## Multiple Antibiotic Resistance in *Pseudomonas putida*Associated with Overproduction of a Membrane Protein

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Porins are major outer membrane proteins which produce non-specific aqueous channels across the membrane that permit the diffusion into the bacterial cells of hydrophilic compounds including sugars, amino acids, and antibiotics. In some gram-negative organisms, antibiotic resistance can be induced by mutational loss of channel that causes a decrease in outer membrane permeability. By using Tn5phoA-1 transposon mutagenesis, mutants of Pseudomonas putida were isolated which showed a marked increase in their resistance to single or multiple antibiotics norfloxacin, streptomycin, and tetracycline. Total and membrane protein pattern analysis demonstrated the appearance of a protein of apparent molecular weight of 50 kDa when a mutant Pseudomonas putida KH1027 was grown in the presence of  $100\,\mu\,\mathrm{g/ml}$  streptomycin,  $20\,\mu\,\mathrm{g/ml}$  norfloxacin, and  $50\,\mu\,\mathrm{g/ml}$ tetracycline, respectively. This finding suggested that the 50 kDa membrane protein is associated with the decreased membrane permeability to antibiotics. To determine whether this protein was overproduced by null mutation in repressor protein like MexR in the MexAB-OprM pump system of P. aeruginosa, attempts were made to map the transposon insertion site using mexR primer of P. aeruginosa. The failure to amplify the bigger size fragment with the known insertion of TnphoA-1 element suggested that overexpression of 50 kDa protein was not achieved by inactivation of the MexR like repressor.

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