platensis* and 2 green (*Chlorella pyrenoidosa* and *Scenedesmus quadrisporia*) were tested in compliance with the agar well diffusion method for their antibacterial and antifungal agent production on various organisms that incite diseases of humans and plants (*E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Penicillium herquei*, *Fusarium moniliforme*, *Helminthosporium* species, *Alternaria brassicae*, *Saccharomyces cerevisiae*, *C. albicans*) (Rania et al., 2008). Antibacterial activity of methanolic extracts from 32 macroalgae (13 Chlorophyta and 19 Phaeophyta) from the Atlantic and Mediterranean coast of Morocco were evaluated for the production of antibacterial compounds against *E. coli*, *S. aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *E. faecalis*. (Ibtissum et al., 2009). Khairy et al (2010) examine 3 blue green algal species (*Anabaena flos aquae* (L.) Bory, *Anabaena variabilis* (Kutzting) and *Oscillatoria angustissima* (West and West) against 8 Gram+ve and Gram-ve bacteria (*B. subtilis* 1020, *B. cereus*, *S. aureus*, *Streptococcus faecalis* (G+ve), *E. coli* 1357, *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Vibrio fluvialis* Antimicrobial activity of cyanobacteria isolated from the mangrove forest of Sundarbans have shown Minimum inhibitory concentrations (MIC) of the extracts against *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, and multiple drug-resistant clinical isolates ranged between 0.25-0.5 mg/ml.(Pramanik et al., 2011).
OBJECTIVES

1. Isolation, identification and purification of *Spirulina platensis* for synthesis of silver nanoparticles.
2. Purification of pathogenic strains of isolated bacteria, available in Microbiology Lab.
3. Screening of living and non living strains of *Spirulina platensis* for biosynthesis of AgNPs.
4. Characterization of AgNPs of most potent strains of *Spirulina platensis*.
5. Antimicrobial activity & MIC of biosynthesized strains of AgNPs against pathogenic bacteria.

METHODOLOGY

**Criteria of Selection**

**ALGAL SPECIES** (*Spirulina platensis*)

*Spirulina* is a photoautotrophic prokaryote found in a variety of environments e.g., soil, sand, marshes, brackish, seawater and freshwater. It is abundantly available throughout the year.

**TARGET SPECIES** (Pathogenic bacteria)

The pathogenic bacteria included in the present study will be *E. coli, Staphylococcus aureus, Bacillus subtilis, Bacillus fusiformis* and *Bacillus licheniformis*.

**ISOLATION, PURIFICATION AND CULTIVATION OF SPIRULINA PLATENSIS**

*Spirulina platensis* will be isolated from Dayalbagh region and cultivated in the CFTRI medium. Before experimentation the biomass will be washed thrice in deionized water to remove unwanted materials (Govindaraju *et al.*, 2008)

**MEDIA PREPARATION**

The media used for culturing *S. platensis* is CFTRI medium (4.5 g sodium bi carbonate, 0.5, Di-Potassium, 1.5 Sodium nitrate, 1 potassium sulphate, 1.0 sodium chloride, 0.2 magnesium sulphate, 0.04 Calcium Chloride, 0.01 Iron sulphate.) The media used for culturing the organisms would be Nutrient Agar Media (NAM) (5 g Peptone, 3 g beef extract and 5 g NaCl in a litre solution) for *S. aureus, E. coli, B. subtilis B. fusiformis* and *B. licheniformis*.
A. Isolation of *Spirulina platensis*

*Spirulina platensis* culture will be obtained from Microbiology lab, Department of Botany, Dayalbagh Educational Institute, Dayalbagh, Agra. The culture would be picked up from the stock culture with the help of needle and transferred to petriplates and culture tube containing CFTRI medium and incubated at 28°C for 30 days with (600 – 1600 lux) with a continuous light, 12 hrs per day (Sony *et al*., 2006). Identification will be done using morphological variation, studies and taxonomical approaches according to Anagnostidis and Komarek, 1998 and Desikachary, 1959.

B. Isolation of Microbial Cultures

Microbial cultures (*E. coli, S. aureus, B. subtilis, B. fusiformis* and *B. licheniformis*) used will be obtained from the Microbiology lab., Department of Botany, Dayalbagh Educational Institute, Dayalbagh, Agra. Bacterial cultures would be picked up from the stock cultures with the help of inoculation needle and transferred to the petridishes containing NAM medium directly and incubated at 37°C for 24 – 48 hours. In a petridish when bacterial colonies appeared on NAM medium, it would be transferred to other dishes or slants for experiment. Confirmation of bacterial culture would be carried out using “Bergey’s Manual of Systemic Bacteriology” (Claus and Berkly, 1986).

C. Screening of different strains of *Spirulina platensis* for biosynthesis of AgNPs.

Silver nitrate will be purchased from A.B. Company, Agra, India. Before experimentation, the algal biomass will be washed twice in distilled water to remove the unwanted materials. Silver nanoparticles formations will be carried out by taking 1 g of wet biomass in a 250 ml Erlenmeyer flask with 10⁻³ M aqueous AgNO₃ for different times (2, 4, 7, 8 days) and incubated at room temperature. The pH will be checked during the course of reaction and it was found to be 5.6 (Tsibakhashvili, 2010). Non living strains of *Spirulina platensis* will be collected from Jalmahal (Jaipur), Ramgarh (Jaipur) and Rajkot (Gujrat) from Pushpa Srivastava. Silver nanoparticles synthesis using non living culture of *Spirulina platensis* will be carried out by taking 5 mg of *Spirulina* powder in 50 ml of aqueous AgNO₃ solution of 10⁻³ molar concentration. (Govindaraju *et al*., 2008). The time of addition of extract into the aqueous AgNO₃ solution will be considered as the start of the reaction. Under continuous stirring conditions, after 10 min., the light yellow color of AgNO₃ solution will gradually changed to brownish yellow color indicates the formation of silver nanoparticles. The principle of preparation of silver nanoparticles by using microorganism is a bioreduction.
process; the silver ions are reduced by the extracellular reductase enzymes produced by the microorganisms to silver metal in nanometer range (Fu et al., 2006). The reaction involved in this process is shown in Fig-I.

**Fig-I: Reaction showing preparation of silver nanoparticles.**

It is concluded from protein assay of microorganisms that the preparation of silver nanoparticles is an NADH-dependent reductase. The enzyme reductase gains electrons from NADH and oxidizes it to NAD+. The enzyme is then oxidized by the simultaneous reduction of Silver ions forming silver metal in nanoform. In some cases a nitrate-dependent reductase is responsible for the bioreduction process whereas in the case of rapid extracellular synthesis of nanoparticles the reduction happens within few minutes, therefore a complex electron shuttle materials may be involved in the biosynthesis process (Moghadham et al., 2010). Screening of different strains will be done on the basis of intensity of color change during the course of experiment.

**D. Characterization of most potent strains of Spirulina platensis**

The bio-reduction of AgNO₃ ions in solution will be monitored by periodic sampling of aliquots (0.1 ml) of aqueous component and measuring UV-vis spectra of the solution. The nanoparticles will be characterized and confirmed by FTIR, XRD and HR-TEM analysis (Chandran et al., 2006)

**E. Anti-microbial assay**

(1) **Agar well diffusion method:** The silver nanoparticles synthesized using *Spirulina platensis* would be tested for anti microbial activity by agar well diffusion method against pathogenic bacteria. *E. coli*, *S. aureus*, *B. subtilis*, *B. fusiformis* and *B. licheniformis* will be sub-cultured on nutrient agar medium. Wells of 10 mm diameter will be made on nutrient agar plates using gel puncture. Each strain was swabbed uniformly onto the individual plates
using sterile cotton swabs. Using a micropipette different concentration of the sample of nano particles solution (10 µl, 20 µl and 50 µl) will be poured onto each well on all plates. After incubation of 37°C for 24 hours, the different levels of zone of inhibition of bacteriae will be measured. (Govindaraju et al, 2010).

(2) MIC (Minimal Inhibitory Concentration): This concentration was found with lowest possible zone of inhibition. The paper discs in different concentrations were placed on the surface of medium containing bacterial culture. The zone of inhibition will be measured at the diameter and recorded as the MIC.

**IMPORTANCE OF WORK**

Elemental silver occurs naturally. It is considered non-toxic, non-allergic, is not cumulative and is not known to harm either wildlife or the environment. Antibiotic drugs can be used to kill the pathogens attacked by silver nanoparticles but bacteria and viruses are becoming increasingly resistant to drug therapies. Silver nanoparticles kill all types of fungal infections, bacteria and viruses, including antibiotic resistant strains. No drug based antibiotic is effective on all types of bacteria. Additionally, research to date has shown that bacteria have been unable to develop any immunity to silver.

*Spirulina platensis* (SP), a blue-green alga (photosynthesizing cyanobacterium) possesses diverse biological activity due to high content of highly valuable proteins, indispensable amino acids, vitamins, beta-carotene and other pigments, mineral substances, indispensable fatty acids and polysaccharides. *Spirulina* is gaining more attention in the field of medical science because of its nutraceutical and pharmaceutical importance. It has been demonstrated that small amounts of *Spirulina* reduced HIV-1 replication while higher concentration totally stopped its reproduction.

Thus, the *Spirulina* mediated synthesis of Ag nanoparticles and their antibacterial activity communicated in this work gains its importance in its medical application. The present study is likely to explore the unexploited bioefficacy of *Spirulina* synthesized silver nanoparticles as antibacterial agent. The proposed work is likely to add following information to the present state of knowledge to the area of relevance:

*Outcome of new nano phyco product possessing antibacterial activity.*

*Establishment of bioactive constituents responsible for bioefficacies.*

*Provides scope for further research leading to structure of nanodrug reactivity relation.*
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* Original not seen.