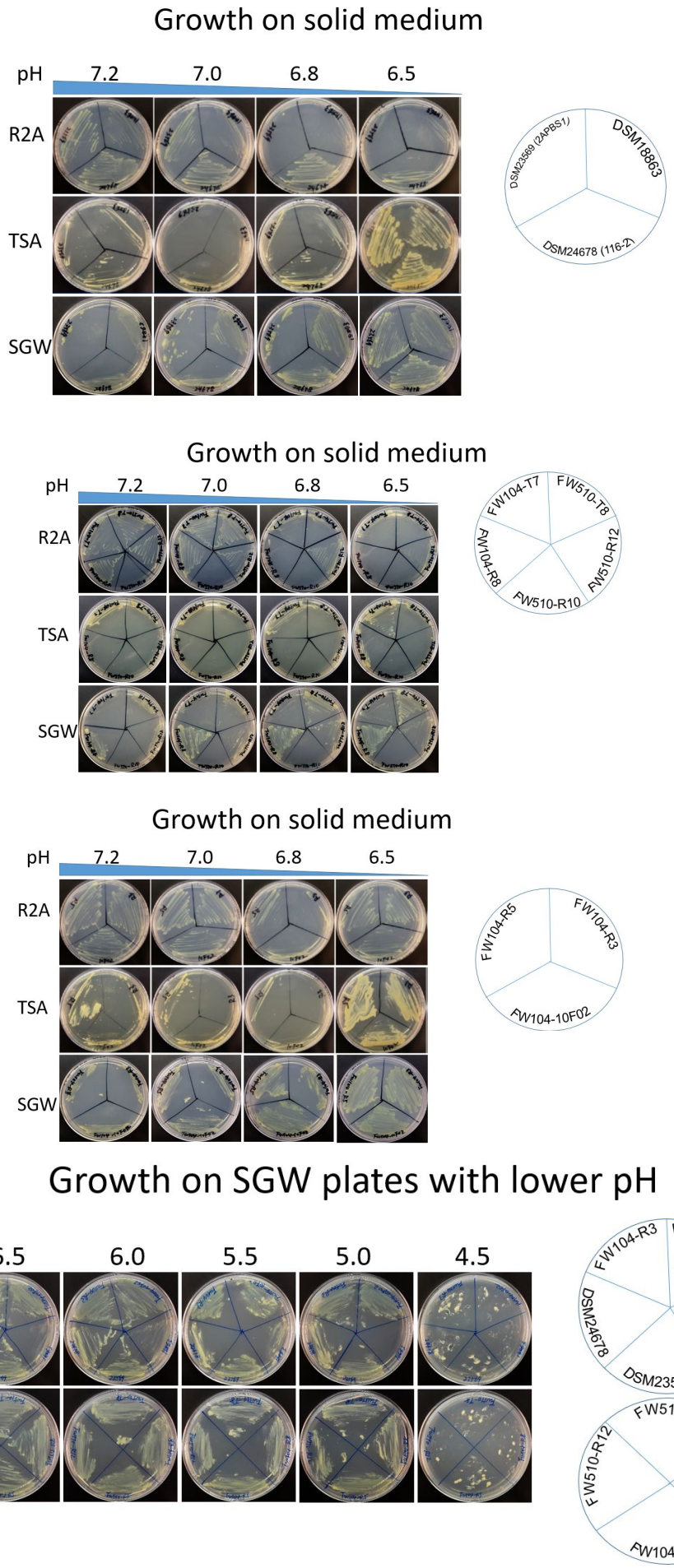


Figure S1. Growth test for each strain grown on R2A, TSA or SGW solid plates with different pH conditions. The FW510-R10 could not grow in any type of tested solid plates, therefore it was excluded in the following experiments.

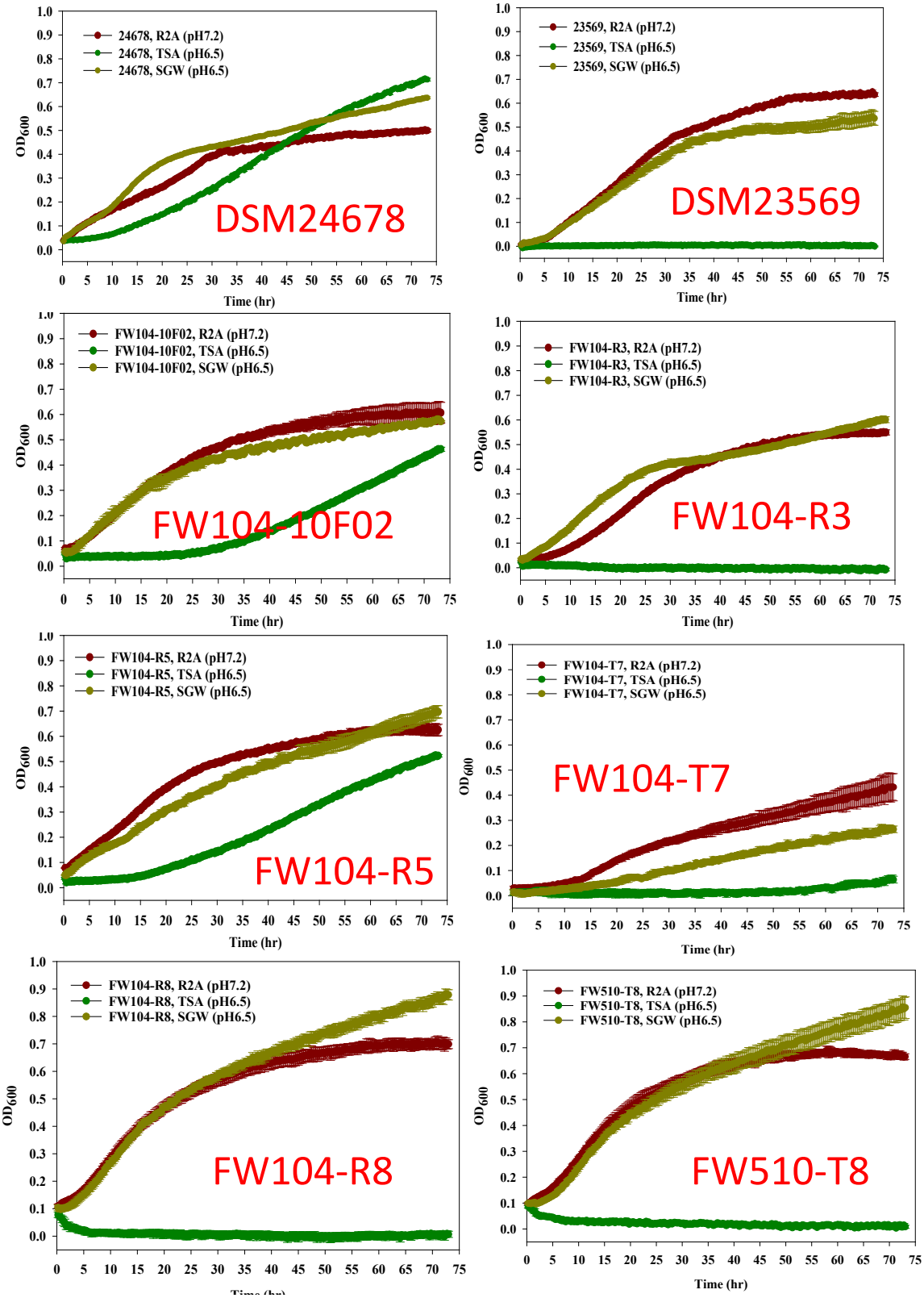
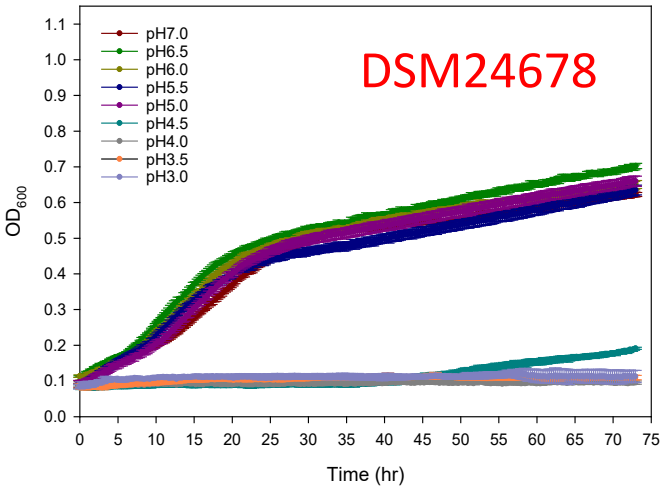
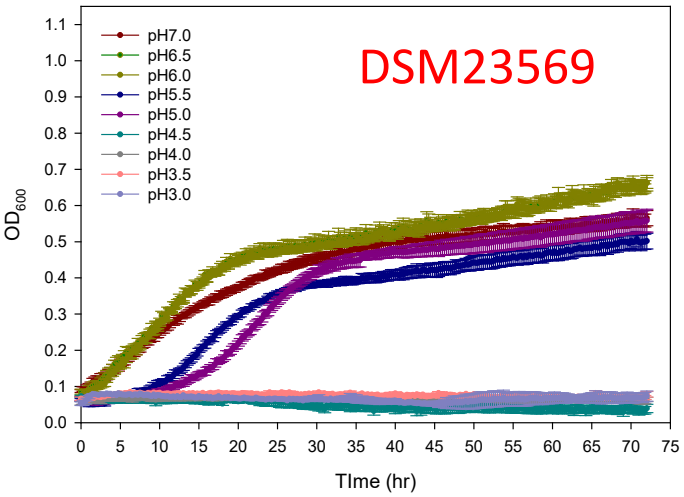
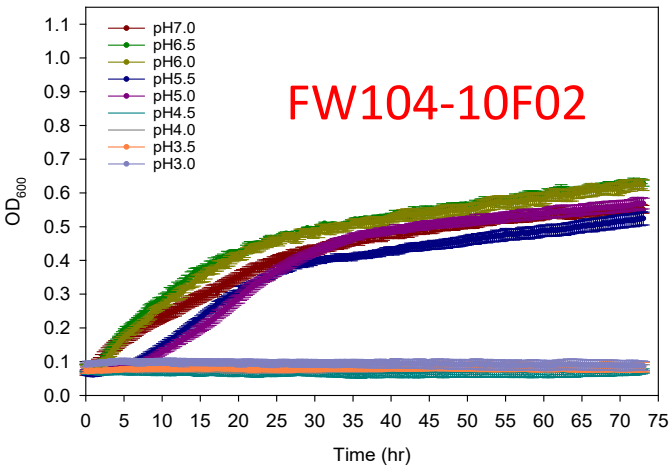
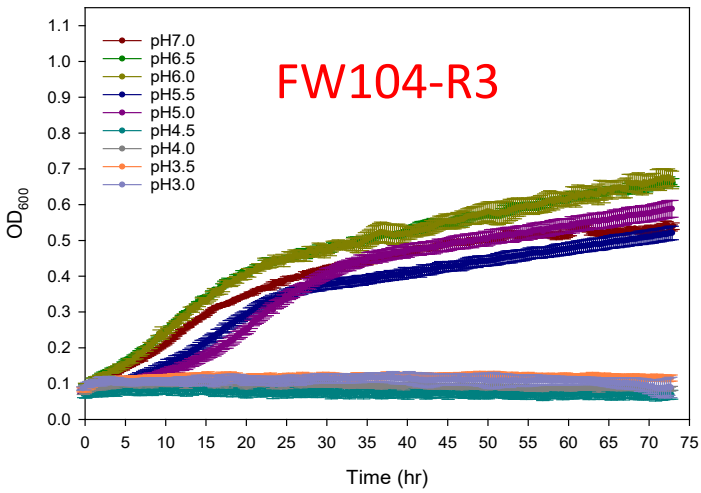


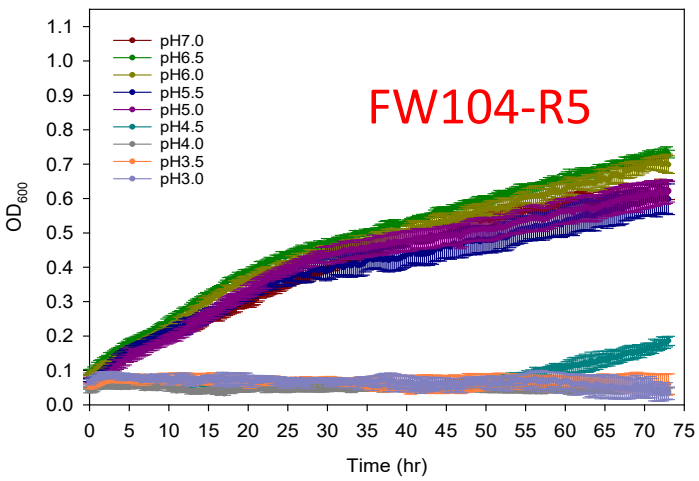
Figure S2. Growth analysis for each strain grown in R2A (pH 7.5), TSA (pH 6.5) or SGW (pH 6.5) liquid medium. Since the FW510-R10 could not grow on any type of tested solid medium, it was excluded from this liquid medium test experiment. Data are presented as the mean of biological replicates (n=4) and error bars represent SD.

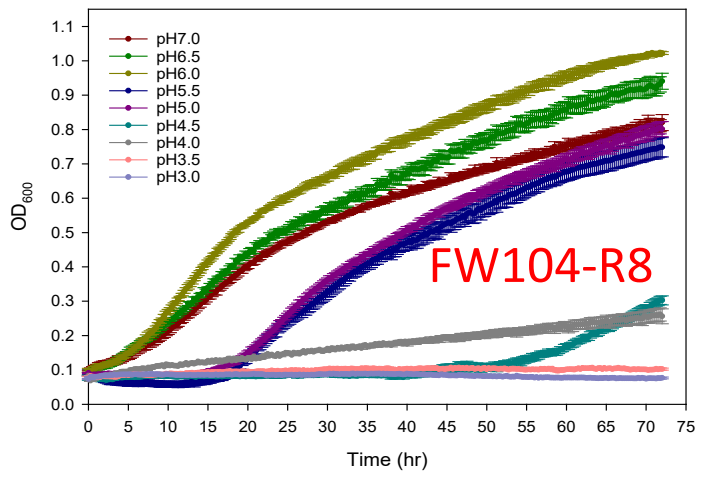












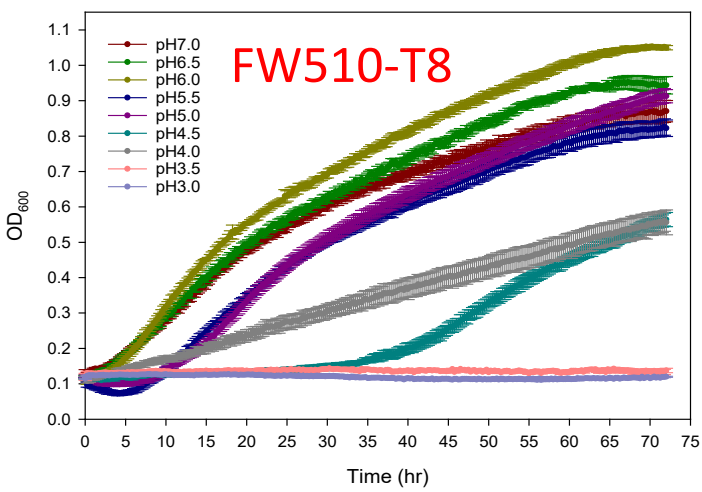
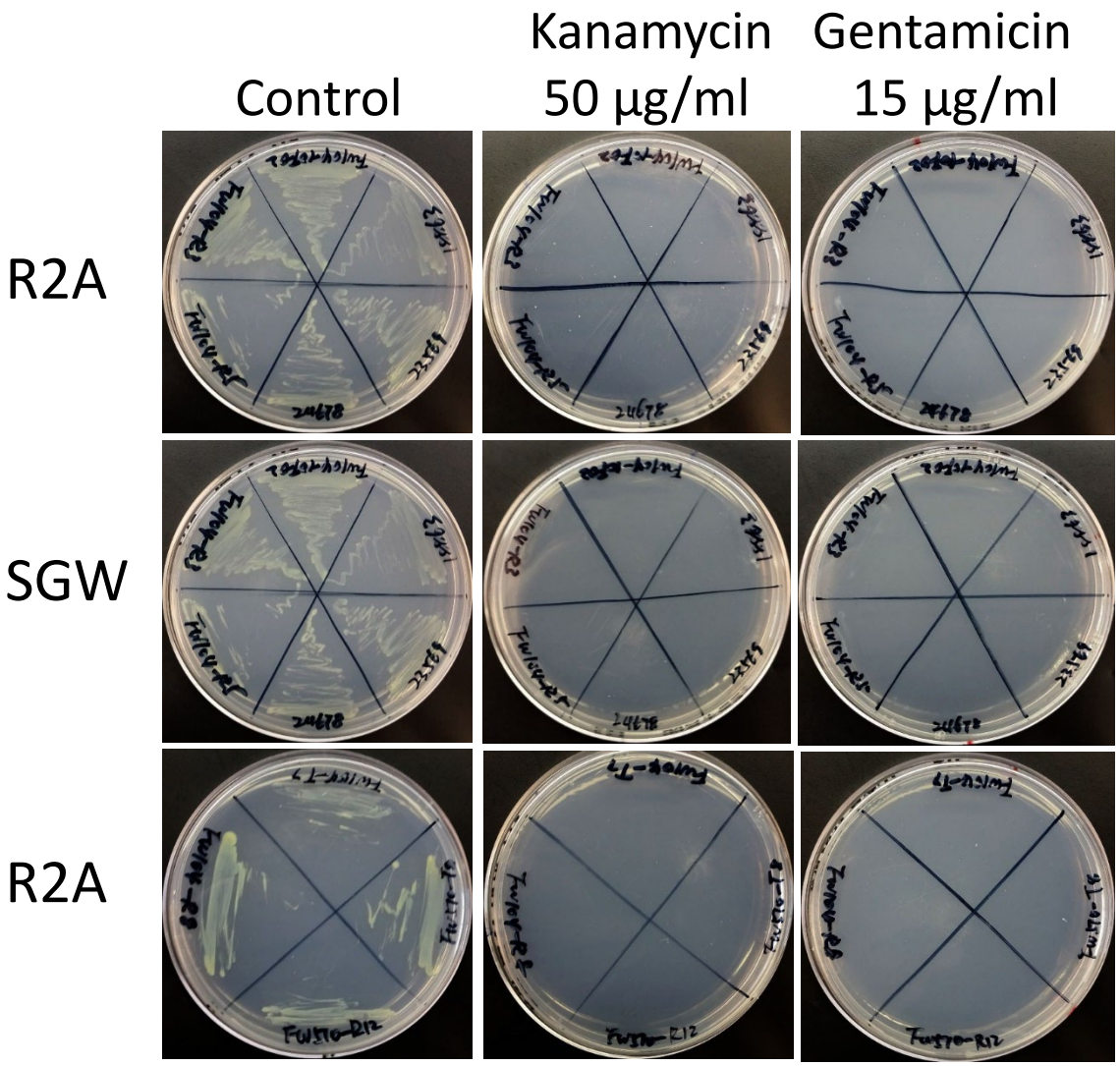


Figure S3. Growth analysis for each strain grown in SGW liquid medium with different pH conditions. Since FW104-T7 and FW104-R12 did not grow very well and FW104-R10 did not grow on the R2A/TSA/SGW plates (Figure S1, S2 & Table S1), these three strains were excluded from the growth profile experiment. Data are presented as the mean of biological replicates (n=4) and error bars represent SD.



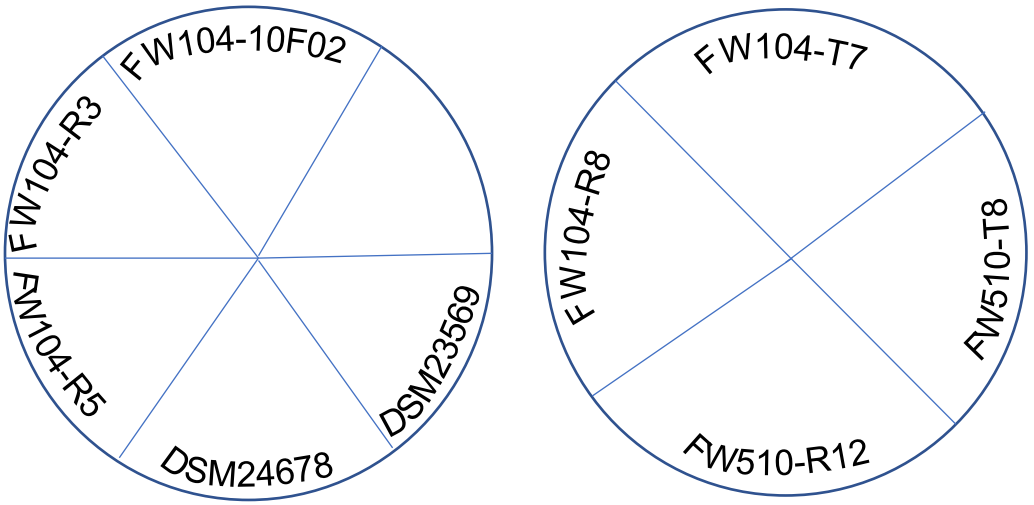


Figure S4. Antibiotic sensitivity test for each strain grown on R2A (pH 7.2) or SGW (pH 6.5) medium.

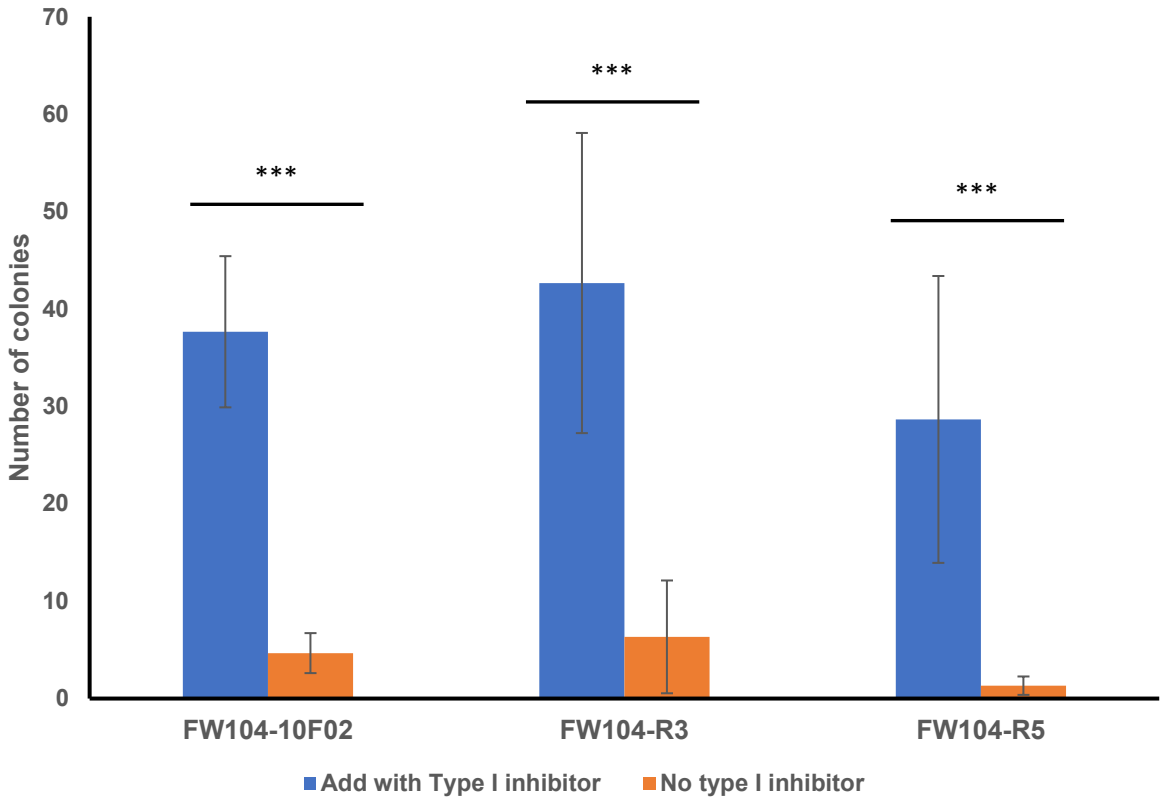
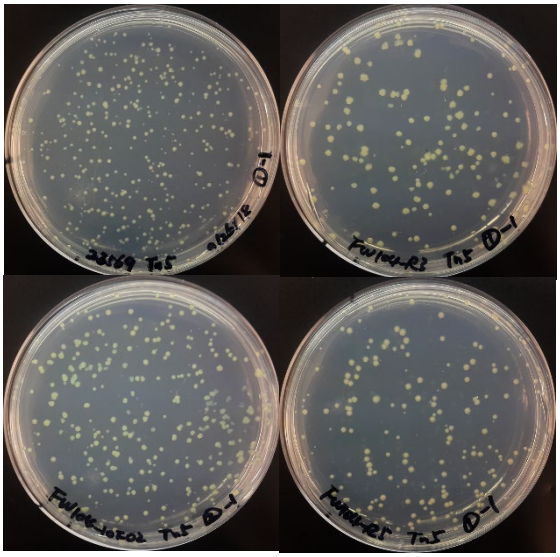


Figure S5. Effects of type one restriction inhibitor on transformation efficiency for different strains. The vector pPROBE-GT and SGW medium are used for this test. Significance is indicated by *, $0.010 < p \leq 0.050$, **, $0.001 < p \leq 0.010$ and ***, $p < 0.001$ determined by unpaired two-tailed t test. The bars represent the average $\pm$ SD (n=3).

DSM23569



FW104-R3

FW104-10F02

FW104-R5

Figure S6. Electroporation of the barcoded Tn5 transposon delivery vector into different strains (DSM 23569, FW104-10F02, FW104-R3 and FW104-R5). For transformation,1μg of Tn5 transposon delivery vector was used.

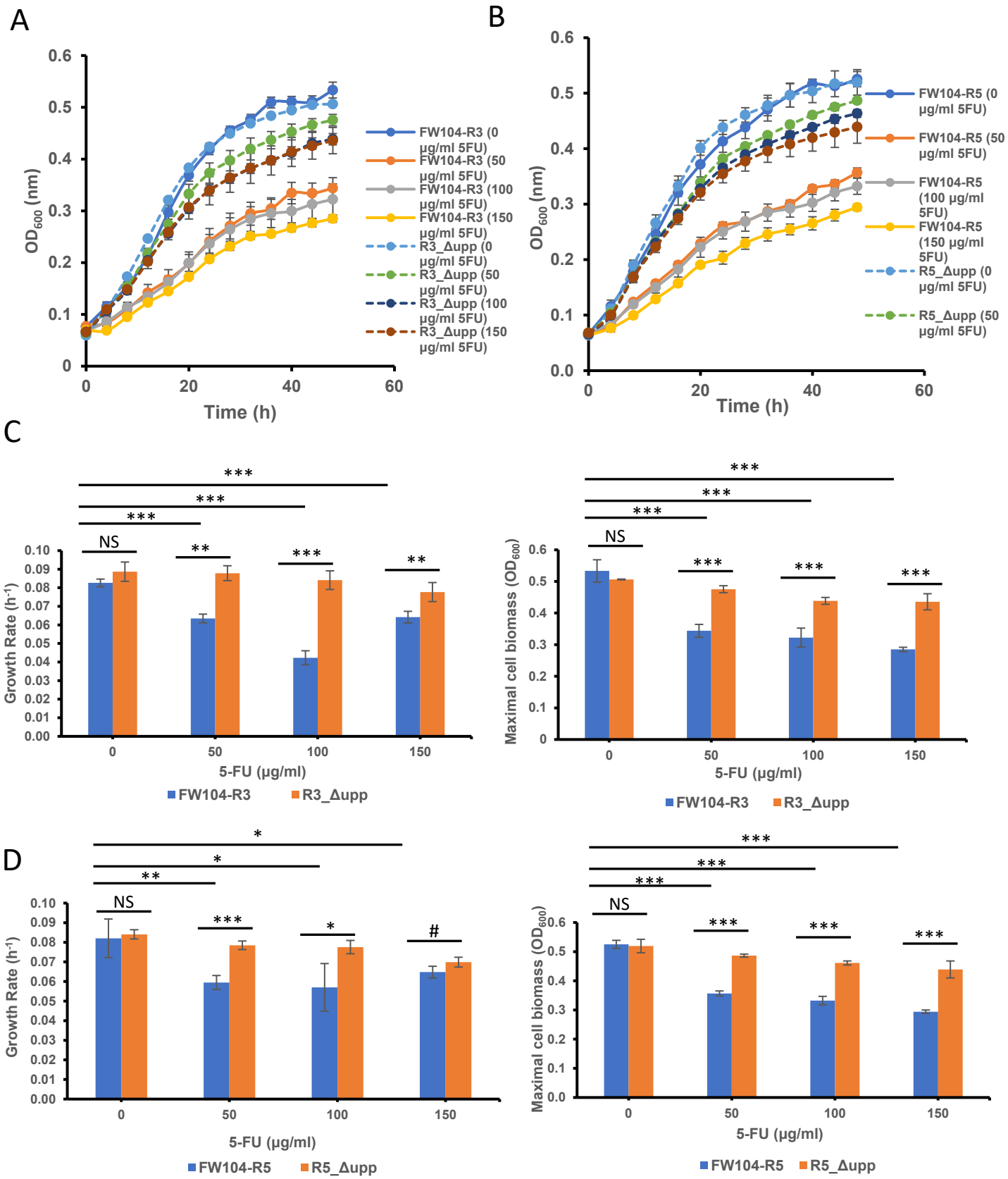


Figure S7. Deletion of *upp* increased resistance to 5-FU in the FW104-R3 and FW104-R5 strains. A and B, growth profiles of bacterial strains in SGW medium with different concentration of 5-FU. C and D, growth rate and maximal cell biomass of bacterial strains in SGW medium with different concentration of 5-FU. Significance is indicated by #, $0.05 < p \leq 0.010$, *, $0.010 < p \leq 0.050$, **, $0.001 < p \leq 0.010$ and ***, $p < 0.001$ determined by unpaired two-tailed t test. The bars represent the average ± SD (n=4).

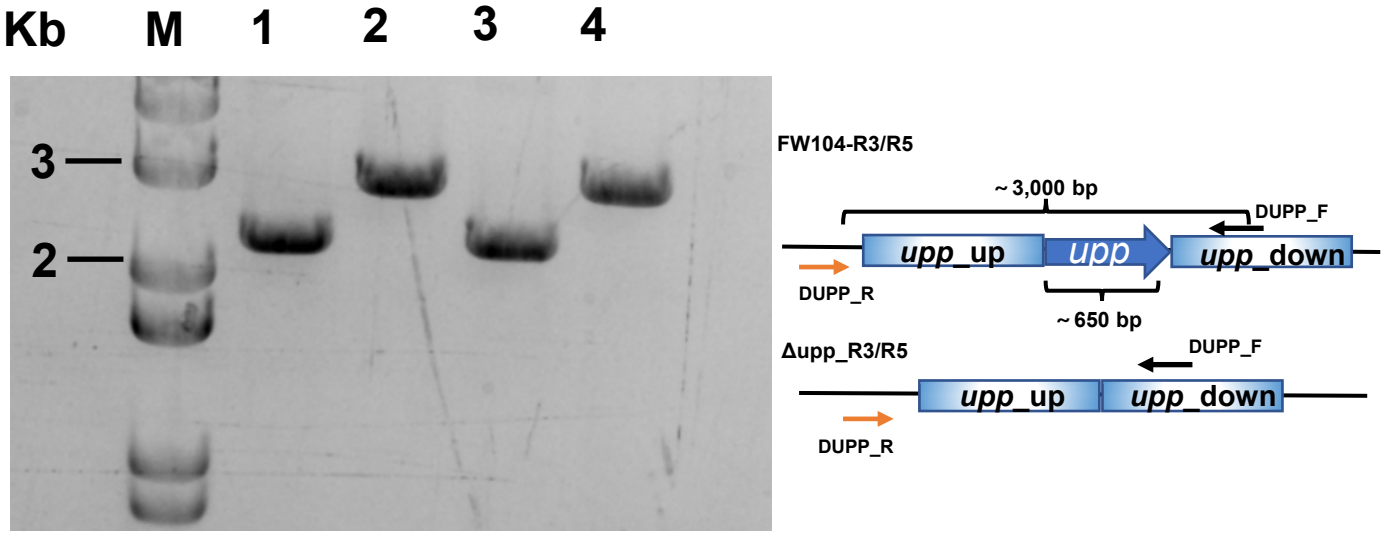


Figure S8. PCR verification of R3/R5_Δ*upp* mutants. Primers (DUPP_F & DUPP_R) are used for verification, in which the reverse primer (DUPP_R) is outside of the left homology region. Lane 1, R3_Δ*upp*; Lane 2 FW104-R3; Lane 3, R5_Δ*upp*; Lane 4, FW104-R5.

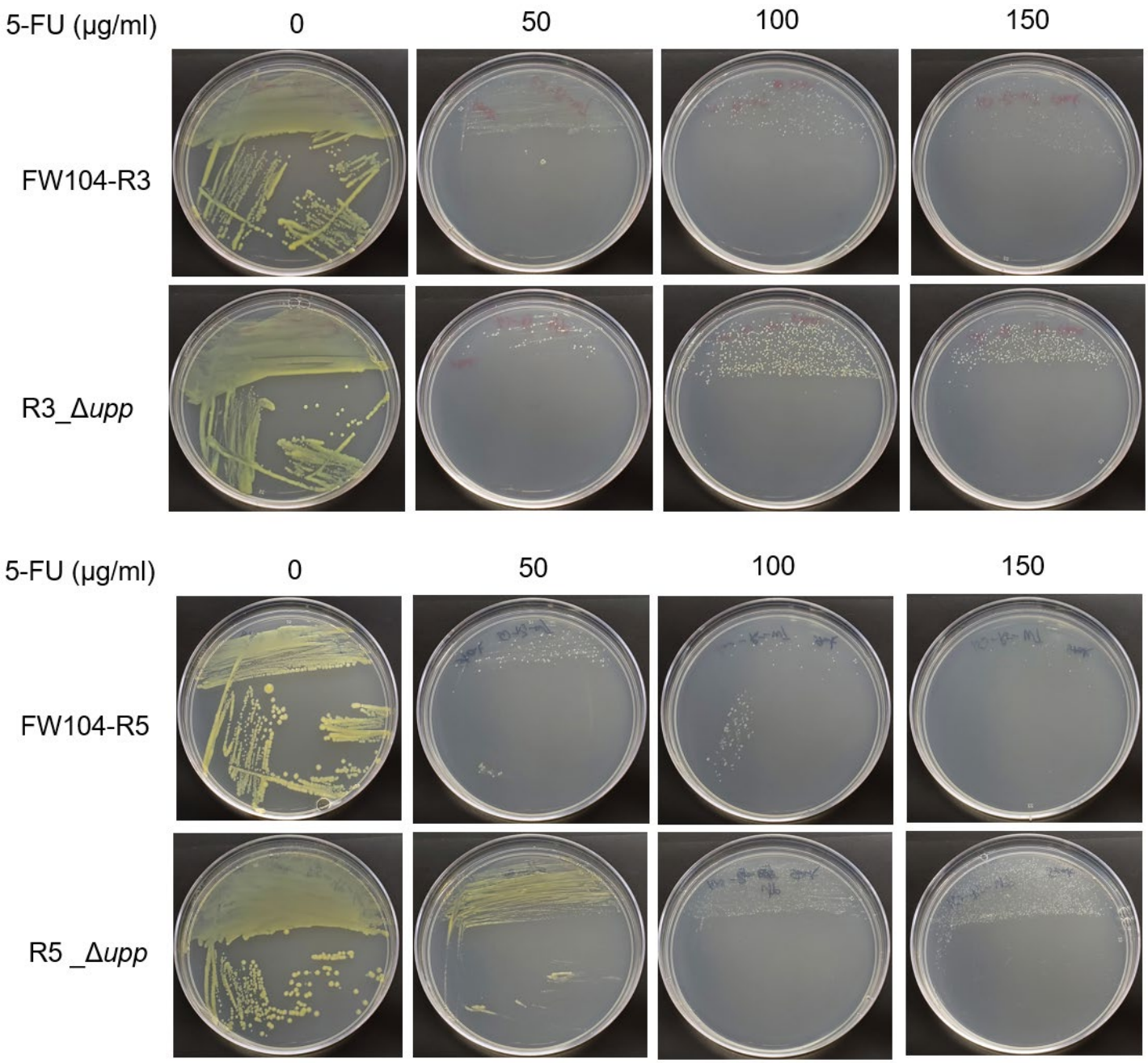


Figure S9. Deletion of *upp* can increase the 5-FU resistance in in FW104-R3 or FW104-R5 strain.

Table S1. Summary of growth test of ten *Rhodanobacter* strains in different media with different pH.

Strain name	R2A				TSA				SGW							
	7.2	7	6.8	6.5	7.2	7	6.8	6.5	7.2	7	6.8	6.5	6	5.5	5	4.5
DSM-23569	++++	+++	-	-	+	+	+	++++	+	+	+	++++	++++	++++	++++	++
DSM-24678	++++	++++	++++	++	+	+	+	++++	++	++	+++	++++	++++	+++	++	++
FW104-10F02	++++	++++	++	+++	++	++	+	++++	++	+++	++++	++++	++++	++++	++++	++
FW104-R3	++++	++++	+	++++	+	-	-	++++	+	++	++	++++	++++	++++	++++	++
FW104-R5	++++	++++	+++	++++	++++	++	+	++++	++	++	+++	++++	++++	+++	+++	++
FW104-T7	+++	++++	+	+	+	+	++	++++	+	+	++	+++	+++	++	++	-
FW104-R8	++++	++++	-	-	-	-	-	+	++	++++	++++	++++	++++	++++	++++	++
FW510-T8	++++	++++	+	-	+	+	+	+	+	++	+++	++++	++++	++++	++++	+
FW510-R10[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FW510-R12	++++	++++	++++	+	-	-	-	-	+	++	+++	++++	++++	++++	++++	+

The growth performance for each strain is based on the plate streaking result (Figure S1). -, no growth; +, few streaks grew; ++, half streaks grew; +++, almost all streaks grew; ++++, all streaks grew.

[a]The strain FW510-R10 was excluded in the following experiments as it did not grow in any type of tested solid plates.