6. SUMMARY

- The diversity, toxicity, growth, crystal protein and gene profiling of Bt were carried out in five natural habitats (forest, agriculture, aquatic, fallow and shifting cultivation) in Mizoram state, Northeast India.

- 107 Bt colonies, which are later placed into 29 strains were isolated from a total of 55 soil samples from five different habitats covering eight districts of Mizoram.

- The isolates were morphologically, biochemically and physiologically characterized and they produced oval spherical and bipyramidal crystals.

- The highest frequency of Bt was recorded in the shifting cultivation (88.88%) while lowest frequency was observed in the forest habitats (31.57%).

- The Bt index of the soil samples from five different habitats in Mizoram was 0.012. Bti was observed at higher frequency (30.84%) and six different undescribed biochemical type combinations were observed (3.73 – 14.0%).

- Eleven different types of crystal protein profile were observed using SDS-PAGE from the isolates of Bt revealing the molecular diversity of this bacterium in different habitats of Mizoram.
• *cry* 4 and *cry* 9 genes were found to be the most abundant in *Bt* isolates of Mizoram soils.

• Among the *Bt* isolates against *Culex tritaeniorhynchus*, SC1 and HP7 showed high toxicity (low LC_{50}), 100% mortality, less time to kill (shorter LT_{50}).

• Ten isolates have 100% mortality along with the standard at a concentration of 1×10^{-6} against *G. mellonella*.

• Culture no.'s MZUbt-6, 15, 21, 22 (RRK, SR1, LP4 and RP1) require less time at a concentration of 1×10^{-6} than standard and promises a great value in IPM.

• In RAPD analysis, it was concluded that *cry* gene content is not involved in determining polymorphism and locus linked genes instead the source or habitat from where *Bt* is isolated determines the polymorphism.

• Phylogenetic analysis using 16s rRNA sequence showed that it is highly conserved; and is a reliable method for species identification as well as authenticity of its use in molecular biology.
1. Luria Bertani (broth)
   - Tryptone : 10 g
   - Yeast extract : 5.0 g
   - Sodium chloride: 5.0 g
   - Distilled water : 1000 ml
   - pH : 7.0 -7.2

2. Luria Bertani (agar)
   - Tryptone : 10 g
   - Yeast extract : 5.0 g
   - Sodium chloride: 5.0 g
   - Distilled water : 1000 ml
   - Agar : 1.5 %
   - pH : 7.0 -7.2

3. T₃ medium (Travers et al., 1987)
   - Tryptone : 3.0 g
   - Tryptose : 2.0 g
   - Yeast extract : 1.5 g
   - Sodium phosphate : 0.05m (pH 6.8)
   - Manganese chloride : 0.005 g
   - Distilled water : 1000 ml
   - Agar : 15.0 g

4. Nutrient agar
   - Tryptone : 5.0 g
   - Yeast extract : 2.50 g
   - Glucose : 1 g
   - Agar : 15 g
   - Distilled water : 1000 ml
   - pH : 7.0
5. Methyl red agar (1 liter)

- Peptone : 5 gram
- Beef extract : 3 gram
- Sodium chloride : 3 gram
- Agar : 2%
- Methyl red : 10 ml.
- pH : 7.0-7.2

0.2 gram of methyl red was dissolved in 10.0 ml distilled water.

6. MR-VP Broth

- Peptone : 0.5gm
- Di-potassium hydrogen phosphate : 0.5gm
- Glucose-10%solution : 5ml (filter sterilized)
- Distilled water : 100ml
- pH : 7.6

Barrit’s Reagent

Solution-A
- Alpha-napthol : 50.0gm
- Ethanol (absolute) : 95.00ml

Dissolve the alpha-napthol in ethanol with constant stirring.

Solution-B
- Potassium hydroxide
- Creatinine
- Distilled water

Dissolve the potassium hydroxide in 75ml of water. The warm solution was cooled, creatinine added and stirred. Remaining water was added and stored.

7. Composition of Starch hydrolysis media

- Peptone : 0.5gm
- Beef extract : 0.38gm
Soluble starch - 0.2gm
Agar - 1.5gm
Distilled water - 100ml
pH - 7.0

8. Nitrate Broth
   Beef extract - 0.3gm
   Peptone - 0.5gm
   Potassium nitrate - 0.1gm
   Distilled water - 100ml
   pH - 7.6

Reagents

Solution-A
   Alpha-napthalamine - 0.5gm
   Acetic acid(5N)30% - 100ml

Solution-B
   Sulfanilic acid - 0.8gm
   Acetic acid(5N)30% - 100ml.

The reagents were stored in brown bottles.

9. Christensen’s Urea Agar
   Peptone : 1 g
   Dextrose : 1 g
   Sodium chloride : 5 g
   Potassium phosphate, monobasic : 2 g
   Urea : 20 g
   Phenol red : 0.012 g

To prepare the urea base, dissolve the first six ingredients in 100 ml of distilled water and filter sterilize (0.45-mm pore size). Suspend the agar in 900 ml of distilled water, boil to dissolve completely, and autoclave at 121°C and 15 psi for 15 minutes. Cool the agar to 50 to 55°C. Aseptically add 100 ml of filter-sterilized urea base to the cooled
agar solution and mix thoroughly. Distribute 4 to 5 ml per sterile tube (13 x 100 mm) and slant the tubes during cooling until solidified. It is desirable to have a long slant and short butt. Prepared media will have a yellow-orange color. Store the prepared media in the refrigerator at 4 to 8°C until needed. Once prepared, do not reheat the medium as the urea will decompose.

10. Tryptophan broth

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Tryptone</td>
<td>10.0 g</td>
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<tr>
<td>L-Tryptophan</td>
<td>1.0 g</td>
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<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
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<tr>
<td>Ferric chloride</td>
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<td>Distilled water</td>
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<td>Conc. HCl</td>
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<td>pH</td>
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11. Arginine Dihydrolase Broth

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12. Peptone Broth

<table>
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<tbody>
<tr>
<td>Peptone</td>
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<tr>
<td>Sodium chloride</td>
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Kovac’s Reagent

Amyl/isoamyl alchol 150.0 gm
Para-dimethyl aminobenzaldehyde - 10.0gm
Concentrated HCl - 50.0ml

13. Skimmed Milk Agar
   Skim milk powder - 100gm
   Peptone - 5gm
   Agar - 15gm
   Distilled water - 1000ml
   pH - 7.2

14. Ammonium salts basal medium for fermentation test
   Ammonium dihydrogen phosphate 1.0 g
   Potassium chloride 0.2 g
   Magnesium sulphate 0.2 g
   Agar 10.0 g
   Distilled water 1000 ml

15. Nutrient Yeast Salt Medium (NYSM)
   Glucose : 10 g
   Peptone : 5.0 g
   Sodium chloride : 5.0 g
   Beef extract : 3.0 g
   Yeast extract : 0.5 g
   Magnesium chloride : 0.203 g
   Calcium chloride : 0.102 g
   Manganese chloride : 0.01 g
   Distilled water : 1000 ml
   pH : 7

16. Esculin
17. Salicin fermentation test

Salicin 10% stock solution (10g sugar was dissolved in 100 mL distilled water) filter sterilized.

Peptone water
Peptone  1g
NaCl  0.5g
Distilled water  100 mL
pH  7
phenol red indicator  0.01%

5 ml of peptone broth mixed with 0.5 ml of Salicin stock solution after sterilization of peptone broth.

18. Sucrose fermentation test

Sucrose 10% stock solution (10g sugar was dissolved in 100 mL distilled water) filter sterilized.

Peptone water
Peptone  1g
NaCl  0.5g
Distilled water  100 mL
pH  7
phenol red indicator  0.01%

5 ml of peptone broth mixed with 0.5 ml of sucrose stock solution after sterilization of peptone broth.

19. Egg yolk powder agar (for Lecithinase production test)
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Egg powder</td>
<td>0.5%</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5%</td>
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<tr>
<td>Agar</td>
<td>1.5%</td>
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<tr>
<td>pH</td>
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