

ABSTRACT

Khewra salt mines of Pakistan are known for extremely saline conditions which is highly favorable for salt loving microbiota, halophiles. This study was conducted with aim to isolate halophilic or halotolerant bacteria from this hypersaline environment and characterize them. Soil, water, rocks and salt drippings were taken as samples from Khewra mine and its vicinity. Total twenty five samples were collected and processed for growth. Twelve samples did not show growth while thirteen samples grew on high salt media. Isolates were sub-cultured and purified to characterized by microscopy, biochemical test, API testing and macroscopic characters. All isolates were checked on different parameters, 0-30% sodium chloride concentration, pH 7-9 and temperature 25-40°C. Growth conditions were optimized by these parameters. DNA isolation of these strains was carried out by organic extraction method for bacteria and treated with RNase. Rnase treated DNA were run through gel elcetorphoresis against 1Kb genomic marker. Polymerase chain reaction was used to amplify with universal primers. Amplified product was sent for gene sequencing. For molecular characterization and phylogenetic analysis 16s rRNA sequencing was done.

Antimicrobial sensitivity testing was done for all isolates. Gram positive isolates were tested against coamoxiclav (AMC), Ceftazidime (CAZ), Cefixime (CFM), Ampicilline(AMP), Penicillin(P), Ceftriaxone(CRO), Ciprofloxacin (CIP), Cefotaxime (CTX), Amikacine (AK), Vancomycin (VA), Gentamicin (CN), Lenezolid (LZ), Oxacilline(FOX), Oxacilline(FOX), Cefuroxime (CXM) while Gram negative bacteria were tested against co-amoxiclav (AMC), Ceftazidime (CAZ), Salbactam (SCF), Tazobactam (TZP), Meropenem (MEM), Ceftriaxone (CRO), Ciprofloxacin (CIP), Cefotaxime (CTX), Amikacine(AK), Imepenem (IPM), Levofloxacin (LEV), Cefuroxime (CXM), oxcifloxacin (MXF) and Cefepime (FEP). Isolate AJ18-y, AJ18-w and AJ21-y were identified as member of *Staphylococcus* spp. Isolate AJ14-w and AJ22 were identified as *Bacillus* spp. Isolate AJ14-y was identified as *Stentophmonas maltophilia* on basis of API 20NE testing. Isolate AJ15 showed growth code for *Hafnia alvei* upon API10S testing. Isolate AJ16, AJ19 and AJ25 were *Serratia marcesens* in