CHAPTER 2. LITERATURE OVERVIEW

2.1 Seaweeds (Marine macroalgae)

Marine macroalgae also referred to seaweeds, resemble plants and they generally grow attached to the rocks or some hard substrates in coastal zones [21]. The empirical classification of seaweeds since the mid-19th century has been done into four different groups on the basis of colour. The groups include blue-green algae which belong to phylum *Cyanophyta* consisting an approximate of 15000 species, red algae belonging to phylum *Rhodophyta* including an approximate of 6000 species, brown algae belonging to phylum *Ochrophyta* consisting of about 1750 species and green algae which belong to phylum *Chlorophyta* including an approximate of 1200 species. At a certain point in their life cycle, all seaweeds either exist as unicellular, as spores or zygotes and can possibly exist as planktonic for a short period of time. In seaweed floras, blue-green algae are abundantly found and are more prevalent in temperate rocky and sandy seashores. Seaweeds are predominant all over the world and none of them has been found to be poisonous [22, 23].

Edible seaweeds were extensively consumed as fresh, dried or ingredients in foods in most of the Asian and non-Asian countries. The photosynthetic mechanism of seaweeds is similar to that of terrestrial plants however, the efficiency of converting solar energy to biomass is higher in seaweeds owing to their simple cellular structure and the aqueous environment in which they are submerged. The aqueous environment is beneficial for the seaweeds due to the continuous supply of water, CO₂ and several other nutrients. Since seaweeds contain a rich amount of proteins, vitamins (vitamin A, E, C and Niacin) and minerals and they are believed to be the food supplements for the 21st century. Red and green algae contain a higher concentration of vitamin B12, B1, folic acid, pantothenic acid and folinic acids compared to brown algae [24].
Since the 4th and 6th century, seaweeds have been consumed as a food source in Japan and China respectively. An English Physician in 1750’s utilized ash from kelp (Phaeophyceae) to treat goitre. Kelp contains a rich amount of iodine and has also been utilized in treating obesity in the 19th century whereas agar has been utilized as a laxative. The presence of iodine makes seaweeds a rich iodine source and their crude extracts have been utilized in brewing for clarification. *Chondrus crispus*, a red seaweed, is known for the hydrocolloid carrageen and has been recognized in Ireland since 1810. Another hydrocolloid named Alginic acid was initially discovered by Charles Stanford in 1880s exists in all brown seaweeds. In the late 1920s and early 1930s, California and Scotland have developed a large-scale alginate industry respectively. China has been cultivating *Laminaria japonica* since the 1950s. In industrial and food industries, an increasing amount of applications were found in hydrocolloids in those years. Hydrocolloids are non-crystalline substances giving a viscous solution when dissolved in water and are composed of large molecules. Agar, alginate and carrageenan belong to water-soluble carbohydrates and are utilized to form different gels of variable firmness. These hydrocolloids enhance the viscosity of aqueous solutions [25]. They are also utilized in making water-soluble films and are used as stabilizing agents in ice creams, since they have the ability to inhibit the formation of crystals which gives a smooth texture to ice creams [26].

### 2.1.1 Seaweed sources

Seaweeds are believed to be a polyphyletic group since the three groups of multicellular algae (green, red and brown algae) do not share a common multicellular ancestor [27]. Moreover, several blue-green algae (*Cyanobacteria*) which have the ability of tuft-formation are occasionally believed to be seaweeds (the term seaweed is an informal term which lacks a proper definition). There are two specific environments where seaweeds dominate which include the availability of seawater and the availability of light which is adequate to carry out photosynthesis. One more requisite includes the presence of a strong attachment point and hence seaweeds
commonly occupy the coastal zones where rocky shores inhabit most of the seaweeds compared to sand and shingles. Seaweeds inhabit a range of ecological niches from the sea top to few meters deep. Several coastal seaweeds inhabit the areas several miles from the sea while as a few species of red algae are included among the deepest living seaweeds. Several species like Sargassum have developed as full planktons and float freely depending on the gas-filled sacs which helps in maintaining the adequate depth while as some other species have adapted to tidal rock pools. In order to fully adapt to such conditions, seaweeds must withstand the frequently changing temperatures, salinity as well as occasional drying. Green seaweeds mostly inhabit sandy beaches and they mostly dominate the inter-tidal regions. The presence of chlorophyll is responsible for the green colour and is an essential pigment to perform photosynthesis and hence the green seaweeds need an appropriate amount of light which makes them inhabit less shadowed and not too deep areas [27, 28].

2.2 Endophytes and endophytic actinomycetes

Endophytes are those microbes which inhabit the healthy tissues of the plants intercellularly and/or intracellularly and do not cause any apparent signs of disease [29]. Endophytes are prevalent all over the world and they colonize all plants. Endophytes have been isolated from nearly all the plants studied till date. The association between an endophyte and a plant can be obligate or facultative where the host plants do not get any harm. The interactions between an endophyte and a plant are complex ones which involve mutualism and antagonism [30]. The growth of the endophytes is strictly limited when associated with any plant and they eventually adjust to their living environment [31]. Endophytes have the ability to synthesize numerous compounds that help in promoting the growth of the plants and also maintain the stable symbiotic association between the two [32]. Studies on the endophytic bacteria, fungi and/or actinomycetes have been presenting a challenge since their isolation from plant tissues has begun. Isolation of endophytes involves
the surface sterilization of the plant tissue followed by culturing on a media which is suitable for bacteria, fungi or actinomycetes [33].

Actinomycetes are saprophytes and are essential in breaking down the organic matter into more digestible form. They are also known for their ability to synthesize metabolites which are very useful for humans, animals and plants. Since the last 75 years, several strains of actinomycetes have been isolated from different substrates and have displayed an astonishing biosynthetic potential in culture [34]. Actinomycetes prevail in both the terrestrial and marine environment. Terrestrial substrates have been the predominant source of diverse actinomycetes, however, due to a decline in the number of novel compounds from the terrestrial environment it became crucial to search for new actinomycetes from unexplored habitat and hence the marine environment came into the picture. Since, there is an extreme difference between the conditions that prevail in the marine environment compared to terrestrial ones, it is surmised that the actinomycetes isolated from marine sources might be able to produce different types of novel bioactive compounds [35].

2.2.1 Bioactive compounds from actinomycetes

Actinomycetes represent a genus of biologically important prokaryotes with a dynamic potential of synthesizing bio-actives [34]. Actinomycetes belonging to the genus *Streptomyces* alone serve as a source of a vast number of biologically active compounds. Recently, endophytic actinomycetes have gained a considerable attention and are being isolated from several sources including crop plants and medicinal plants. It has been proven that endophytic actinomycetes improve and promote the growth of the plants in which they reside and also help in reducing the disease symptoms through the production of secondary metabolites. Production of secondary metabolites by endophytic actinomycetes acts as a defence mechanism against plant pathogens. Bioactive compounds from *Streptomyces* possess different biological activities including antimicrobial, antitumor, herbicidal, antiprotozoal, immunosuppressive, etc. [36].
Marine actinomycetes produce the secondary metabolites with different chemical structures which may lead towards the synthesis of novel drugs. Different enrichment and selective methodologies can be applied in order to isolate rare marine actinomycetes with the ability to synthesize novel bioactive compounds. Some of the secondary metabolites from marine actinomycetes with diverse chemical structures include terpenes and terpenoids, polyketides, peptides, caprolactones, polycyclic xanthones, quinones, alkaloids, esters, lactams, etc. [37]. The synthesis of a meroterpenoid, Azamerone by a novel marine bacterium from the genus Streptomyces has been reported [38]. A polycyclic polyketide, Abyssomicin C has been reported from a marine Verrucosispora strain which is far superior in inhibiting the folic acid biosynthesis compared to some familiar sulpha drugs [39]. Bonactin isolated from a marine-derived Streptomyces sp. BD21-2 has been reported to be active against several strains of bacteria [40]. In short, actinomycetes as a whole have got an enormous potential in synthesizing a wide range of bioactive compounds.

2.2.2 Actinomycetes and antibiotics

Actinomycetes are widely recognized due to their diverse range of applications and two-thirds of the currently developed antibiotics come from actinomycetes. Few of those antibiotics include anthracyclines, chloramphenicol, tetracycline, nucleosides, erythromycin, vancomycin, gentamicin, etc. All these antibiotics are very useful in treating a large number of diseases such as respiratory disorders, leprosy, tuberculosis and various other deadly infections [41]. Genus Streptomyces alone has got the potential of synthesizing a wide spectrum of antibiotics and moreover, the source of antibiotics until 1974 was limited to this genus only [42].

Numerous studies have been going on regarding the potential applications of marine actinomycetes as novel antibiotic producers. A novel antibiotic, Abyssomicin C from a marine Verrucosispora strain has been reported to inhibit the folic acid
biosynthesis in addition to a potential antibacterial agent against Gram-positive bacteria in combination with clinical multi-drug resistant *S. aureus* and vancomycin-resistant *S. aureus* [39]. Another novel antibiotic, Essramycin from a *Streptomyces* strain has been reported with antibacterial properties against various bacterial species [43]. Along with the production of novel antibiotics with potent antibacterial properties, marine actinomycetes are continuously being studied for the antibiotics with antifungal properties. A novel antibiotic, Chandrananimycin A from *Actinomadura* species has been reported to act as a potent antifungal and antialgal agent. The antibacterial potential of this particular antibiotic against *S. aureus* and *B. subtilis* has been reported together with anti-cancerous properties [44].

### 2.2.3 Enzyme production by actinomycetes

Actinomycetes have got an overwhelming potential to synthesize a wide range of industrially important enzymes such as amylases, cellulases, hemicellulases, lignolytic enzymes, lipases and xylanases. Marine actinomycetes which differ physiologically, biochemically and molecularly from the terrestrial actinomycetes have got the potential to synthesize different biologically active enzymes [45]. A brief description of different enzymes synthesized by actinomycetes, in general, are given below.

#### 2.2.3.1 Amylases

In order to perform the extracellular digestion, actinomycetes release amylases to the external environment of cells. Bacteria and fungi also secrete amylases to perform extracellular digestion. α-amylase is a biotechnologically valuable enzyme which has a great role to play in food, fermentation, textile and paper industries. α-amylase is a hydrolysing enzyme which hydrolyses starch and glucose to release glucose and mannose as end products. Previous reports on the synthesis of amylases by actinomycetes have been reported, for instance, six
different isolates of actinomycetes isolated from South Indian Coastal region have been reported to show amylolytic activity [46].

2.2.3.2 Cellulases

These enzymes belong to a group of glycosyl hydrolases with cellulolytic activities and are categorized into various families based on their sequence homologies. In general, cellulases are divided into four different groups of enzymes including exoglucanases, endoglucanases, cellulobiohydrolases and β-glucosidases. Extensive studies have been performed on three different strains of cellulase producing actinomycetes including Cellulomonas fimii, Microbispora biospora and Thermobifida fusca [47]. There are two different types of cellulase systems in microorganisms, one complexed and the other non-complexed. Complexed systems (also known as cellulosomes) are the characteristic feature of anaerobic bacteria. Non-complexed cellulase systems (also known as free systems) are present in most of the aerobic bacteria including actinomycetes. This system involves the extracellular secretion of enzymes through specific pathways [48, 49].

2.2.3.3 Hemicellulases

Hemicellulases are related to a family of glycosyl hydrolases and glycosyltransferases. Hemicellulases carry out the digestion of hemicellulose which is composed of xylan and mannan as the main components. This digestion further enhances the cellulose hydrolysis. Thermobifida fusca has been extensively studied for the production of cellulolytic enzymes [47, 50].

2.2.3.4 Lignolytic enzymes

Lignin breakdown is carried out by a complex of three principal enzymes which include Laccases, manganese peroxidases and lignin peroxidases. Laccases are extracellular inducible enzymes which belong to oxidoreductases
utilizing oxygen both as an oxidizing agent and a cofactor. Manganese peroxidases are active at low redox potentials while as lignin peroxidases particularly breakdown the high redox potential compounds. Both manganese and lignin peroxidases are collectively known as heme peroxidases. Actinomycetes including *Streptomyces thermoviolaceus*, *Streptomyces viridosporus* T7A, and *Thermomonospora fusca* BD25 (recently reclassified as *Thermobifida fusca*) have been reported to secrete a few proteins with low peroxidase activity. These proteins have been isolated and partially characterized [50, 51].

### 2.2.3.5 Xylanases

Xylan accounts for about 30-35 % of the dry weight of land growing plants. In order to perform any biological or a chemical process which requires the constituents of xylan, the digestion of xylan is important. The breakdown of this tough layer of xylan is carried out by xylanases, which constitute a group of enzymes working together as a unit. The xylanolytic enzyme system is responsible for hydrolysing the xylan. Fungi, actinomycetes and bacteria have been found to be rich in xylanolytic enzyme systems [52]. Previously carried out studies have reported the utilization of actinomycetes in synthesizing xylanases through submerged and solid-state fermentation [53]. The production of xylan through submerged fermentation utilizing wheat bran as a primary substrate by *Streptomyces lividans* has been reported [54]. The synthesis of extracellular xylanase by a strain of *Streptomyces albus* (ATCC 3005) utilizing oat spelt xylan as a primary substrate has also been reported [55].

### 2.2.4 Actinomycetes as a source of enzyme inhibitors

Actinomycetes are capable of synthesizing low molecular weight enzyme inhibitors which inhibit a wide range of enzymes. The first enzyme inhibitor was reported from a *Streptomyces* strain and until now, about 60 other enzyme inhibitors have been reported. Few of the inhibitors such as leupeptins inhibit trypsin, plasmin
and papain, antipain inhibits papain, chymotrypsin, trypsin and cathepsin B. Enzyme inhibitors from actinomycetes have also been reported to possess anti-cancerous properties. One such enzyme inhibitor has been reported from a *Streptomyces* strain with a name Revistin which inhibits reverse transcriptase and another inhibitor named Alistragin is present in the culture filtrates of *Streptomyces griseoviridis* [42, 56].

### 2.2.5 Actinomycetes and anticancer compounds

Cancer is considered as one of the major health problems and a major reason for mortality all over the globe and besides, the second principal reason of cancer death in women is the breast cancer. At present, the treatment of cancer includes therapeutic approaches like surgery, chemotherapy, radiotherapy and immunotherapy. These treatment methods are either given individually or in combination and are helpful in only in some specific cases depending on the stage of a cancer patient. Actinomycetes have not been studied that deeply regarding their potential as a source of anticancer compounds but still, different compounds have been discovered from marine actinomycetes with anticancer properties. The anticancer activity of cell-free extracts of *Streptomyces* sp. (ACT11 and ACT12) on MCF 7 breast cancer cell lines has been reported [57]. A marine actinobacterium, *Salinispora tropica* has been reported to synthesize pure active compounds with suppressive effects on several malignant cell types [58]. A novel compound, Salinosporamide A has been extracted from *Salinispora tropica* [59] is quite distinct from a commercially available proteasome inhibitor anticancer drug, Bortezomib with different mechanisms of inducing apoptosis in multiple myeloma cells [60].

The anticancer and antimalarial effects of Salinosporamide A and its inhibitory effects on parasite development have been reported previously, however, the exact mechanism of inhibiting the erythrocyte development in *Plasmodium* is not known but it is quite possible that it may be due to the interference with proteasome system.
The inhibition of the proteasome system is therapeutically important since it can stop the growth and development of a large number of cells [58].

2.2.6 Nanoparticle synthesis using actinomycetes

Nanoparticles are receiving a significant attention because of their diverse applications in many areas and they are being continuously studied. Nanoparticles have been synthesized from different biological sources including bacteria, fungi and actinomycetes. Marine actinomycetes have acted as an important source in synthesizing a wide range of novel nanoparticles. Previous studies have reported the role of actinomycetes in nanoparticle synthesis. Biosynthesis of silver nanoparticles from actinomycetes has been reported with better antibacterial potential compared to the chemically synthesized nanoparticles [61]. A green approach of silver nanoparticle synthesis from a marine Streptomyces sp.-MS26 has been reported together with the combined effect of silver nanoparticles and different antibiotics against different pathogens [62]. Biosynthesis of gold nanoparticles from a marine Streptomyces cyaneus has been reported which possessed antimicrobial, antioxidant and antitumor activities under in vitro conditions [63].

2.3 Nanotechnology and nanoparticles

Nanotechnology is an old concept which was initially proposed by Richard Feynman in 1959 while addressing the topic entitled “There's plenty of room at the bottom” at the California Institute of Technology. The name nanotechnology has been derived from a Greek word “dwarf” meaning small and is often defined as a collective art of science and technology of small things. At nanoscale dimensions, there is a change in the behaviour of particles and they perform really well. For example, gold at nanoscale dimensions turns out to be a good catalyst for fuel cells and semiconductors. Nanotechnology involves the manipulation of things at their molecular or atomic level with a positive approach of creating unique materials with huge potential. Nanotechnology also has an enormous potential in creating smaller,
inexpensive and faster devices with improved performance at nanoscale dimensions. It changes the properties of a material in such a way that the material behaves more efficiently at a nanoscale dimension compared to the same material at a large scale. Most of the vital metabolic processes in living beings occur at the nanoscale range and one such example is haemoglobin, which helps to carry oxygen across the bloodstream is approximately 5 nanometers in diameter. Scientists have been making continuous efforts to imitate nature’s nanoscience and have been successful in few cases. Products like sunscreens and stain resistant dresses have already been commercialized and a continuous progress is being made to create more useful nanomaterials. The invention of STM and AFM has revolutionized the growth of nanotechnology and have opened new opportunities of imaging at nanoscale dimensions with an atomic resolution [64].

Nanoparticles are classified based on their size and diameter [65] and the magnetic properties of nanoparticles make them a potential candidate in the biomedical field. A continuous interest has been growing in recent years towards nanoparticles and their diverse applications in the biomedical field including targeted drug delivery, hyperthermia, photoablation therapy, bioimaging and biosensors. For instance, iron oxide nanoparticles are gaining a considerable attention since they possess excellent properties including chemical stability, non-toxicity, biocompatibility, high saturation magnetisation and high magnetic susceptibility which accounts for their biomedical applications including bioimaging, hyperthermia, drug delivery, cell labelling and gene delivery [66].

Nanoparticles can be categorized into different nanosystems which include metallic nanoparticles, bimetallic or alloy nanoparticles, metal oxide nanoparticles and magnetic nanoparticles.

**Magnetic nanoparticles:** Magnetic nanoparticles are gaining a considerable attention because of their applications in biotechnology, biomedical, material science, engineering, and environmental areas. A large amount of attention is being
paid in synthesizing different types of magnetic nanoparticles. In the biomedical field, magnetic nanoparticles find their applications as therapeutic agents in treating cancer, however the biomedical applications require the superparamagnetic behaviour of particles at room temperature. Moreover, in a physiological setting, the therapeutic, biological and diagnostic applications of magnetic nanoparticles require their stability in water at pH 7 [67].

Iron oxide nanoparticles possess unique chemical and magnetic properties and are at different oxidation states and with different polymorphic crystalline structures. Maghemite (γ-Fe₂O₃) and magnetite (Fe₃O₄) are the most biocompatible forms of iron oxide but the most commonly used form of iron oxide in the biomedical field is magnetite (Fe₃O₄). Research on iron oxide nanoparticles and their different biomedical applications including the treatment of hyperthermia has been carried out [66]. Cobalt and Manganese doped ferrites are the most used and play a promising role in the biomedical field [68].

**Metallic nanoparticles:** For over a century, metallic nanoparticles have captivated the interest of researchers and are gaining a considerable interest for their enormous potential in nanotechnology particularly the biomedical and engineering fields. The improvisation of the properties of metallic nanoparticles through modification paves a way for applications in different fields. Various imaging devices including MRI, CT, PET, ultrasound, surface-enhanced Raman-spectroscopy (SERS), and optical imaging have been developed over time which helps in providing the state of any disease [69].

Gold nanoparticles are studied in detail for their biomedical applications due to their chemical inertness and surface functionalization of gold which is due to the negative charge present on its surface. Hence, it is utilized in biosensors, bioimaging and photothermal therapy. The ease of functionalization of gold with different organic molecules allows its coupling with ligands, antibodies or drug for active or passive drug delivery. The chemical inertness of gold adds to its biocompatibility both in vitro
and in vivo. Similar to gold, silver and its compounds are also used for medicinal purposes since decades. The unique properties of silver nanoparticles including high electrical conductivity, thermal conductivity, chemical stability, catalytic activity, antibacterial and enhanced optical properties have widened their field in various areas with electronic, photonic, antimicrobial and disinfectant applications. The extraordinary antimicrobial properties of silver nanoparticles allow them to be used in wound dressings and device coatings and moreover, silver nanoparticles are increasingly being used in the biomedical field for biosensors, photothermal therapy and drug delivery [66]. Copper nanoparticles are being utilized as antimicrobial agents as well as their interaction with other particles helps to improve the antimicrobial efficacy. Compared to other metallic nanoparticles, copper nanoparticles are highly reactive and all these properties including their small size allow them to be used in different areas. When exposed to air, copper nanoparticles form copper oxide and to prevent this oxidation, silica and carbon are utilized to coat copper nanoparticles. Metallic copper nanoparticles have been used in place of silver nanoparticles and other noble metals as anti-infective agents and they also find applications in water treatment [70].

**Bimetallic or alloy nanoparticles:** Bimetallic nanoparticles are gaining a considerable interest from both the scientific and technological perspectives since they involve the modification of physical and chemical properties of metal nanoparticles. Bimetallic colloids, which are an assembly of two types of metals combined into a single unit, possess distinct catalytic, electronic and optical properties than their corresponding monometallic nanoparticles [71]. Some of the examples of bimetallic nanoparticles include iron-cobalt (Fe-Co) nanoparticles, iron-nickel (Fe–Ni) nanoparticles, copper-nickel (Cu–Ni) nanoparticles, iron-platinum (Fe–Pt) nanoparticles, etc. Fe-Co nanoparticles possess good magnetic properties including superparamagnetism, high Curie temperature and high saturation magnetisation, however they are easily oxidised. In the biomedical field, Fe-Co nanoparticles are utilized in MRI. Fe–Ni and Fe-Pt nanoparticles possess high Curie temperature and high saturation magnetisation and are mostly utilized as contrast
agents for MRI. Cu–Ni nanoparticles display good Curie temperatures and magnetic properties and are mainly used for magnetic hyperthermia [66].

**Metal oxide nanoparticles:** They have gained a significant interest in biomedical, bioimaging and biosensing fields due to their unique structure and properties and have developed as vital components in medical transplants, cancer diagnosis and treatment and neurochemical examinations [72]. For instance, TiO₂ nanoparticles possess several unique properties like biocompatibility, chemical stability and optical properties which makes them suitable for different applications. Nanoceria are also gaining interest in the biomedical field and their unique property is the interchangeable oxidation states. In the biomedical field, nanoceria is being used as a biosensor and as an anticancer agent. Porous silica (SiO₂) nanoparticles are also gaining a pace in the biomedical field due to their unique properties and are being investigated for potential drug delivery and biosensors. Zinc oxide (ZnO) nanoparticles and their use in biomedical field for drug delivery and bioimaging has been growing at a fast pace but in order to utilize them for biological systems, they need to be modified because of their dissolution in water and acidic solutions and moreover, the utilization of ZnO nanoparticles in imaging requires them to be doped because the bandwidth of ZnO is in the UV region and the UV rays cannot enter the tissues and are harmful to cells and tissues [66]. Copper oxide (CuO) nanoparticles have also been attracting a significant attention owing to their bactericidal and biocide properties and they also display several applications in the biomedical field. Although CuO nanoparticles are beneficial in the biomedical field, the toxicity that is displayed by these nanoparticles restricts their use in the medical field. CuO nanoparticles can be toxic for mammalian cells along with vertebrates and invertebrates and have the capability of inducing ROS and damage DNA and mitochondria [73].

### 2.4 Methods of synthesis of nanoparticles

There are different methods that can be utilized in synthesizing nanoparticles
and all those methods are broadly classified into two main categories i.e. Bottom-up and top-down method. Both these methodologies are further divided into various subclasses depending on the operation, reaction condition and adopted protocols.

### 2.4.1 Bottom-up approach (Constructive)

This method involves the building up of materials starting with atoms then groups and finally nanoparticles. Among bottom-up methods, sol-gel is generally an ideal method for nanoparticle synthesis because of its simplicity [74]. Spinning involves the synthesis of nanoparticles through spinning using a spinning disc reactor (SDR). Nitrogen, or an inert gas, is mostly utilized to fill the reactor so as to avoid any chemical reactions due to the presence of oxygen [75]. In the CVD method of nanoparticle synthesis, a reaction chamber is utilized inside which gaseous molecules (thin film) are merged through their deposition on a substrate [76]. Pyrolysis is the most frequently used method of largescale synthesis of nanoparticles in industries and involves the burning of a precursor (either a liquid or a vapour) with flame [77]. Biosynthesis of nanoparticles refers to the green and environment-friendly method of synthesizing nanoparticles which are non-toxic and biodegradable. Biosynthesis of nanoparticles utilizes bacteria, fungi, actinomycetes or plant extracts along with some precursors and hence limits the use of chemicals [78].

### 2.4.2 Top-down approach (destructive)

This method involves the conversion of a massive substance to nanoscale dimensions. Mechanical milling involves an inert atmosphere for the synthesis [79]. Nanolithography is used to synthesize nanoparticles of a required shape [80] and LASiS condenses a plasma plume that produces nanoparticles [81]. Sputtering involves surface deposition through the collision of ions followed by annealing [82]. Thermal decomposition involves the endothermic chemical decomposition of chemical bonds through heat. The decomposition temperature of any element is that
particular temperature where it chemically decomposes and that decomposition leads to the synthesis of nanoparticles [83].

2.5 Applications of nanoparticles

Nanoparticles are gradually gaining a considerable interest owing to their enormous applications in different fields. Researchers have started using nanoparticles in drug and gene delivery systems, antibacterial agents, biosensors, cancer therapies, catalysts, detection of proteins and much more. Extensive research has been published regarding the applications of nanoparticles. Few examples describing these applications are given below.

2.5.1 Drug delivery

The significant factor which is required in developing a novel drug delivery system and to attain the highest therapeutic effect is the precise and secure targeted drug delivery. The smaller size of nanoparticles allows them to avoid different cellular barriers. Nanocarriers enhance the diffusion of hydrophobic compounds making them appropriate for parenteral administration. Nanocarriers also enhance the stability of various therapeutic agents including peptides and oligonucleotides [84]. Magnetosomes from *Magnetospirillum gryphiswaldense* have been investigated for *in vitro* toxicity on mouse fibroblasts and have been found non-toxic to mouse fibroblasts [85]. Bacterial magnetic particles have been investigated for their impact on mouse immune response and have shown no toxic effects and hence can be the emerging candidates for novel drug or gene carriers for tumour therapies [86]. Low cardiac toxicity of doxorubicin (DOX) loaded bacterial magnetosomes has been reported together with their ability to inhibit the tumour growth [87]. The *in vitro* and *in vivo* delivery of β-galactosidase plasmids utilizing polyethylenimine (PEI)-associated magnetotactic bacterial nanoparticles (MTB-PEI-NP) has been reported [88]. Gold nanoparticles can be readily altered with different ligands and functional groups and display low toxicity, high surface area and stability. Hence, gold nanoparticles can be
emerging candidates for novel drug deliveries. Gold nanoparticles display numerous biomedical applications, however the reports on the use of biosynthesized gold nanoparticles as gene delivery agents are less [84].

Silver nanoparticles have been widely utilized for their novel therapeutic applications and moreover, they also display antibacterial, antifungal, antiviral and anti-inflammatory properties. Different concentrations of silver nanoparticles synthesized from *B. licheniformis* showed anti-angiogenic activity and reduced the cell survival [89].

Copper nanoparticles also have the potential drug loading and efficient photoluminescence capability which makes them as potential carriers for drug deliveries. Copper nanoparticles can act as the carriers of anticancer drugs where the degradation of DNA through the action of copper nanomaterials by means of singlet oxygen production can take place. Chemical modifications of copper nanoparticles to produce active molecules can make them suitable for interacting with more macromolecules [83].

Nanoparticle-mediated delivery of drugs to specific target sites is being anticipated to cut down the utilization of anticancer drugs and with improved specificity, greater efficiency as well as lesser toxicity. In the next few years, nanotechnology will rise to a whole new level with growing therapeutic and diagnostic applications [90].

### 2.5.2 Antimicrobial activity

With an unprecedented rise of antibiotic-resistant pathogenic microbes, the treatment of the infections has become difficult due to the hindrance posed by drug-resistant microbes. More than 70 % of the bacterial infections display resistance towards any one of the antibiotics that are commonly used for treating the infection [91]. For centuries, metals including silver, copper, gold, titanium and zinc are being
utilized since they are known to possess antimicrobial properties. In recent times, nanotechnology has provided enormous opportunities in different areas of science and technology. Metal nanoparticles including silver, copper, gold, titanium, magnesium have been recognized for their antimicrobial activity. The larger surface/volume ratio and the lesser size of nanoparticles compared to their respective metals leads to a change in their properties including heat treatment, mass transfer, the rate of dissolution and catalytic potential [92].

In accordance with the literature, silver nanoparticles represent the most prevalent inorganic nanoparticles utilized as antimicrobial agents. Silver nanoparticles act on drug-resistant pathogens and they are believed to damage the outer bacterial membrane. Researchers also believe that silver nanoparticles are responsible for inducing pits and gaps in the bacterial membranes leading to cell fragmentation. Silver nanoparticles and their antimicrobial efficiency upon size reduction have been studied on *Bipolarissor sorokiniana* and *Magnaporthe grisea*. Silver nanoparticles have also been utilized to control the growth of soilborne fungi which often do not produce spores. Silver nanoparticles were found very active in controlling the growth of spore-forming fungal plant pathogens and may possibly display less toxicity compared to synthetic fungicides [92, 93]. Silver nanoparticles have been tested for their antimicrobial effect against 4 different foodborne pathogens viz. *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Vibrio parahaemolyticus* and it has been stated that silver nanoparticles can act as alternative disinfectants and cleansing agents of equipment and surfaces in food-related settings [94].

Gold nanoparticles are considered extremely valuable for their antibacterial properties since they are less toxic, highly functional, have polyvalent effects, display photothermal activity and are easily detected. Most of the antibiotics and antibacterial nanomaterials generally act through the production of ROS in causing cell death, however, gold nanoparticles do not act through the ROS-related mechanism. The antibacterial effect of gold nanoparticles has been attributed to two
factors, viz. (1) attachment to the bacterial membrane and a modification in membrane potential together with a reduction in the ATP level (2) inhibition in tRNA-ribosome binding [95]. The antimicrobial efficiency of gold nanoparticles was demonstrated in *E. coli, S. aureus, P. aeruginosa, Micrococcus luteus, Aspergillus fumigates* and *Aspergillus niger* [96].

Copper is an easily accessible metal and an essential trace element in a majority of living organisms. Copper particles in nanoscale dimensions are utilized in gas sensors, high-temperature superconductors, solar cells and wood preservatives. Copper in its metallic form has been utilized as an antibacterial agent since decades [97]. The antimicrobial activities of silver and copper nanoparticles on *B. subtilis, S. aureus* and *E. coli* showed that copper nanoparticles are more effective in controlling the growth of *B. subtilis* compared to silver nanoparticles and the reason suggested is the higher affinity of copper nanoparticles to surface amines and carboxyl groups. Silver nanoparticles, on the other hand, were more effective on *E. coli* and *S. aureus* compared to copper nanoparticles [98]. An effort to enhance the antimicrobial properties by grafting copper nanoparticles on the surface of multiwall carbon nanotubes (MWCNT) has been put forth and it has been stated that carbon nanotubes increased the surface area of copper nanoparticles which was the reason for higher activity of Cu-MWCNT system compared to pure copper nanoparticles and MWCNT on *E. coli*. The efficiency (% kill) of Cu-MWCNT system was found higher (75 % ± 0.8) compared to pure copper nanoparticles (52 % ± 1.8) in *E. coli*. The possible antibacterial mechanism of Cu-MWCNT system could be the release of copper ions which after entering the bacterial cell resulted in the disruption of biochemical processes [99]. Chitosan-stabilized copper nanoparticles were found to be effective against bacteria and a yeast [100].

Owing to the enormous development in the field of nanotechnology and the wide range of applications that nanoparticles display, there needs to be a check on the toxicity that nanoparticles can display. The main toxic effects of nanoparticles that are of concern include neurological and respiratory damage, circulatory damage
apart from various other toxic effects. While as numerous nanoparticles appear non-toxic yet some of them display toxicity which needs to be studied. The antimicrobial effect of nanoparticles and the abolition of the bacterial infections could be a valuable development in the health sector [101, 102].

2.5.3 Biosensors

For constructing a biosensor, the two important properties of nanoparticles including surface area and free energy are important in the immobilization of biomolecules as these properties help in maintaining the strong interactions. The coating of biomolecules on larger materials often results leads to denaturation and also damages their bioactivity but the adsorption of the same biomolecules on nanoparticle surfaces helps in retaining their bioactivity owing to the biocompatibility of nanoparticles. Meanwhile, a majority of nanoparticles carry charges which helps them to adsorb different biomolecules through electrostatic interactions. The immobilization of proteins by gold nanoparticles has been reported as a result of covalent bonding. Compared to other nanoparticles, gold nanoparticles are possibly the most commonly used nanoparticles for immobilization of proteins [103]. In the early 90s, gold nanoparticles have been utilized for immobilizing different types of enzymes and in the development different enzyme electrodes which retained the enzymatic activity [104]. A successful immobilization of horseradish peroxidase on gold nanoparticles has been reported. The smaller sized nanoparticles were observed to be more appropriate for enzyme immobilization [105]. A reagentless amperometric immunosensor with long-term stability by immobilizing an antibody to control Japanese encephalitis by immobilizing a related antibody on gold nanoparticles were developed [106].

2.5.4 Nanoparticles as catalysts

The large surface area and the specific characteristics of nanoparticles have allowed them to be broadly used as reductants and/or catalysts in order to enhance
the reaction rates [107]. For instance, the catalytic function and the good dispersing ability of magnetic nanoparticles have resulted in their utilization as reaction rate enhancers in microbiological processes. Fe3O4 nanoparticles together with the coated microbial cells of Pseudomonas delafeldii have been utilized to perform the desulfurization of dibenzothiophene and no reduction in the desulfurization efficiencies of P. delafeldii was observed [108].

2.5.5 Nanopharmaceuticals

Nanopharmaceuticals can be utilized in detecting diseases at a very early phase and the diagnostic applications could build upon conventional procedures using nanoparticles. Nanopharmaceuticals is a developing field which involves the nanoscale functioning of the drug particles or therapeutic delivery systems. The delivery of an appropriate dose of a specific active agent to a specific disease site is still challenging in the pharmaceutical industry. The huge potential of nanopharmaceuticals can tackle this failure as it involves the site-specific targeting of active agents. Nanopharmaceuticals can also reduce the toxic side effects of the system and in that way can result in better patient compliance. There is an enormous pressure faced by pharmaceutical industries to deliver high quality and successful products and hence, nanotechnology can provide a boost in enhancing the formulation and targeted discovery of drugs. Nanopharmaceuticals have enormous potential in the production of cost-effective drugs and the success rate in research and development and thus can reduce the time for both drug discovery and diagnostics [109].

2.6 Microbial pathogenesis

A pathogen is referred to a microorganism with an ability to cause a disease in any animal, plant or an insect. The term pathogenicity refers to the disease-causing capability of any microorganism inside a host. Microbes are able to express their pathogenicity through their virulence, a term which signifies the degree of
pathogenicity of the microbe. Some of the important microbes and their pathogenicity is briefly described below.

2.6.1 Pathogenicity of ESBL producing bacteria

ESBL production by different bacteria has resulted in an unprecedented increase in resistance towards antibiotics. Beta-lactamases are defined as those bacterial enzymes which have the capability to hydrolyze the beta-lactam ring that is usually found in all the beta-lactam antibiotics which eventually leads to antibiotic resistance. ESBLs constitute a diverse and challenging group of enzymes that are being looked upon closely by clinical laboratories. Presently, ESBLs are one of the diverse group of enzymes and are most often the variants of broad-spectrum TEM and SHV beta-lactamases. These enzymes have the ability to hydrolyze most of the penicillins, in addition to cephalosporins which confers resistance towards these agents. Only those ESBLs which are able to hydrolyze the substrates (penicillins and cephalosporins or monobactams) at measurable rates pose a challenge in the clinical practice [110]. The ability of bacteria to produce ESBLs led to resistance towards third-generation cephalosporins. ESBL producing bacteria are linked to high morbidity and mortality rates, particularly among those patients which are under intensive care. Hence, precise laboratory detection is vital in order to avoid any failures with antimicrobial therapies [111]. ESBLs are encoded by three major genes including blaTEM, blaSHV and blaCTX-M [112] and are being recognized at a fast pace. These bacteria are on a rise since the past few years and are a cause of many diseases such as diarrhoea, septicaemia, pneumonia and urinary tract infections [113]. ESBLs are either plasmid or chromosomally mediated but the plasmid-mediated ESBLs are predominantly found among Enterobacteriaceae. These enzymes are widely spread throughout the world and furthermore, E. coli and K. pneumoniae contain a significant percentage of ESBLs. A continuous modification in ESBLs leads to a change in the amino acid sequence present near the active site of these beta-lactamases. There are 400 different variants of ESBLs that have been identified so far and all those variants are classified into different groups [114].
ESBL production has become a major hurdle in treating the infections caused by Gram-negative bacteria. These bacteria are spreading at a very fast pace and are a matter of concern due to their adverse effects on human health. The most important reason behind the increased resistance of ESBLs towards antibiotics is that these enzymes undergo continuous mutations which result in an expansion of substrate profile. Gene encoding CTX-M-15 which is a major factor for ESBL production is prevalent worldwide and is more prevalent in South India [115].

2.6.2 Pathogenicity of an opportunistic pathogen *P. aeruginosa*

With the discovery of the phenomenon of bacterial QS that is employed by the bacteria in order to regulate their pathogenic and virulence mechanisms has attracted the researchers in developing various novel therapeutic agents. QS is a system of communication developed by several bacteria in order to coordinate group behaviours. QS controls a series of extracellular virulence factors and biofilm formation in numerous pathogenic bacteria including *P. aeruginosa* [116]. *P. aeruginosa* is a ubiquitous microorganism and an opportunistic pathogen targeting the patients with overlying conditions including AIDS, cancer, patients immunocompromised by cytotoxic drugs, cystic fibrosis, blood, skin and genito-urinary infections. This organism is a major and a severe cause of nosocomial infections. *P. aeruginosa* has the capability to synthesize various extracellular compounds including elastase, alkaline protease, rhamnolipids, pyocyanin, exotoxin A, and hydrogen cyanide [117, 118]. All these compounds are crucial for *P. aeruginosa* to regulate the virulence. QS system in *P. aeruginosa* controls the production of these extracellular factors together with the biofilm formation. QS also acts as a defence system in protecting *P. aeruginosa*. A decrease in the production of virulence factors through inactivation of the QS system can provide many ways to fight the infection caused by drug-resistant bacteria [119]. From the past 15 years, a deep research is being going on in order to find different QS inhibitors. Some of those inhibitors possess diverse structures such as furanone derivatives, different heterocycles, drugs such as macrolide and non-macrolide antibiotics, salicylic acid
etc [120]. Apart from these inhibitors, some natural substances such as plant extracts have been reported to possess a great capability in inhibiting QS systems of Gram-negative bacteria [121].

2.6.3 Pathogenicity of *Enterococcus* species

The past few decades have experienced the emergence of multi-drug resistance enterococci leading causes of various drug resistant infections and have become a serious health-related issues including UTI, endocarditis and bacteraemia. The virulence factors are responsible for antibiotic resistance, toxin production, biofilm formation, etc. The antibiotic therapy of such infections is difficult since enterococci display resistance to several drugs. *Enterococci* may also develop or acquire resistance to other antibacterial agents including chloramphenicol, ciprofloxacin, erythromycin, tetracycline, trimethoprim and vancomycin. The resistance displayed by *Enterococci* against antibiotics poses a threat to the effectiveness of new antibiotics. New approaches including active or passive immunization may need to be taken into consideration [122, 123].

2.6.4 Pathogenicity of *Streptococcus* species

The genus *Streptococcus* includes numerous pathogenic species which cause diseases in humans and animals and have evolved characteristic repositories of protein and non-protein toxins. *S. pyogenes* is an important species of this genus which is a gram-positive extracellular bacterial pathogen. Group A streptococci (GAS) are a cause of various pyogenic infections ranging from mild to severe, involving the mucous membranes, tonsils, skin, and deeper tissues, which may lead to a fatal outcome due to the production of exotoxin B (SpeB) [124, 125]. *S. pyogenes* pathogenesis is facilitated by a massive repository of extracellular virulence factors. The persistence of the organism at the early stage of infection is mediated by a series of antiphagocytic molecules [126].
2.6.5 Pathogenicity of *Salmonella typhimurium*

In humans, the infections caused by *Salmonella* strains depend on certain factors which include the type of strain involved as well as the physiological condition of the host. *Salmonella* strains mostly affect the children and aged people. In comparison to healthy individuals, immunocompromised patients are more sensitive towards *Salmonella* infections. Most of the *Salmonella* strains are pathogenic due to their potential of invasion, replication and survival inside the human host which leads to severe consequences. The remarkable feature displayed by *Salmonella* while invading a non-phagocytic human host cell includes the induction of its own phagocytosis in order to take a control of the host cell machinery. The persistence of *Salmonella* within the host is the main factor for its virulence and those strains without this capability are non-virulent. *S. typhi* and *S. paratyphi* A, B and C infections lead to enteric fever [127, 128]. *Salmonella* infections are a serious threat all over the world and since these strains have developed resistance towards the first line of antibiotics, new antimicrobial agents of choice have been introduced which include fluoroquinolones and extended-spectrum cephalosporins. These new antimicrobials are utilized in treating the infections due to the multidrug-resistant strains of Salmonella [128, 129].

2.6.6 Pathogenicity of *Proteus mirabilis*

*Proteus mirabilis* is a rod-shaped Gram-negative bacterium mostly known for its swarming motility and urease activity. This organism is a frequent source of catheter-associated urinary tract infections (CAUTI) that are often polymicrobial. *P. mirabilis* resides in a diverse range of habitats including soil, water sources, and sewage, but this organism is mostly found as a commensal of the gastrointestinal tracts of humans and animals. This bacterium is capable for causing a wide range of infections in humans including those of wounds, the eye, the gastrointestinal tract, and the urinary tract, but it is mostly noted for infections of the catheterized urinary tract, known as CAUTI. *P. mirabilis* associated UTIs and CAUTIs are usually
complicated resulting in urolithiasis and permanent renal damage. This bacterium is an agent of catheter biofilm formation, rapidly fouling the surface of a newly inserted urinary catheter. In this process, surface organelles such as fimbriae and other adhesins appear to play an important role. Urease enzyme also contributes dramatically to this process [130, 131].

Currently, there are no licensed vaccines available for this organism, and moreover, multidrug-resistant isolates of *P. mirabilis* are increasing at a fast pace. Therefore, efforts to generate effective vaccines or therapeutic treatments are required.

### 2.6.7 Pathogenicity of MRSA

MRSA is the primary cause of hospital and community-associated infections and this organism is resistant towards the whole class of β-lactam antibiotics, including methicillin and penicillin. Hospital-associated MRSA strains are generally multi-drug resistant, hence leaving the treatment options on the use of less efficient drugs for example vancomycin. MRSA, similar to several other *S. aureus* strains, synthesize virulence factors including toxins, adhesion proteins, immune evasion and other virulence determinants. In *S. aureus* pathogenesis, surface proteins have several important roles. These proteins play a role in the cell wall metabolism of this bacterium apart from facilitating the binding to host tissue, internalization and immune evasion. These proteins also help in the aggregation and the biofilm formation of this bacterium [132, 133].

### 2.6.8 Pathogenicity of a fungal pathogen *Candida albicans*

*Candida* species are the most predominant in causing several health-related issues including serious infections and they pose a serious threat in clinical practice. The rate of fungal infections is dependent on the immunological condition of host, application of broad-spectrum antibiotics, transplants, continued use of intravascular
and urethral catheters, use of corticosteroids and parenteral nutrition [134]. *Candida albicans* is considered to be the most pathogenic *Candida* species and so far, this species remains the most persistent *Candida* species that is isolated clinically [135]. Different species of *Candida* cause candidemia [136] and produce harmful enzymes to thrive on human mucous membranes [137].

*C. albicans* is also able to produce biofilm and the biofilm formation by *C. albicans* is not a simple gathering of cells, but rather highly structured microbial communities, which is being assumed to carry out different functions such as to ease the process of the influx of nutrients and waste disposal. A complex three-dimensional structure is formed by mature *C. albicans* during biofilm formation [138].

### 2.6.9 Conclusion

The marine environment is a treasure of various important living organisms and seaweeds are one among those treasures. Seaweeds inhabit various endophytic organisms including bacteria, fungi and actinomycetes. Endophytic actinomycetes have an enormous potential to synthesize novel bioactive compounds including antibiotics, anticancer agents and enzymes. These bioactive compounds are continuously being studied in order to find a solution to the worldwide health-related issues. Actinomycetes also have the potential in assisting the synthesis of nanoparticles including copper, gold, silver, etc. and moreover, synthesis of nanoparticles from biological sources is more efficient compared to the physical and chemical methods because the biologically synthesized nanoparticles are environment-friendly and they also reduce the use of toxic chemicals. Nanotechnology has provided a new platform to tackle health-related issues because the nano range size of particles helps them to penetrate deeper in the cells which kill the pathogenic bacteria more efficiently. Nanoparticles can also be tagged with different molecules in order to reach the specific target sites leading to an enhancement in the mode of action. Metallic copper has been in use since decades because of its antimicrobial properties. Since copper is a low cost and a readily
available material, hence the synthesis of copper nanoparticles is economic and beneficial. Copper nanoparticles are active against *Enterobacteriaceae* and *P. aeruginosa* in addition to the drug-resistant *C. albicans*. These microorganisms cause several grave infections which can lead to death. Since, drug-resistant pathogenic microbes are continuous increasing at a fast pace, there is a need to find an alternative so that the problem of increasing antibiotic resistance comes to an end. The activity of copper nanoparticles against various pathogenic microorganisms can make them an alternative to various present-day antibiotics to which the microorganisms display resistance. Copper nanoparticles can be commercialized to replace the less effective antibiotics and this process requires a proper modification because copper nanoparticles can also display toxicity which needs to be minimized.