IVET-Based Identification of Genes Modulated by Quorum-Sensing Signal Molecule in *Rhodobacter sphaeroides* 2.4.1.

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Rhodobacter sphaeroides 2.4.1 is a free-living, photoheterotrophic gram-negative bacterium that possesses a cell density-dependent regulatory system, generally termed quorum-sensing (QS). The *cerR-cerI* gene pair, which is a member of the *luxR-luxI* family, has been identified in this bacterium. However, biological role of the QS system in this organism is unclear, and structural genes controlled by the QS system remain to be systematically elucidated. To identify the genes controlled by QS, we constructed a genomic library of the bacterium in pSG2, a suicide vector containing promterless *lacZ* (pl-lacZ) and promoterless kanamycin-resistant gene (pl-km) as reporters (Fig. 1).

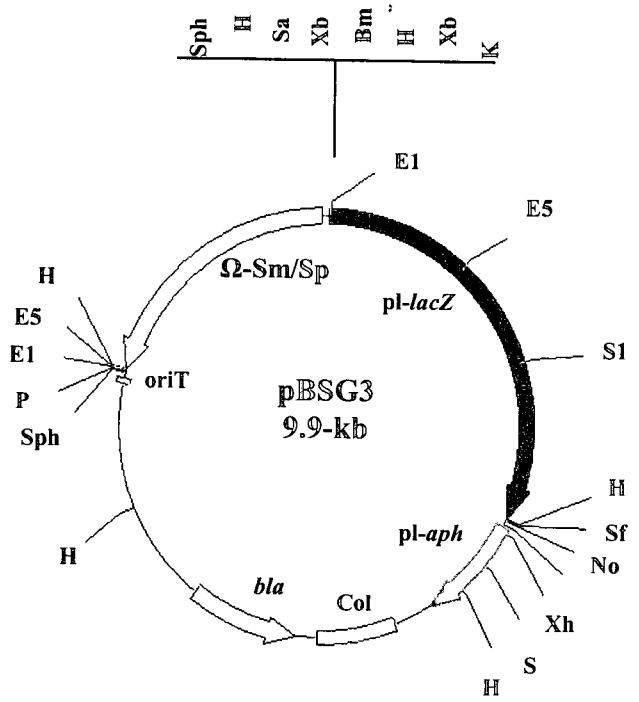

Fig. 1. Genetic map of the suicide vector pSG2.

More than 20,000 library clones were constructed, each of which contains a 3-5 kb genomic DNA fragment, and were introduced into *R. sphaeroides* strain AP3T that is a *cerI*-null mutant strain. About 20,000 exoconjugants were obtained which contain pSG2-derived random reporter fusions in the genome generated by single crossover between inserts in pSG3 derivatives and the identical region in the genome of AP3T. Each of fusions was tested for the inducibility of the expression of reporter genes upon the addition of exogenous synthetic acyl-HSL (RAI-I) molecules. In this screening, we obtained 23 independent clones

(Table 1). Among these clones, 21 clones showed >2-fold induction of β-galactosidase activities, and two showed >2-fold reduction of the activities in response to synthetic RAI-I. Two clones, which showed the most significant induction levels, were cloned and their physiological roles are being investigated.

Table 1. β–Galactosidase activity and sequence homology of selected clones

Clone number	Miller unit without HSL	Miller unit with HSL	Identity (similarity)	Homolog protein (species)
#1[a]	3.2	53.3	91% (91%)	Hypothetical protein (*Rhodobacter sphaeroides*)
#57	7.8	17.1	59% (69%)	Hypothetical protein (*Chloroflexus aurant*)
#198	7.1	13.2	91%	Hypothetical protein (*Rhodobacter sphaeroides*)
#209	3.8	14.4		No homology with known function
#779	6.5	11.7	50% (62%)	Alginate O-acetylation protein (*Helicobacter pylori*)
#74	36.6	30	100%	Hypothetical protein (*Rhodobacter sphaeroides*)
#193	112.7	49.8	40% (48%)	Putative (*Human papillomavirus* type)

[a] Sixteen other clones were isolated, which have the reporter gene fused at this gene.