R. solanacearum / Culture preservation

Protocols for *Ralstonia solanacearum* culture preservation

Source webpage (see links at the end of the document)

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Water suspensions

R. solanacearum can be stored for several years in distilled or deionized water (or tap water boiled to eliminate chlorine) without significant loss of virulence or change in phenotype. Cultures should be streaked first on TZC medium and well-isolated fluidal colonies restreaked on CPG plates because some strains are sensitive to the formazan pigment produced for TZC. Two loopfuls of bacteria from a composite of about six individuals 48 to 72 h-old colonies are transferred to 5 to 8 ml of sterile water in screw cap test tubes. Suspensions should be turbid (10^7 to 10^8 CFU/ml). Suspensions should be stored near 20ºC (15ºC min. and 28ºC max.) and restreaked every six months; repurify the culture if nonnucid become numerous. Cultures have maintained viability for 8 to 10 years in water.

Cryostorage

Freezing (less than -70ºC) *R. solanacearum* in a cryoprotectant is the most convenient method of long term storage with minimal phenotypic changes. Prepare screw-capped freezer vials for use by adding 0.6 ml of 20% (v/v) glycerol in water to each, capping loosely, and autoclaving for 20 min. Allow the tubes to cool to room temperature, cap tightly, and store at 4ºC until needed. To stock a strain, add 0.5 ml of CPG broth to each of two vials. Transfer to each vial the cells from one half a heavily streaked two-day-old (at 30ºC) CPG or TZC plate; thoroughly mix, seal tightly, and place in the ultralow freezer. One vial serves as the working stock and the second vial as a backup stock in a separate ultralow freezer to reduce loses if one of the freezers fails. To recover a strain, remove a vial from the ultralow freezer and quickly, while the contents remain frozen, use a sterile wooden applicator or hypodermic needle to scape off a small quantity of the sample from the surface of the frozen stock and streak this onto a TZC plate.

Lyophilization

R. solanacearum tolerates lyophilization very well, and this method was used for decades by Dr. Luis Sequeira to maintain his collection. To prepare the suspension medium for freezing, autoclave separately 14% (w/v) Bacto peptone and 14% (w/v) sucrose in water and then combine equal volumes of the sterile solutions. Remove cells from a fresh CPG plate and make a very dense (e.g., 10^10 CFU/ml) suspension in a small quantity of the supspension medium. Use a Pasteur pipette to dispense the bacterial suspension into the bottom of two or more sterile lyophilization ampules (about 10% of the total volume in the ampule) keeping the neck area of the ampule clean. Freeze the samples in a dry ice-ethanol bath and attach the ampules to a freeze-drying machine. When lyophilization is complete, flame seal the neck of each ampule under vaccum. Store the ampules at room temperature protected from the light. Some lyophilized cultures are known to have remained viable for 30 years.

Reference

Denny, TP; Hayward, AC. 2001. Gram-negative bacteria: Ralstonia. Pages 151-174 in: Laboratory guide for identification of plant pathogenic bacteria, 3rd ed. Schaad, NW; Jones, JB; Chun, W, eds. APS Press, St. Paul, M. N.
CABI/EPPO. 1999.

Internet links

***R. solanacearum* / Culture preservation webpage:**
http://plantpath.ifas.ufl.edu/rsol/CulturePreservation.html

***R. solanacearum* / bacterial wilt dedicated website:**
http://plantpath.ifas.ufl.edu/rsol/