**Figure 1.** *Serratia marcescens* Strain TS1 showing red pigmented colonies on *Serratia* medium ATCC

**Figure 2.** *Serratia marcescens* strain TW1 showing red pigmented colonies on *Serratia* medium ATCC

**Figure 3.** Microscopic observation of strain TS1 showing gram negative rod shaped bacteria.

**Figure 4.** Microscopic observation of strain TW1 showing gram negative rod shaped bacteria.

**Figure 5.** Flagella staining of strain TS1 showing peritrichious flagella under microscopic observation.

**Figure 6.** Flagella staining of strain TW1 showing peritrichious flagella under microscopic observation.
Figure 7. Gelatin liquefaction test of strain TSI showing liquefaction in the nutrient gelatin medium where
C—Control tube
L—Test strain TSI showing liquefaction.

Figure 8. Gelatin liquefaction test of strain TW1 showing liquefaction in the nutrient gelatin medium where
C—Control tube
L—Test strain TW1 showing liquefaction.

Figure 9. Deoxyribonuclease test of strain TSI showing a zone of hydrolysis around the colonies in deoxyribonuclease-toluidine blue cephalothin agar.

Figure 10. Deoxyribonuclease test of strain TW1 showing a zone of hydrolysis around the colonies in deoxyribonuclease-toluidine blue cephalothin agar.

Figure 11. Casein hydrolysis test of strain TSI showing a zone of hydrolysis around the colonies in the casein agar.

Figure 12. Casein hydrolysis test of strain TW1 showing a zone of hydrolysis around the colonies in the casein agar.
Plate 13. Dialysis of *Serratia marcescens* TSI and *
*Serratia marcescens* strain TW1 at 4°C for 12 hours
against 5mM Tris-HCl buffer containing 1mM MgCl2
having pH 8.0 in 500ml of cold water.

Plate 14. Diethylaminoethyl Cellulose anion exchange
column chromatography for *Serratia marcescens* TSI
and *Serratia marcescens* strain TW1 at 4°C eluted with
10mM Tris-HCl (pH 8.3) and 10mM sodium phosphate
buffer (pH 6.3).
Figure 15. Determination of Protein content by Lowry method in which BSA was taken as standard. The concentration of protein was obtained as 60µg/0.5ml in *Serratia marcescens* strain TS1 are shown in pink spots and protein concentration for *Serratia marcescens* strain TW1 was obtained 56µg/0.5ml are shown in green spot.
**Figure 16.** Determination of molecular weight of proteases by sodium dodecyl sulphate agarose gel electrophoresis.

**L1**—Molecular marker mass standards: phosphorylase b (97 kDa), tyrosine (85kDa), acid phosphatase (75kDa), bovine serum albumin (66 kDa), glutamic dehydrogenase (50 kDa) and aldolase (40 kDa)

**L2**—Protease sample of *S. marcescens* TW1 (50kDa)

**L3**—Protease sample of *S. marcescens* TS1 (56kDa)

**Figure 17.** Isoelectric focusing electrophoretogram, pH 2.0 to 11.0 stained with coomassie blue.

**M**—Isoelectric focusing standards: amylglucosidase (pI 3.6), trypsin inhibitor (pI 4.5), carbonic anhydrase II (pI 6.5), lentil lectin (pI 8.3) and ribonuclease A (pI 9.6)

**L1 & L2**—*Serratia marcescens* TW1 showing pI 6.5

**L3 & L4**—*Serratia marcescens* TS1 showing pI 6.4
Figure 18. Effect of Temperature on protease activity was examined in 100mM Tris-HCl buffer having pH 8.0 at 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C and 65°C for 30 minutes. The *Serratia marcescens* strain TS1 showed maximum activity at optimum 40°C is shown in blue and the *Serratia marcescens* strain TW1 showed maximum activity at optimum 45°C is shown in pink colour.

Figure 19. Effect of pH on protease activity was examined in various buffers such as Glycine-HCl buffer having pH 2.0 to 3.5, Acetate buffer having pH 4.0 to 5.5, Phosphate buffer having pH 6.0 to 7.0, Tris-HCl buffer having pH 7.5 to 9.0 and Carbonate-Bicarbonate buffer having pH 9.5 to 11.0 at 30°C for 30 minutes. The pH optimum of *Serratia marcescens* strain TS1 was at pH 8.5 Tris-HCl buffer is shown in blue colour and pH optimum of *Serratia marcescens* strain TW1 was at pH 6.5 in Phosphate buffer shown in brown colour.
Figure 20. Effect of metal ions on protease activity was examined in 8.3mM of MgSO$_4$, MnCl$_2$, CaCl$_2$, CuSO$_4$, FeSO$_4$, HgCl$_2$ and ZnCl$_2$ in 0.1M Tris-HCl buffer having pH 7.5 at 25$^\circ$C for 30 minutes. The metal ions HgCl$_2$ and 20mM Na$_2$EDTA have inactivated the protease of both *Serratia marcescens* TS1 shown in blue colour and *Serratia marcescens* TW1 is shown in brown colour.

Figure 21. Effect of inhibitors on protease activity was examined in 20mM EDTA, 8.3mM Iodioacetic acid, 8.3mM Dithiothreitol, 8.3mM Leupeptin, 1% of 2-β Mercaptoethanol, 1% of Tween 20 and 3% of ethanol in 0.1M Tris-HCl having pH 7.5 at 25$^\circ$C for 30 minutes. The protease of *Serratia marcescens* strain TS1 was inactivated completely by 20mM EDTA and not by other inhibitors as shown in blue colour while as the protease of *Serratia marcescens* strain TW1 were inactivated by 20mM EDTA and not by other inhibitors as shown in brown colour.
Figure 22. Raising of antibodies towards protease of *Serratia marcescens* TW1 by injecting 0.5ml of purified protease along with incomplete Freund’s adjuvant through the subcutaneous route to the New Zealand white Rabbit having 2.3kg of body weight bearing ethical number NCP/IAE/KSR/04/09.

Figure 23. Ouchterlony double immunodiffusion analysis. The 1.2% (wt/vol) agarose gel was prepared in 0.05M borate buffer having pH 8.5. The distance from the peripheral wells to the centre well is approximately 5.5mm and each cut of the well is 4mm in diameter.  
P—Central well contained 20µl of antiserum  
S1—Cultural broth supernatant of *Serratia marcescens* strain TW1  
S2—Ammonium sulphate fraction 20µl of protease of *Serratia marcescens* strain TW1.  
S3—Dialysate protease 20µl of *Serratia marcescens strain* TW1  
S4—DEAE cellulose purified 20µl of protease of *Serratia marcescens* strain TW1
Figure 24. Washing test of protease *Serratia marcescens* strain TS1.

I. A. Blood stained cotton cloth.  
B. Washed with detergent wheel only  
C. Washed with both wheel detergent and protease of *Serratia marcescens* TS1

II. A. Chocolate stained cotton cloth,  
B. Washed with wheel detergent only,  
C. Washed with both protease of *Serratia marcescens* TS1 and wheel detergent.

III. A. Egg yolk stained cotton cloth.  
B. Washed with wheel detergent only,  
C. Washed with both protease of *Serratia marcescens* TS1 and detergent.

Figure 25. Agarose gel electrophoresis of Genomic bacterial DNA.  
L1--DNA marker of 2.0kb  
L2-- Strain TS1 0.92kb  
L3-- Strain TW1 0.87kb

Figure 26. Polymerase Chain Reaction product of DNA.  
L1-- DNA marker having molecular weight 1500bp  
L2, L4-- *Serratia marcescens* TS1 showed 920bp  
L3, L5-- *Serratia marcescens* TW1 showed 870bp