3. AIM AND OBJECTIVES

The study has the following Objectives

1. To isolate and identify a chitinase deficient, protease producing microorganism from soil

2. To determine the efficiency of microbial deproteinization of raw shrimp shells using *Serratia marcescens*

3. Study of enzyme kinetics and characterization of purified protease from *Serratia marcescens*


5. Physio-chemical characterisation of microbially deproteinized chitin in comparison with commercial chitin

6. Transformation and expression of protease plasmids in *E. coli* and study of deproteinization kinetics of transformed bacteria.

7. To study the utility of chitin as a growth enhancer in plants

8. To determine the biological properties of deproteinized chitin viz antimicrobial activity, membrane formation etc

### 3.1 SCHEMATIC OVERVIEW OF THE PROJECT

<table>
<thead>
<tr>
<th>Experimental Systems</th>
<th><em>Serratia marcescens</em></th>
<th>Shrimp Shell Waste</th>
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</thead>
</table>

#### PHASES

1. Isolation and Identification of chitinase deficient microorganism

2. Deproteinization of shrimp shell waste by *S. Marcescens*

3. Enzyme kinetics of purified protease from *S. Marcescens* and its characterization

#### EXPERIMENTS

- **a)** Isolation of microorganism
  - 1. Screening of protease activity
  - 2. Screening of chitinase activity

- **b)** Identification of Microorganism
  - 1. Gram’s staining
  - 2. Biochemical test (IMVIC test)

- **c)** Molecular Characterization using 16SrRNA

- **d)** Protease assay

- **e)** Chitinase assay

- **a)** Partial Purification of shrimp shell waste.

- **b)** Deproteinization by *S. Marcescens*

- **c)** Chitin Estimation & Protein Reduction

- **d)** Protease Assay

- **a)** Purification of Protease by Column chromatography.

- **b)** Molecular Weight determination SDS PAGE (Gel Eval 1.22 software)

- **c)** Optimization of pH, Temp, Substrate Concentration.

- **d)** Optimization of Deproteinization media for the Production of Proteases.

- **e)** Protease Assay in Deproteinization media

- **f)** Effect of pH & Temp on Deproteinized media
**Chapter 3**

**Aim and Objectives**

**Phase - II**
1. Deacetylation of Shrimp Shell wastes using *Lactobacillus Spp.*
2. Qualitative analysis

**Phase - III**
1. Isolation & Characterization of Plasmid from *S. Marcescen*.
2. Restriction digestion, cloning & transformation of *serratia* Plasmid.
3. Deproteination efficiency of recombinant strain

**Phase - IV**
1. Assessment of Application of Chitin in Agriculture
2. Antimicrobial Activity
3. Water Purification