Cultivations with Kleenol 30

Glycerol stocks of *A. radioresistens* 50v1 and *A. johnsonii* 2P08AA were separately streaked onto LB agar plates and incubated at 32 °C for ~24 h. Isolated colonies were inoculated into 2.0 mL 0.2x M9 containing 25 μ M Fe²⁺ and 0-2.0% v/v Kleenol 30 (or 0-40.0 μ L). Pre-cultures were initiated by addition of 20.0 μ L 90% v/v ethanol to yield final concentrations of 150 mM or 1.0% v/v ethanol. Pre-cultures were grown to late log phase (OD ~0.6 at ~12 h). Target cultures were prepared using fresh media (2.00 mL) which were inoculated with 20.0 μ L (1:100 dilution) of the respective pre-cultures and initiated by addition of 1.0 % v/v ethanol.

Temporal changes in OD were followed for 0-15 h. Growth kinetics were characterized by regression analysis (Microsoft Excel) using a modified version of the Gompertz equation (Begot et al., 1996), which describes a non-linear bacterial growth model, and yields the parameters of growth rate (k), lag time (L), and an estimate of the maximum change in relative biomass ($log(N/N_0)$). All regressions were minimized by least squares analysis. Control cultures containing no inoculate showed no growth (OD \leq 0.002), as did cultures containing inoculate but no ethanol (OD \leq 0.002), which indicated negligible biological contamination and accumulation of abiotic particles during cultivation.

Survivals of late-log phase cultures (OD ~0.4) were assessed by plating onto LB agar plates. In control experiments, plate counts using 0.2x M9 agar plates supplemented with ethanol (just prior to use) yielded irreproducible results, when compared to LB agar plate, likely due to variances in adsorption of ethanol (under our conditions). For the plate count assays, therefore, aliquots (100 μ L) of the cultures (in 0.2x M9/Fe) were transferred to 2.5 mL microcentrifuge tubes, decimally diluted by 10⁶-fold using 0.2x M9, and spread (20 μ L) onto LB agar plates using sterile plastic cell spreaders. All plates were sealed with parafilm and incubated at 32 °C for 24 h. Plates with \leq 300 colonies were enumerated and expressed as colony forming using per mL of the parent culture (cfu mL⁻¹).