The work presented in this thesis is based on the results of the investigative studies carried out in the Microbial Genetics Laboratory, University of Delhi South Campus, under the supervision of Prof. Sheela Srivastava. The present study was directed towards the isolation, heterologous expression, purification, and characterization of plantaricins derived from soil metagenome.

Soils contain a high diversity of microorganisms expressing various enzymes and antimicrobial agents potentially useful in agriculture, the chemical industry, and therapeutics. As most of the organisms (>99%) are unculturable by standard laboratory techniques, methods have been developed to access this reservoir of natural products by means of nucleic acid analysis. Total DNA from an environmental sample is extracted and new genes or activities are sought by screening.

Several microorganisms, especially lactic acid bacteria (LAB) exhibit strong antimicrobial properties. A previous study has shown the potential of plantaricin produced by strain LR/14. The plantaricin LR/14 has a broad spectrum inhibiting many Gram-positive and Gram-negative bacteria including some pathogenic and food spoilage bacteria. The low yield after purification was main limitation of the work. The present investigation is an effort to obtain antimicrobial gene(s) from soil metagenome and to gain a higher yield of metagenome-derived plantaricin production in a heterologous host, *E. coli*. Next objective was to purify and characterize gene products, and use them as bio-preservatives. The work presented here, as is customary, has been divided into following sections: Introduction, Materials and Methods, Results, Discussion, Summary and Conclusions, References and Annexures.

Introduction comprises the review of literature related to the metagenomic analysis including isolation of DNA, construction of library, and screening of clones for specific function. It also explains the advantage of heterologous expression and the role of different components present in plantaricin operon.
This section also includes the importance of therapeutic values of bacteriocin and their usage as biopreservative. With the background provided by the information available in this section, the objectives pursued during the present investigation have been delineated.

**Materials and Methods** comprises the list of all the materials used during this study with a comprehensive account of the different protocols used in the present investigation.

**Results:** In order to provide a distinctive focus, the findings of the work are divided into three chapters. **Chapter 1** presents strategies for isolation of metagenomic DNA from soil sample, construction of library, and screening of clones for antimicrobial function. **Chapter 2** embodies the identification of heterologous expression, purification, characterization, processing and large scale production of plantaricins derived from soil metagenome. Finally **Chapter 3** deals with the use of hurdle technology and the effect of plantaricins against Gram-negative organisms.

**Discussion** describes the relevance and contribution of this work to the scientific world. All the major findings have been analyzed and this information has been interwoven with known knowledge from the available literature. The new information generated has been highlighted and further lines of study that can be pursued as a result of such information have also been proposed.

**Summary and Conclusions** gives the overview of the work done and the major outcomes have been highlighted.

The literature referred and cited during the course of this study is listed alphabetically in **References.** This section is followed by **Annexures,** which is a compilation of the components of all the media/solutions/buffers used during this study.

The present work, directed towards the isolation, heterologous expression, purification, and characterization of plantaricins derived from soil metagenome has succeeded in fulfilling all the objectives. And also gives a new line of studies, to look for novel microbial function from soil metagenome.
These findings provide the directions for further work, and the answer to which will be equally exciting. Thus, this study should be considered as the beginning of a new scientific endeavor.