Importance of biofilm formation for promoting plant growth under salt stress in *Pseudomonas putida* KT2440

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Abstract

An underutilized experimental design was used to isolate adapted mutants of the model bacterium *Pseudomonas putida* KT2440. The experimental design consisted of subjecting a random pool of mini-Tn*5* mutants of *P. putida* KT2440 to several rounds of selection in the rhizosphere of soybean irrigated with NaCl solution. Isolated adapted mutants (MutAd) showed a mutation in a gene encoding the membrane-binding protein LapA, which is involved in the early stages of biofilm formation on abiotic surfaces. Two MutAd bacteria (MutAd160 and MutAd185) and a *lapA* deletion mutant were tested to study the effect of this gene on salt tolerance, rhizosphere fitness, extracellular polymeric substances (EPS) production, and plant growth promotion. The inability of the mutants to form biofilm did not hinder attachment to soybean seeds and roots. MutAd bacteria showed an overproduction of EPS when grown under saline conditions, which would compensate for the lack of biofilm formation. MutAd185 bacteria showed increased root attachment and growth promotion of soybean in slightly saline soils. The proposed experimental design would be useful to accelerate bacterial adaptation to the rhizosphere of plants under a given environmental condition, identify genetic mutations that benefit bacterial fitness in that condition, and thus increase their ability to promote plant growth.

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