SUPPLEMENTAL MATERIAL

for

Two systems for conditional gene expression in *Myxococcus xanthus* inducible by isopropyl-β-D-thiogalactopyranoside or vanillate

Supplementary Figures and Tables

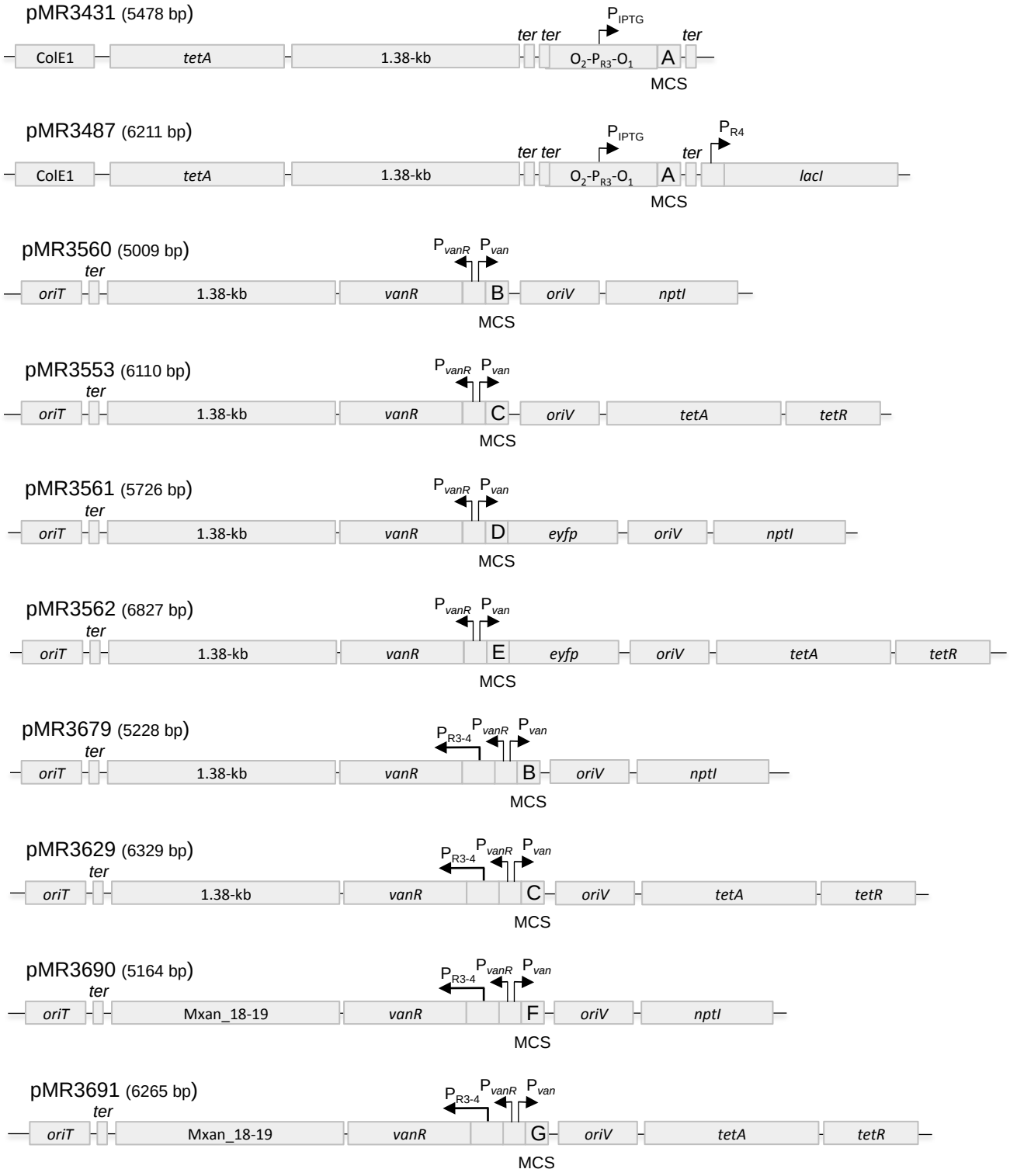

FIG. S1. Schematic of several integrative plasmids for the IPTG- and vanillate-based gene expression systems. Elements used are denoted as follows. P_{IPTG}: the IPTG-inducible promoter composed of the *rrnD* P_{R3} promoter flanked by two *lacO* operators (O_2 and O_1); P_{R4}: the *rrnD* promoter; P_{van}: the vanillate-inducible promoter; P_{vanR}: the

natural promoter for constitutive *vanR* expression; P_{R3-4}: P_{R3} and P_{R4} in tandem; *ter*: transcriptional terminator; *lacI*: gene for *E. coli* LacI repressor; *vanR*: *C. crescentus* VanR repressor gene; 1.38-kb and Mxan_18-19: *M. xanthus* chromosomal DNA segments that allow plasmid integration into the chromosome at distinct sites (see text); ColE1, *oriV*: plasmid replication origins; *oriT*: origin of transfer; *tetA*: tetracycline efflux permease and *tetR*: TetR repressor (for Tc^R); *nptI*: neomycin phosphotransferase I (for Km^R); *eyfp*: enhanced yellow fluorescent protein; MCS, A-G: different multicloning sites (A to G; see Fig. S2 for details).

FIG. S2. Multiple cloning sites (MCS A-G) of P_{IPTG}- and P_{van}-containing plasmids. Shine-Dalgarno motifs or ribosomal binding sites (RBS), fusion *eyfp* tag, and unique restriction sites are indicated.

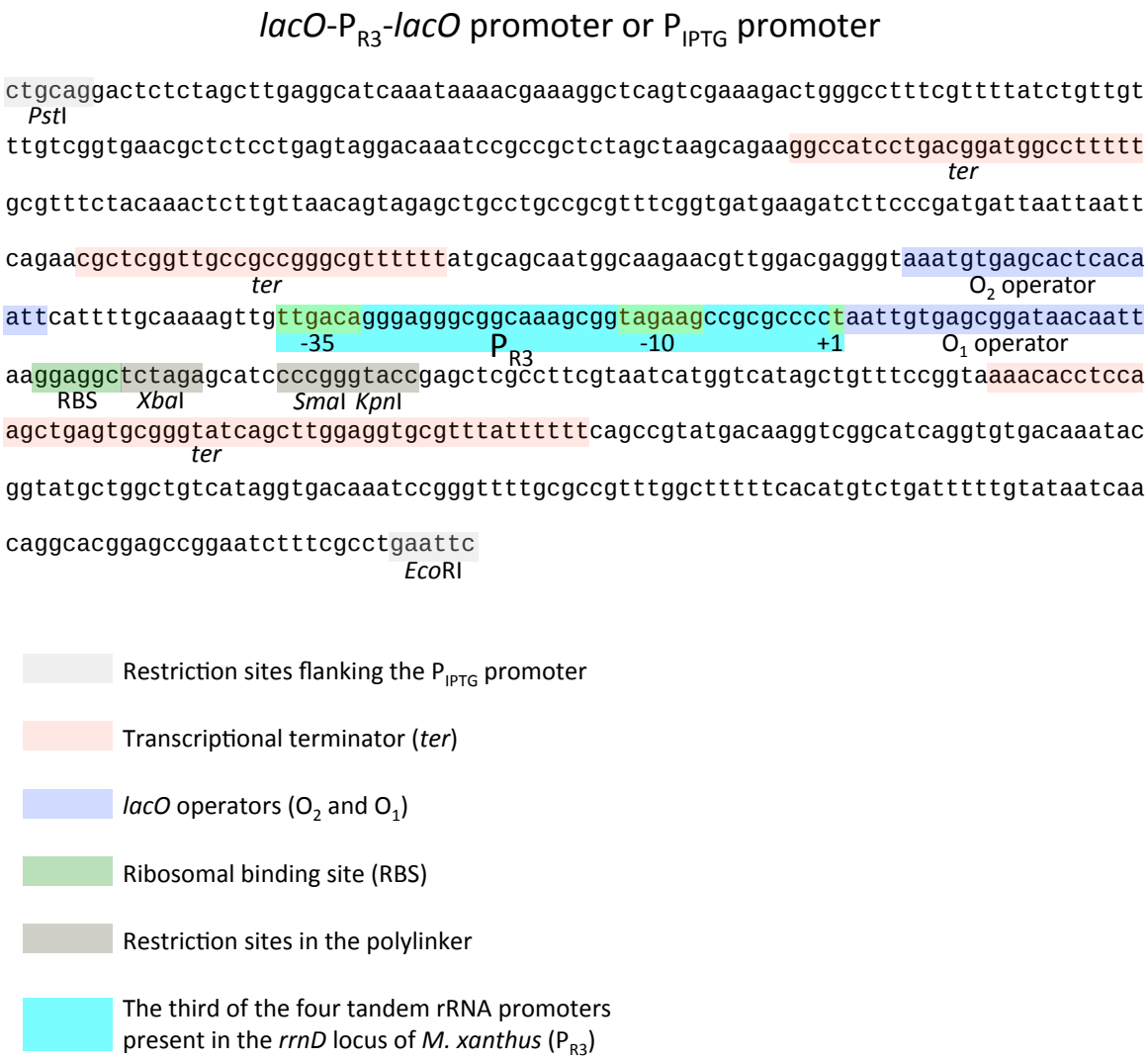

FIG. S3. Complete sequence for the P_{IPTG} promoter.

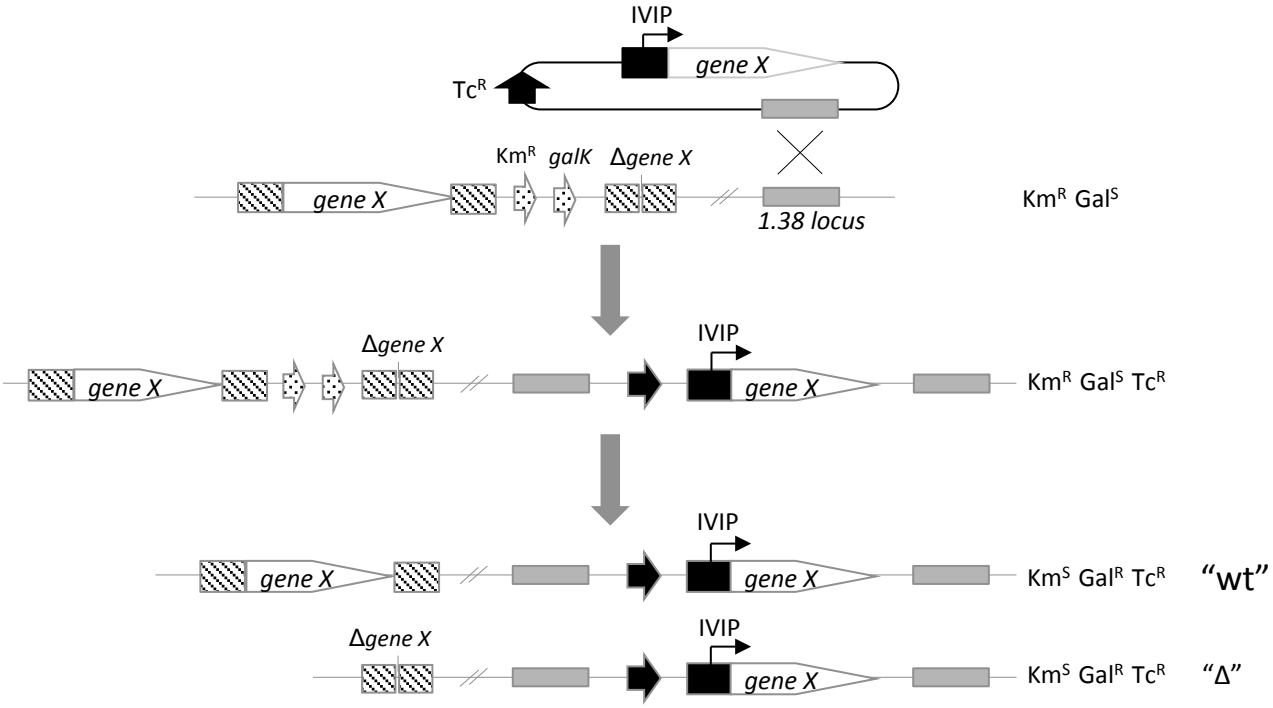

FIG. S4. Strategy for generating strains with conditional expression of essential genes in *M. xanthus*. The gene of interest is represented by *gene X* and the deleted allele by Δ*gene X*. Other elements are as follows. IVIP: inducible P_{IPTG} or P_{van}; Tc^R: tetracycline resistance; Km^R: kanamycin resistance; Gal^R/Gal^S galactose resistance/ sensitivity; 1.38-kb locus: chromosomal locus for plasmid integration via homologous recombination (solid gray boxes); striped boxes: sequences (~1 kb) upstream and downstream of the gene of interest in its natural genomic context. Strains thus generated have a copy of the gene expressed from the inducible promoter (P_{IPTG} or P_{van}) and are denoted "wt" or "Δ" depending on whether they have the wild-type or the deleted allele at the native locus.

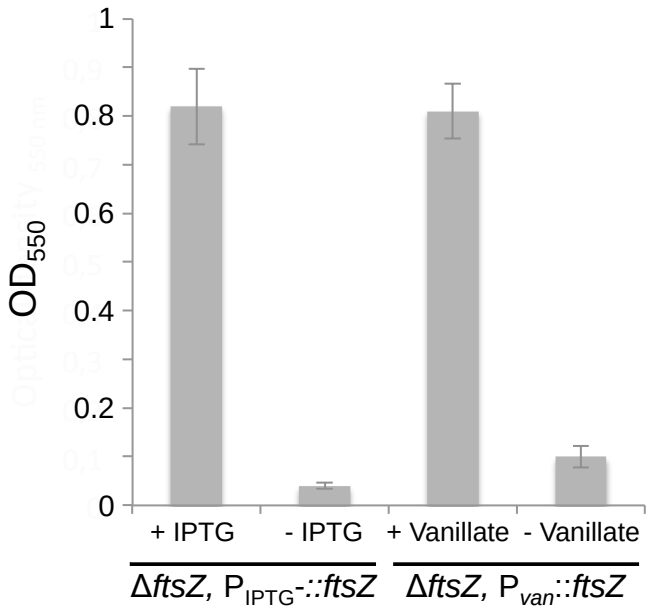

FIG S5. FtsZ depletion abolishes cell growth. Cell densities (OD_{550}) for cultures of strains with a single copy of *ftsZ* under the control of the IPTG- or the vanillate-inducible promoter (strains MR2196 and MR1982, respectively), in the presence and in the absence of the appropriate inducer. A starter culture grown in the presence of inducer to an OD_{550} of 1 was diluted to 0.1 in fresh medium with or without inducer and grown for 24 hours. Cells were then diluted to OD_{550} of 0.1 and grown with or without inducer for a further 24 hours followed by OD_{550} measurement, shown as the mean and standard deviation of three independent measurements.

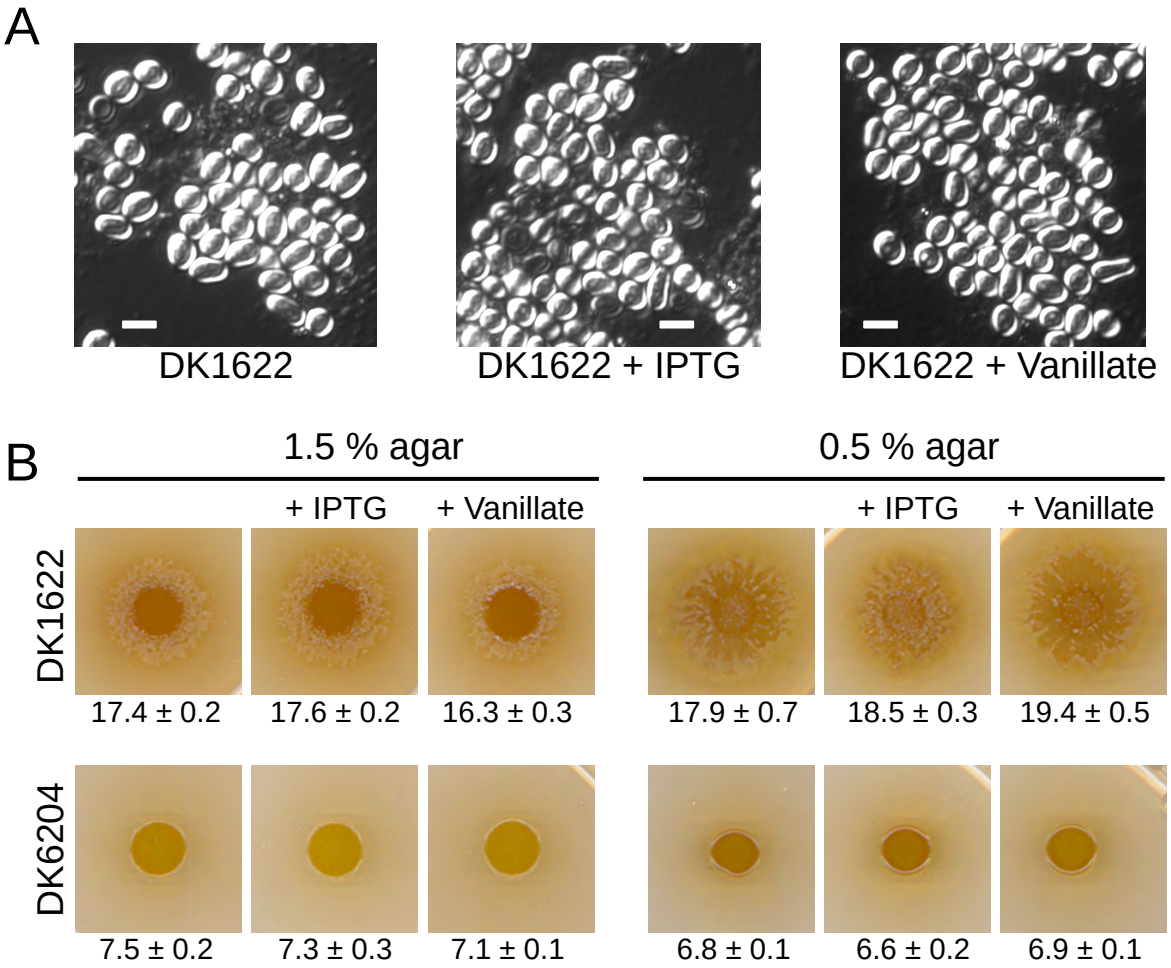

FIG. S6. Motility or sporulation is not impaired by IPTG or vanillate. (A) DIC microscopy images of spores from DK1622 fruiting bodies developed on TPM plates in the absence or presence of 1 mM IPTG or 0.5 mM vanillate. Fruiting bodies were scraped from the plates after 6 days and treated with sonication prior to microscopical observation. Note the round morphological shape that is characteristic of myxospores. Scale bars, 5 μm. (B) Colony spreading motility assays for wild type DK1622 and for the nonmotile A^-S^- mutant strain DK6204 on 1.5% (for A motility) or 0.5% (for S motility) CTT agar plates in the absence or presence of 1 mM IPTG or 0.5 mM vanillate. Images were taken 5 days after DK1622 and DK6204 cultures were spotted on the plates. Mean colony diameters and the corresponding standard deviation from four independent spots (each 6 mm in diameter initially) of each strain and condition are indicated below each spot.

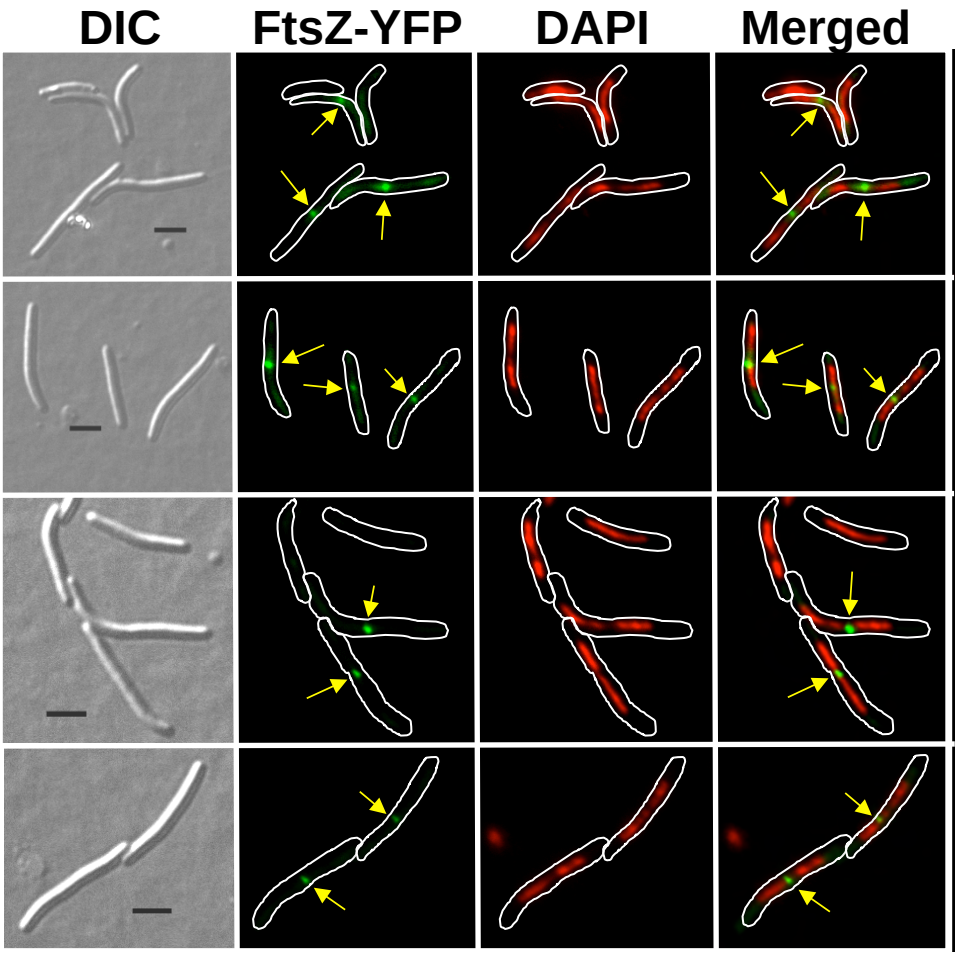

FIG S7. Localization of FtsZ-YFP after induction with vanillate. In each row, panels from left to right correspond to images obtained by DIC, FtsZ-YFP fluorescence (green), DAPI fluorescence (red), and a merged image of the last two (green and red) for strain MR2479 after 1 hour of induction with 0.5 mM vanillate. For the fluorescence images the cell outline is shown drawn with a white line. Scale bars, 5 μm. Yellow arrows point FtsZ-YFP foci.

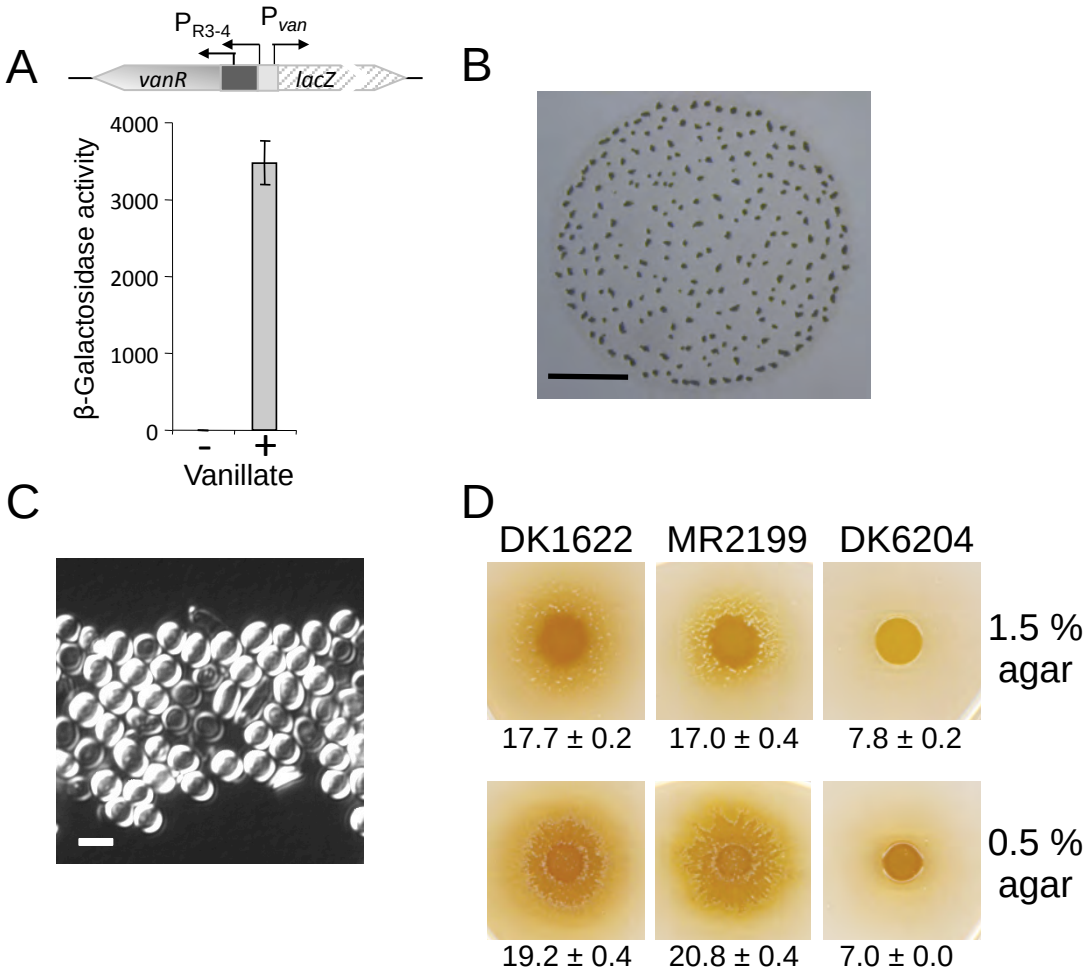

FIG S8. DNA integration at the Mxan18_19 chromosomal locus does not affect P_{van}-*lacZ* reporter gene expression, aggregation, sporulation, or motility. (A) Expression of the P_{van}::*lacZ* reporter gene for strain MR2491 (with P_{van}::*lacZ* integrated at the Mxan18_19 chromosomal locus and the VanR repressor expressed from P_{R3-4}) after a 15- to 20- hour growth in the absence or in the presence of 0.5 mM vanillate. Mean values and standard deviation of the mean are from three independent experiments. Fruiting body aggregates (B) and DIC image of spores (C) for strain MR2199, bearing the P_{van}::*ftsZ-yfp* construct at the Mxan18_19 locus, 6 days after triggering starvation-induced development on TPM agar. Scale bars, 2 mm (B) and 5 μm (C). (D) Colony spreading assays for A motility (top panels, 1.5 % agar) and S motility (bottom panels, 0.5% agar) for MR2199 and, as controls, for the wild-type strain DK1622 and the A^-S^- nonmotile mutant strain DK6204. Images were captured 5 days after cultures were spotted on the indicated CTT-agar plates. Mean colony diameters and the corresponding standard deviation (estimated as in Fig. S6B from six independent spots, each 6 mm initial diameter) are shown below each spot.

SUPPLEMENTARY TABLES

Table S1. DNA oligonucleotides used in this study.

Name	Site	Sequence 5´-3´(restriction site underlined)	Reference
1_FtsZ.for	*Nde*I	agaggacccatatggaccagttcgatca	This study
2_FtsZ.rev	*Kpn*I	ttttggtacccggcagttccgtctggccct	This study
9_LacZ.for	*Nde*I	tctagaaggaaacacatatgaccatgatta	This study
20_rRNA-1.for	*Nco*I	aaaaaccatggggacgagtcgagggagtca	This study
21_rRNA-3.rev	*Nco*I	aaaaaccatggaccgcctccccgtcttcttc	This study
33_Ori.for	*Nde*I	gctgtcggggcacatatggcgccagacgat	This study
34_Ori.rev	*Xba*I	gcatgcgggaagtctagacgccgcgccgac	This study
cdnL-BamHI	*Bam*HI	aaaggatcctcaggccagattgaagatctt	This study
cdnL-NdeI	*Nde*I	aaaaaaaacatatgcagaccagcttcaag	This study
ftsZ-EcoRI	*Eco*RI	aaaagaattcttacggcagttccgtctggccc	This study
ftsZ-NdeI	*Nde*I	aaaaaaaacatatggaccagttcgatcagaacaag	This study
lac274-BamHI	*Bam*HI	aaaaggatccaggcgaaagattccggctccg	This study
lacI_NdeI_DR111	*Nde*I	aaaacatatgtcactgcccgctttccagtc	This study
lacI_XhoI_DR111	*Xho*I	aaaactcgaggtgaaaccagtaacgttatac	This study
lacZSEQ2	---	acggttaacgcctcgaatc	This study
lacZ-XbaI	*Xba*I	aaatctagaatcacacaggaaacagctatg	This study
prRNA3-XhoI	*Xho*I	aaactcgaggcctccccgtcttcttctgcccgg	This study
prRNA5-RI	*Eco*RI	aaagaattccggaagccagcaggacggcg	This study