CHAPTER -1

INTRODUCTION
1.1 INTRODUCTION:

Nature supports the survival of living organisms on the earth with great biodiversity both in terrestrial and aquatic life. The biological diversity refers to the existence of different kinds of lives on earth, such as plants, animals and microorganisms, whose secondary metabolites are rich source of drugs especially the antibiotics which help in the improvement of human health by acting against many infectious pathogens. As per the World Health Organization (WHO) report, many individuals from the developing countries have been suffering from infectious diseases resulting in an increase of death rate every year (Lee et al., 2009). On the other hand, pathogens have become resistant to existing drugs. Hence, there is a need to identify novel potent compounds which are capable of acting against pathogens.

Natural bioactive compounds are of great importance as they possess valuable physicochemical properties such as specific interactions with multiple biological targets. These properties are hardly found in synthetic bioactive molecules. (Feher et al, 2003; Payne et al., 2007; Schneider et al., 2008). These antibiotics have great value in the field of therapeutic medicine (Kaltenpoth, 2009; Thumar, 2010) as they have ability to kill or inhibit various disease causing pathogens by specific biological action of the pathogen at an even very low concentration, antibiotics are also considered as non-toxic, non-allergic and non-harmful to the host (Prapagdee et al., 2011).

Most of the bioactive compounds are isolated from natural sources, which have extensive therapeutic applications in medicine as antibiotics, antiproliferatives, antioxidants, immunomodulators etc. These natural bioactive compounds have various applications in agriculture, food industry and pharmaceuticals (Thumar,2010; D’Hondt et al., 2014).

1.2 ANTIBIOTICS:
The first antibiotics were of natural origin, e.g. penicillins produced by fungi in the genus Penicillium, Currently, antibiotics are obtained by chemical synthesis, such as the sulfa drugs (e.g. sulfamethoxazole), or by chemical modification of compounds of natural origin. Many antibiotics are relatively small molecules with a molecular weight of less than 1000Da. The classical definition of an antibiotic is a compound produced by a microorganism which inhibits the growth of another microorganism (Mayer et al., 1986). Antibiotics can be grouped by either
their chemical structure or mechanism of action (Kummerer, 2009). They are a diverse group of chemicals that can be divided into different sub-groups such as β-lactams, quinolones, tetracyclines, macrolides, sulphonamides etc. Antibiotics are a chemically diverse group of compounds produced by microorganisms that have microstatic or microcidal activity. They function by a variety of mechanisms to disrupt microbial metabolism. The discovery and clinical use of antibiotics just over 50 years ago coupled with improvements in immunization drastically reduced human suffering and deaths from infectious diseases (Nwosu, 2001). In addition to antibiotics, many semi-synthetic derivatives of antibiotics and synthetic antimicrobial agents are used in clinics and animal husbandry. What is now apparent from the medical and nonmedical use and abuse of antibiotics is a growing body of evidence of antibiotic resistance in microorganisms. Evidence of resistance came soon after clinical use of antibiotics and has been accelerating so rapidly that the threat of resurgence of infectious diseases as a major human threat (Nwosu, 2001; Martinez and Fernando, 2014).

1.2.1 History of antibiotics development:

Since centuries, search for the antibiotics has begun, based on germ theory of the disease, the theory that has linked microbes as the leading cause for several ailments. However, later the new prospect of modern antibiotics was identified with the discovery of enzyme lysozyme and the antibiotic substance called “penicillin” by Alexander Fleming (1928).

During the World War II in 1930s, there is a necessity of treating wound infections that lead to the continuation of extensive research on penicillin and other antibiotics, as untreated or prolonged wound healing would lead to several complications or death (Manring et al., 2009). The term “antibiotic” was coined by Selman Waksman (1941) where the author has defined that antibiotic is any small molecule produced by a microbe that antagonizes the growth of other microbes. Further, Glusker and Hodgkin (1994) crystallized penicillin and determined its structure by X-ray crystallography. Subsequently, more antibiotics including clavacin, neomycin, actinomycin, streptomycin, candididin, grisein and fradicin were discovered at later stages.
1.2.2 Need for finding new antibiotics:

The important factors which led to the search for new antibiotics include the development of new infectious diseases and multi-drug resistance pathogens (Spellberg, et al., 2008, Jones, et al., 2008).

1.2.2.1 Development of new infectious diseases:

Microorganisms can survive in the new habitat. During the adaptation to the new habitat, the harmless microorganisms may turn into harmful strains, which may enter to the human host (Cowen, 2008) and cause new diseases. For example: In case of bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are responsible for major widespread of various diseases among them *Staphylococcus aureus* causes most of the hospital associated infections (Hancock, 2007). In case of fungal infections, *Candida albicans, Aspergillus flavus* causes nosocomial fungal infections like candidiasis and aspergillosis in adults (George and Alangaden, 2014). These infectious diseases are highly destructive to the social lives and very limited antibiotics are available to treat infections (Nikaido, 1994). To overcome these problems, there is a need for new antibiotics which can be used to treat various microbial infections. Hence, the development of new drugs with broad spectral activity and without side effects with shorter span of treatment are required to fight effectively against infectious diseases (Demain and Sanchez, 2009).

1.2.2.2 Multi-drug resistant pathogens:

Antibiotic resistance is one of the adaptations of microorganisms to survive against the action of antibiotics to which they were once sensitive (Bancroft, 2007). This property also leads to a major problem in the cure of infectious diseases (Goossens et al., 2005; Rogers, 2008; Roberts, et al., 2009; Pearson and Carol, 2008; McCallum et al., 2010). Most of the pathogenic microorganisms are resistant to at least one of the currently existing antibiotics (Cragg and Newman, 2001; Demain and Sanchez, 2009). For example, pathogenic strains of *Staphylococcus aureus* were susceptible to penicillin in the initial periods and later they acquired resistance to penicillin action (Pray, 2008).

Some of the common drug-resistant pathogens are carbapenem-resistant *Enterobacteriaceae* (CRE), multi-drug resistant *Acinetobacter* (AMDR), vancomycin-resistant

### 1.3 CLASSIFICATION OF ANTIBIOTICS:

Antibiotics classification based on the source depends upon the ability of a microorganism to produce a particular antibiotic. Most of antibiotics are secondary metabolites of three important types of microbes - bacteria, filamentous fungi and actinomycetes. Antibiotic production is not species specific and more than one antibiotic may be produced by the same type of species or strain. For example, antibiotics like streptomycin (an amino glycoside), griseoviridin (lactones), novobioicin (a complex aromatic moiety containing glycoside), cyclohexamide (acetate derived aromatic compound) and viridogrisein (a depsipeptide) show differences in their structures but they are produced by the same type of microbial strains. Contrastingly, various species of microbes produced similar types of antibiotics. For example, both *Streptomyces* and *Pseudomonas* species were produced by cycloserine antibiotic and likewise, penicillin from lower fungi *Cephalosporium* and *Streptomyces* species. The production of chemically differentiated antibiotics is due to the difference in the chemical composition or physical state of media used for cultivation of the organism, the range of aeration of the culture and the variation in their incubation periods (Banga *et al.*, 2008; Kavitha and Vijayalakshmi, 2009; Sallam *et al.*, 2010). An over-view of this system indicates that much systematic work has to be done with regard to the relationship between taxonomic studies of the organisms and chemical nature of the antibiotics produced by them (Manivasagan *et al.*, 2009; Kariminik and Baniasadi, 2010; Sibanda *et al.*, 2010). Antibiotics show differences in their chemical structures, type of activity (bacteriostatic or bactericidal), route of administration, antimicrobial spectrum. Based on the properties, antibiotics are classified as follows:

#### 1.3.1 Antibiotics classification based on the spectrum of activity:

Based on the spectrum of activity, antibiotics are classified on the mode of action against the microorganisms. Antibiotics might have a static (inhibitory) or a decimate (killing or cidal)
effect on a wide variety of microorganisms. In bacteriostatic effect, antibiotics inhibit the growth of microorganisms, without killing them whereas in bactericidal effect the antibiotic kills the microorganisms. If the antibiotics are active against both gram-positive and gram-negative bacteria species they are considered as broad spectrum antibiotics (Vandamme, 1994; Maier et al., 1999; Atta and Ahmad, 2009; Mohd-Fuat et al., 2010; Usha et al., 2011; Atta et al., 2011) whereas, the antibiotics which are active either against gram-positive or gram-negative bacteria are known as narrow spectrum antibiotics (Ting et al., 2009). If the antibiotics are effective against only a specific microorganism are considered as limited spectrum antibiotics (Russell, 1996). This classification helps to find out an unknown antibiotic in comparison with a known antibiotic by estimating the range of antimicrobial activities.

1.3.2 Antibiotics classification of based on chemical structure:

This classification is based on their chemical nature of the antibiotics. This kind of antibiotic classification helps to know about the relationship between their chemical structure and functional groups (Lancini, 1982). Further the antibiotics were classified into five functional groups such as inhibitors of cell wall synthesis, inhibitors of protein synthesis, inhibitors of membrane function, inhibitors of nucleic acid synthesis and Antimetabolites (Berdy and Magyar, 1968).

1.3.2.1 Inhibitors of cell wall synthesis:

The cell contents of the bacteria were surrounded by inner plasma membrane and outer peptidoglycan cell wall. Specific anti-bacterials interferes with the synthesis of the cell wall by weakening the peptidoglycan layer. Therefore the structural integrity of bacteria is not constant (Mitsuhashi, 1982) thus the bacterial growth was blocked by inhibiting the cell wall synthesis. For example Beta-lactam antibiotics (functional group is lactam ring) such as penicillin acts on peptidoglycan layer thus inhibiting the cell wall synthesis (Norrby et al., 1986, Jordan et al., 2008).

1.3.2.2 Inhibitors of protein synthesis:

Enzymes and cellular structures are primarily made of proteins. The survival and multiplication of the bacterial cells mainly depends on protein synthesis process (Katz and
Some of the antibacterial agents targets protein synthesis of the bacteria through attaching either to 50s or 30s subunits of the intracellular ribosomes. However, this action will disrupt the bacterial cell metabolism. Therefore it inhibits the growth or multiplication of the bacteria thus accelerates the bacterial death. For instance protein synthesis inhibitors includes ketolides, macrolides, Aminoglycosides and Tetracyclines (Arthur, 1993).

1.3.2.3 Inhibition of membrane functions:

Based on the regulation and segregation of extra and intra cellular flow of substances, the bacterial cell membranes will act as a significant barrier. The cell membrane structure is found in both prokaryotic and eukaryotic cells, by the disruption of the cell membranes leads to leakage of significant solutes which are necessary for the survival of the bacteria, in turn which directly effects the growth of the microorganisms. Example: Membrane functions inhibitors like polymyxins, cyclic polypeptides which are bactericidal in nature (Davis et al., 1986).

1.3.2.4 Inhibitors of nucleic acid synthesis:

Quinolones belongs to important class of antibiotics and it plays a major role in the synthesis of DNA by inhibiting the topoisomerase enzyme, especially topoisomerase II (DNA gyrase) enzyme, that usually enters the nucleus during DNA replication. By inhibiting the nucleic acid synthesis it directly interferes with growth of the bacteria (Walsh, 2003). Example: Quinolones and furanes.

1.3.2.5 Antimetabolites:

Antimetabolites interfere with one or more enzymes which are essential for DNA synthesis. Antimetabolites acts as substrate for actual metabolites which are useful in regular metabolism, as it affect synthesis of DNA (Berdy and Magyar, 1968).

Example: Sulfonamides, Trimethoprim.
1.4 SCREENING OF ANTIBIOTICS:

Antibiotic screening is carried out by checking their activity against different test microbes like bacteria, fungi, yeasts, etc. The most frequently used test microorganisms are *Saccharomyces cerevisiae*, *Candida albicans*, *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, multi-drug resistant pathogenic microorganisms etc.

According to Okami and Hotta (1988), the screening of antibiotics generally consists of isolation and cultivation of organisms, development of suitable assay methods, chemical characterization and identification of antibiotic principle. Depending upon the intracellular or extracellular nature of antibiotics, various isolation methods have been developed. Simple solvent extraction method or treatment with ion exchange resins or adsorption onto activated charcoal are commonly used for the extracellular antibiotic extraction (BerniceSharon and Kalidass, 2014; Hemdan, *et al.*, 2015). However, intracellular antibiotic extraction was achieved by grinding of cell mass or mycelium using polar solvents. Extraction processes help in the collection of closely related compounds in the crude form. The crude extract have to subject for further purification using chromatographic techniques to obtain pure compound (Burgess, *et al.*, 1991).

1.5 IDENTIFICATION AND CHARACTERIZATION OF ANTIBIOTICS:

Determining the structure of antibiotic was a crucial step in drug discovery. Various criteria needed for the identification of antibiotics includes nature of the antibiotic, producing organism, mode of action and its physico-chemical properties.

The identification of the antibiotic activities was carried out by using paper or thin-layer chromatography (TLC) and bioautography studies (Hamill, 1977). To check the purity of antibiotic, liquid chromatography mass spectrometry (LCMS) or high performance liquid chromatography (HPLC) can be used (Augustine *et al.*, 2005). The structure of purified antibiotic compound can be analyzed by using mass spectrum (MS), ultra violet (UV) spectrum, infra red (IR) spectrum and $^{13}$C and $^1$H nuclear magnetic resonance (NMR) analysis. This data can be used to search available databases containing reported structures to recognize the unknown antibiotic compounds (Laatsch, 2010).
1.6 LACTIC ACID BACTERIA:

The term Lactic acid bacteria (LAB) was gradually accepted in the beginning of the 20th century (Carol et al., 2010). Lactic acid bacteria is having direct influence on the organoleptic, shelf-life and nutritional characteristics, because of this phenomenon LAB has long history of application in fermented foods (Leroy et al., 2004; Yang et al., 2014). LAB is generally gram positive, non-sporing, acid tolerant, usually nonmotile, catalase negative, non respiring cocci or rods, and fastidious organisms. Lactic acid bacteria produce lactic acid as an end product through fermentation of carbohydrates along with other metabolites like ethanol, acetic acid, hydrogen peroxide, different plant growth promoters etc.

LAB is generally recognized as safe (GRAS) organisms, Lactic acid bacteria is generally used in the production of fermented foods because for their ability to produce desirable change in colour, texture, taste and flavor (Yang et al., 2014). These lactic acid bacteria are mostly known to inhibit food borne pathogens and food spoilage microorganisms by producing different antimicrobial substances such as bacteriocins, lactic acid, and several antibiotic compounds, because of these characteristic features LAB is generally utilized for enhancing the safety and extended shelf-life of food products (O’Bryan et al., 2014). Bacteriocins produced by LAB are nothing but extracellular bacterial proteins secreted by the cells (Bharti et al., 2015). These bacteriocins of LAB can be easily degraded by the human gastrointestinal proteases because of this important feature LAB generally gained importance in the recent years in food preservation (Cleveland et al., 2001).

1.6.1 Classification of lactic acid bacteria:

Lactic acid bacteria are generally classified based on morphology, range of sugar utilization and mode of glucose fermentation. Lactic acid bacteria consisting of different number of bacterial genera within the phylum Firmicutes. The genera includes Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Enterococcus, Oenococcus, Lactococcus, Carnobacterium, Lactosphaera, Vagococcus, Mellilococcus, Tetracenococcus and Weissella are recognized as LAB (Figure 1.1) (Stiles and Holzapfel, 1997; Holzapfel et al., 2001; Jay, 2000; Ghahremani et al., 2015). Lactic acid bacteria can be generally divided into two groups based on the sugar fermentation pathways, those organisms which produce exclusively lactic acid as end
product are termed as homo fermentative lactic acid bacteria, and organisms which produce acetic acid, ethanol, CO$_2$ in addition to lactic acid are termed as hetero fermentative LAB. All members of *Pediococcus*, *Lactococcus*, *Streptococcus*, *Vagococcus*, along with some *lactobacilli* are grouped as homofermenters.

*Carnobacterium*, *Oenococcus*, *Enterococcus*, *Lactosphaera*, *Weissella* and *Leuconostoc* and some Lactobacilli come under heterofermenters. The hetero fermentative LAB gain more importance in food industries by producing aromatic and flavour components such as diactyl and acetaldehyde. The classification of lactic acid bacteria into different genera is based on morphology, mode of glucose fermentation, configuration of lactic acid produced, ability to grow at high salt concentrations, growth at different temperatures, and acid or alkali tolerance.

![Classification of Lactic Acid Bacteria family.](image)

**Figure 1.1: Classification of Lactic Acid Bacteria family.**

1.6.2 Lactic acid bacteria as probiotics:

Lactic acid bacteria are generally considered as major group of probiotic bacteria. In recent years the use of Lactic acid bacteria as “Probiotics” is gaining more importance since mainly *lactobacillus* sps and *bifidobacteria* sps may have several therapeutic functions (Berg, 1996; Oberg et al., 1998). According to WHO(World Health Organisation) probiotics is defined as “ Live organisms when administered in adequate amounts confer health benefits on the host”(WHO,2002).LAB is known as most important non pathogenic bacteria that play a key role
in producing vitamins, preservation of foods, and also protects mankind from various diseases such as cancer due to their antimicrobial action. These bacteria are known from centuries for their importance in food preservation. Lactic acid bacteria prevents food spoilage by natural fermentation. This family of bacteria is widely used in food industries because of their responsibility in food conservation by the production of wide range of antimicrobial metabolites which are generally considered as natural food ingredients consumed by mankind (Alfonzo et al., 2013). The probiotics concept was first defined by fuller in 1989 as “a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance”. LAB constitutes an integral part of the healthy gastrointestinal (GI) microecology and is involved in the host metabolism (Fernandes et al., 1987). Fermentation has been specified as a mechanism of probiotics (Gibson and Fuller, 2000). LAB along with other gut microbiota ferments different substrates like lactose biogenic amines and allergenic compounds into short-chain fatty acids and other organic acids and gases (Gibson and Fuller, 2000; Gorbach, 1990; Jay, 2000). Lactic acid bacteria involves in synthesizing bacteriocins, enzymes, antioxidants and vitamins (Fernandes et al., 1987; Knorr, 1998). Along with these different kinds of properties, intestinal lactic acid bacteria plays an important mechanism for the metabolism and detoxification of foreign substances which are entering into the body (Salminen, 1990).

1.7 ANTIMICROBIAL METABOLITES FROM LACTIC ACID BACTERIA:

Lactic acid bacteria are known to produce numerous antimicrobial compounds that are active against various pathogens (Jeevaratnam et al., 2015). The preservation action of LAB is due to combined effect of antimicrobial metabolites produced by LAB during fermentation (Havelaar, 2009). These antimicrobial metabolites create acidic environments which are unfavorable for food spoilage organisms and pathogens. Antimicrobials compounds which are produced by lactic acid bacteria (LAB) can be divided into two types they are:

- Lower molecular mass compounds having molecular mass <1000 Da.
- Higher molecular mass compounds having molecular mass >1000Da such as bacteriocins.
- All antimicrobial compounds (non-bacteriocins) which are produced by LAB are of low molecular mass (Collins, 2009).
Table 1.1: Antimicrobial compounds of lactic acid bacteria species (Khalid, 2011).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Organisms</th>
<th>Sensitive organisms</th>
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<tbody>
<tr>
<td>Lactic acid</td>
<td>Lactic acid bacteria</td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gram negative bacteria</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Hetero fermentative Lactic acid</td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td>acid bacteria</td>
<td>Gram negative bacteria</td>
</tr>
<tr>
<td>Reuterin</td>
<td>Lactobacillus ruteri</td>
<td>Protozao, fungi, Gram positive bacteria, Gram negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bacteria, Gram negative bacteria</td>
</tr>
<tr>
<td>Benzoic acid, Mevalonolactone, Methylhydantoin</td>
<td>Lactobacillus plantarum</td>
<td>Fungi, Gram negative bacteria</td>
</tr>
<tr>
<td>Reutericyclin</td>
<td>Lactobacillus reuteri</td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td>Aciodolin, Acidophilin</td>
<td>Lactococcus acidophilus</td>
<td>Gram negative bacteria</td>
</tr>
<tr>
<td>Bulgaricas</td>
<td>Lactobacillus bulgaricus</td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gram negative bacteria</td>
</tr>
</tbody>
</table>

Several studies have indicated that LAB play a positive role in modulating the host immune system and display of antimicrobial activities against common food-borne pathogens and in preventing and treating diarrhea (Mduduzi et al., 2016).
1.8 **THE GENUS Enterococcus faecium:**

*Enterococcus* was first described in 1899 as a new streptococcus of enteric origin, and further streptococcus was further classified in mid 1930s based on Biochemical and physiological properties into four divisions, they are: viridans, pyogenic, lactic and *Enterococcus*. The genus *Enterococcus* belongs to family: *Enterococcaceae*, order: *Lactobacillales*, phylum: *Firmicutes*. They are gram positive, catalase negative, facultative anaerobic, non spore forming cocci that occurs mostly as single cocci and also in short chains with a fermentative ability resulting in production of L(+) Lactic acid as major end product. And the phylogenetic analysis shows *Enterococcus* is closely related to genera *Lactococcus* and *Streptococcus*. The genera of LAB with which *Enterococcus* are grouped are identified by a low G+C content of <50 mol% (Klein *et al.*, 1998). The Characteristics of *Enterococci* used in their identification includes growth at 5°C to 50°C and for the most part survival growth for 30 min at 60°C, growth in media containing 6.5% Nacl, hydrolysis of esculin in presence of bile salts, survival at extreme pH upto 9, other characteristics exhibited by most the *Enterococci* species include hydrolysis of leucine-b-naphthylamide (Teixeira *et al.*, 2007).

1.8.1 **Natural habitats of Enterococci:**

These *Enterococci* are diverse and versatile group of bacteria with several intrinsic characteristics that allow them to grow under variable conditions and having remarkable adaptability to a variety of conditions. These *Enterococcus* species are widely distributed in nature such as soil, water, food stuffs and dairy products and on the intestinal mucosal surfaces of the humans and animals. The presence of *Enterococci* play an important role in fermented food products (Hugas *et al.*, 2003), such as olives (omar *et al.*, 2004), in which *Enterococcus faecium* have been isolated from the brines of olives (Lavermicocca *et al.*, 1998). As alternative the antimicrobial activity of *Enterococcus faecium* (Barbosa *et al.*, 2014) or their bacteriocins has attracted a great deal of attention as a novel approach to contribute to potential applications as functional starter cultures in the food preservation (Settanni *et al.*, 2008). *Enterococcus* are found to be in all the intestines of humans and animals. *Enterococcus* genus includes 28 species, out of their *Enterococcus faecalis* and *Enterococcus faecium* are the most commonly present species in guts of the human intestine and only limited number of *enterococcal* species is of importance for the ecology of the GIT or the food microflora, it
includes *E. faecalis*, *E. faecium*, *E. durans/hirae*, *E. gallinarum* and *E. casseliflavus* (Klein, 2003).

### 1.9 ADVANTAGES OF MICROORGANISMS FOR THE ISOLATION OF BIOACTIVE COMPOUNDS:

- Short doubling time of microbes, favours the cultivation on large scale in short time.
- Simple media modifications such as nutrient limitation and pretreatments have been found to increase the production of the bioactive compounds by microbes.
- Microbial bioactive compounds are cost effective by using low cost solid substrates as nutrient medium by solid state fermentation we can increase the yield of bioactive compounds.
- In general most of the microbial bioactive metabolites are extracellular in nature and are directly secreted into the fermentation medium and are easy for downstream processing.

### 1.10 AIM AND OBJECTIVES OF THE STUDY:

There is great demand in search of novel antibiotics to treat drug resistant pathogens, hence there is a necessity to identify new antimicrobial compounds. The main objectives of the present research work are:

- Isolation and screening bioactive compound producing novel lactic acid bacteria species from different soil and dairy samples of Visakhapatnam, Tirupati, Hyderabad and Chennai.
- Morphological, physiological and biochemical characterization studies for isolated potent lactic acid bacteria isolate.
- Taxonomical identification of potent lactic acid bacteria isolates by 16S rRna and phylogenetic studies.
- Optimization of antibacterial compound production under submerged fermentation by the altering the medium components and cultural conditions by using OFAT and Response surface methodology.
• Purification, characterization and structural elucidation of the pure antibiotic compound from the promising isolate.

• Determination of antibacterial and other biological properties of the compound.

• Insilico analysis of the antibiotic activity.