Utilization of Amadori Opines by *Agrobacterium* spp. : Ecological and Evolutionary Implications

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Opines are produced by crown gall tumor cells, which are induced by pathogenic *Agrobacterium* spp. Opines play key roles in the interkingdom interactions between *Agrobacterium* spp. and infected plants; promoting selective growth of the pathogenic bacterium; inducing Ti plasmid conjugal transfer; acting as attractants for agrobacterial strains; and inhibiting growth of certain agrobacterial strains. Among the more than twenty known opines, the chrysopine-family is produced by tumors induced by *A. tumefaciens* Chry-strains, *Ficus* strains, and IIBV7. This family of Amadori-type compounds includes *N*-1-deoxy-D-fructosyl-L-glutamine (dfg), commonly called santhopine, *N*-1-deoxy-D-fructosyl-L-glutamate (dfga), *N*-1-deoxy-D-fructosyl-5-oxo-L-proline (dfop), and chrysopine, the spiropyranosyl lactone of dfg. Chemically, these compounds are closely related to the mannityl opines (Fig. 1). Dfg and dfga are the deoxyfructosyl analogs of mannopine (MOP) and mannopinic acid (MOA), respectively, and chrysopine and dfop are deoxyfructosyl analogs of agropine (AGR) and agropinic acid (AGA),

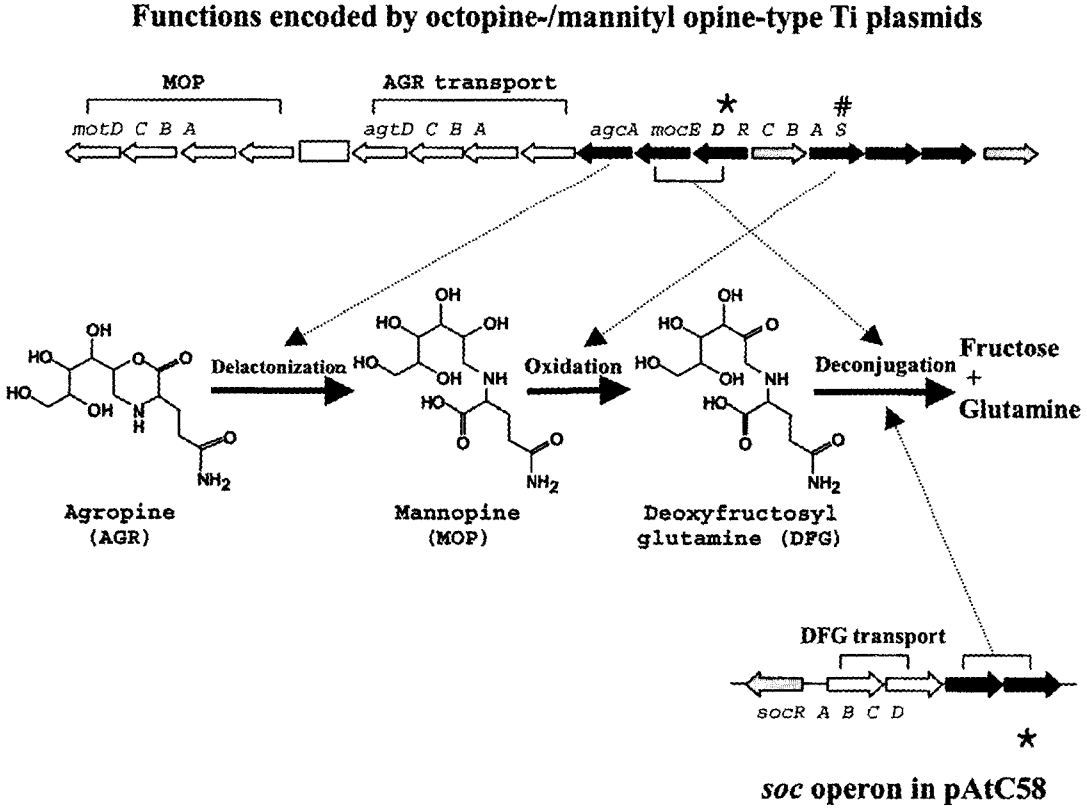

Figure 1. Catabolism of MOP and dfg, and genes associated with the pathway

respectively. These similarities suggest a close relatedness in catabolic pathways and also in the genes coding for the enzymes for their catabolism between the two families of opines. In this regard, dfg is an intermediate in the pathway for catabolism of AGR and MOP coded for by the octopine/mannityl opine-type Ti plasmids such as pTi15955. MOP either converted from AGR or taken up by the MOP-transport system encoded by *mot* genes is oxidized to dfg by MOP oxidoreductase encoded by *mocC* (Fig. 1). The product, dfg, is further degraded by functions encoded by MocD and MocE, the putative dfg deconjugase and a kinase, respectively. The *mot* and *moc* genes cluster to a 25-kb region of the octopine/mannityl opine-type Ti plasmids.

While catabolism of the mannityl opines is coded for by mannityl opine-type Ti and Ri plasmids, a second set of genes for utilization of dfg apparently is located in a large accessory plasmid called pAtC58, can utilize dfg as sole carbon source. Five genes organized in at least two transcriptional groups are responsible for the utilization of this opine; *soc* (santhopine catabolism) *R* and *socABCD*. Nucleotide sequence analysis and analyses of transposon-insertion mutations in the region showed that SocR negatively regulates the expression of *socR* itself and *socABCD*. SocA and SocB are responsible for transport of dfg and MOP. SocA is a homolog of known periplasmic amino acid binding proteins. The N-terminal half of SocB is a homolog of the transmembrane transporter proteins for several amino acids, and the C-terminal half is a homolog of the transporter-associated ATP-binding proteins. SocC and D could be responsible for enzymatic degradation of dfg, being homologs of sugar oxidoreductases and an amidoriase from *Corynebacterium* sp., respectively. The protein products of *socABCD* are not related at the amino acid sequence level to those of the *moc* and *mot* genes of Ti plasmids responsible for utilization of dfg and MOP, indicating that these two sets of genes and their catabolic pathways have evolved convergently from independent origins.

Various isolates of *Agrobacterium* and related soil-bacteria in the family Rhizobiaceae were examined by Southern analysis for the presence of homologs of *socD* and *mocD*, which are essential for degradation of dfg in the two gene sets. Homologs of *mocD* are specific to Ti plasmids in agrobacterial species, and *socD* homologs are present in non-Ti or Ri plasmid replicons in *Agrobacterium*, and also present in some of the isolates of *Rhizobium* and *Sinorhizobium* spp. examined. Genes homologous to *socD* or *mocD* were not detected in isolates of *A. rhizogenes* and *A. vitis* tested. When a clone of *mocC* coding for MOP oxidoreductase (Fig. 1) was introduced into strains containing *socD* homologs, all of the *Agrobacterium* strains and some of the *Rhizobium* strains acquired the capacity to utilize MOP as sole carbon source. Some strains of *Agrobacterium* spp., which lacked homologies to *socD* and *mocD*, gained the ability to utilize MOP upon introduction of *mocC*.

Table. 1 Grouping of *Agrobacterium* isolates according to traits associated with dfg

Group	*A. tumefaciens*	*A. rhizogenes*	*A. vitis*	*A. radiobacter*
A	B6, 15955, R10, A6, A6NC			
B	Chry5, ANT4			
C	C58, T37, IIVB7			K299
D	Bo542			
E	NA147	A4, TR7,8196	K308	
F			K305, AB3 Ag57, Tm4	
G	K108, J73, AB2/73	K599		K84, K112

Based on Southern hybridizations and dfg-utilization traits, the *Agrobacterium* isolates could be divided into seven groups. These results suggest that genes for utilization of Amadori opines are good genetic markers to evaluate evolutionary relationships among Rhizobiacea, and that there exist three or more independently evolved sets of the functions for dfg utilization among various bacterial isolates belonging to the family Rhizobiaceae. We suggest that Amadori compounds naturally occurring in rhizospheres exert natural selections on various soil microorganisms to acquire ability to utilize the compound, leading evolution of genes from numerous genes related to the catabolism of compounds that are chemically similar to the opines. Such genes have propagated by horizontal and vertical gene transfers among Rhizobiaceae.

References

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