A self-transmissible, narrow-host-range endogenous plasmid of Rhodobacter sphaeroides 2.4.1: physical structure, incompatibility determinants, origin of replication, and transfer functions.

A Suwanto and S Kaplan

Department of Microbiology and Molecular Genetics, University of Texas Health Science Center, Houston 77225.

Abstract

Rhodobacter sphaeroides 2.4.1 naturally harbors five cryptic endogenous plasmids (C. S. Fornari, M. Watkins, and S. Kaplan, Plasmid 11:39-47, 1984). The smallest plasmid (pRS241e), with a molecular size of 42 kb, was observed to be a self-transmissible plasmid which can transfer only to certain strains of R. sphaeroides. Transfer frequencies can be as high as 10(-2) to 10(-3) per donor under optimal mating conditions in liquid media in the absence of oxygen. pRS241e, designated the S factor, was also shown to possess a narrow host range, failing either to replicate or to be maintained in Escherichia coli, Agrobacterium tumefaciens, and Rhizobium meliloti. It was further revealed that one of the remaining four endogenous plasmids, pRS241d, was also transmissible at a frequency similar to that of the S. factor. As a cointegrate with pSUP203, S was maintained in E. coli, providing sufficient DNA from which a physical map of S could be constructed. Progressive subcloning of S-factor DNA, in conjunction with assays of plasmid transfer, led to the localization and identification of oriV (IncA), IncB, and the putative oriT locus. The DNA sequence of the 427 bp containing oriTs revealed topological similarity to other described oriT sequences, consisting of an A-T-rich DNA region, several direct and inverted repeats, and putative integration host factor (IHF)-binding sites, and was shown to be functional in promoting plasmid transfer.