In this study, Pseudomonas aeruginosa CCRC11633 and Pseudomonas fluorescens CCRC14347 produced biosurfactants. The result showed that P. aeruginosa had the highest level of biosurfactant secretion among tested strains. Nutrient broth and five different carbon sources (glucose, sucrose, olive oil, soybean oil, n-paraffin) media were tested on shaker-flask scale for culturing P. aeruginosa with olive oil and soybean oil as carbon source, the surface tension of P. aeruginosa fermentation broth were reduced to 35.0 dyne/cm and the surface tension of fermentation broth were reduced to 26.2 dyne/cm. When glycerol-peptone were applied in the medium. We used spectrophotometer to monitor the secretion of rhamnose of the P. aeruginosa, the yield were 6.47 g/L. Use two-stage culture, the yield were 13.21g/L. The optimum cultural condition: with fermentation temperature of 30 ℃, pH7.0, 300mL/L of medium, 5% Glycerol as carbon and 4% peptone as nitrogen source. The biosurfactants produced by P. aeruginosa were identified by TLC as rhamnose, the CMC is 0.11 mL/dL and were stable at high temperature (121 ℃ for 20mins) and different pH value ranges (1-14). Ultrafiltration rather than extraction were better in recovery of rhamnolipid. Anion exchange chromatography on DEAE-Sephacel was chosen for further purification. The rhamnolipid was eluted from the matrix with 0.8M NaCl Tris-HCl buffer and the surface tension of this elution was 26.7 dyne/cm. Ten μ l of rhamnolipid showed growth inhibition to Escherichia. coli, Bacillus cereus, Bacillus subtilis and Staphylococcus aureus. Comply with rhamnolipid concentration increase, the inhibition ring augment for E. coli and Staphylococcus aureus. Using oil-containing medium, biosurfactant producing strain with low surface tension (30.1 dyne/cm) was isolated from Chang-Hwa and Jia-yi area.