Supplementary Materials

Table S1. Oligonucleotides used for RT-PCR experiments.

Name	Sequence (5′ → 3′)
16s_RT_for	TGTCGTCAGCTCGTGTCGTGA
16s_RT_rev	ATCCCCACCTTCCTCCGGT
csgB_RT_for	CATAATTGGTCAAGCTGGGACTAA
csgB_RT_rev	GCAACAACCGCCAAAAGTTT
csgD_RT_for	CCCGTACCGCGACATTG
csgD_RT_rev	CGTTCTTGATCCTCCATGG
bcsA_RT_for	TCGCGATTATCGTCGTCACG
bcsA_RT_rev	GGGTGCTCCAGCGGAATAAA
adrA_RT_for	GGCTGGGTCAGCTACCAG
adrA_RT_rev	CGTCGGTTATACACGCCCG
fimA_RT_for	CGCTTGCGCAGTTGATGCAG
fimA_RT_rev	CCGTCCCCAAGAAGGCAACA
fliC_RT_for	CAACTTACAGCGTATCCGTG
fliC_RT_rev	CGTTCACGCCGTTGAACTG
lptD_RT_for	GATGCGCTCGGTAATGTCC
lptD_RT_rev	CACCTTCCCAGACGTTGGT

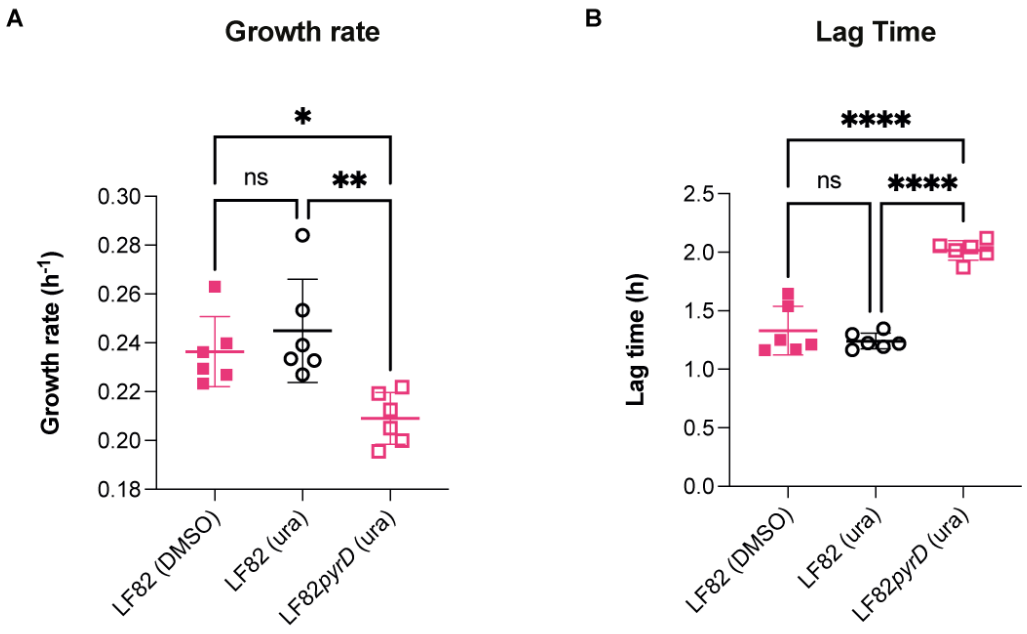

Figure S1. Analysis of growth rates (**A**) and lag time after inoculation (**B**) in Acid Medium for LF82 grown either with 0.25% DMSO, or 0.25mM uracil in DMSO, and for LF82*pyrD::*Tn5 in the presence of 0.25mM uracil. Results of six independent experiments are shown. *, *p*-value < 0.05; **, *p*-value < 0.01; ****, *p*-value < 0.0001, one-way ANOVA with Tukey's test for multiple comparisons.

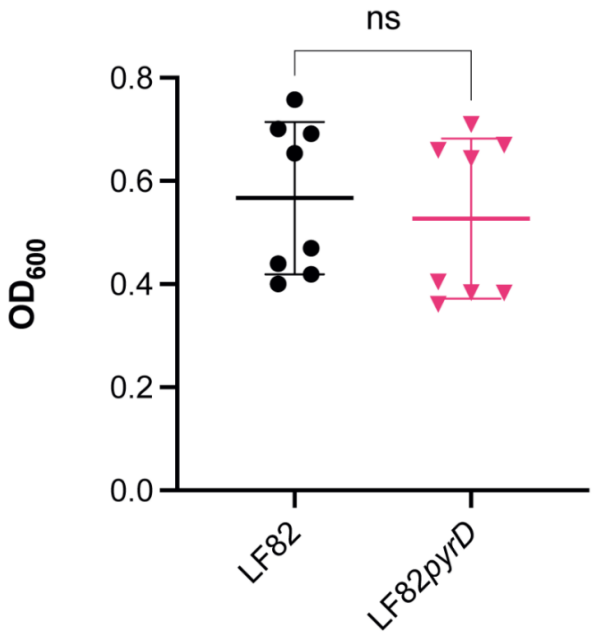

Figure S2. Optical density of overnight cultures of LF82 and LF82*pyrD::*Tn5 grown in YESCA medium in microtiter plates at 30 °C (conditions used for biofilm determination with crystal violet shown in Figure 2). Each point represents an independent biological replicate. ns, not significant (*p*-value = 0.89), Student t test.

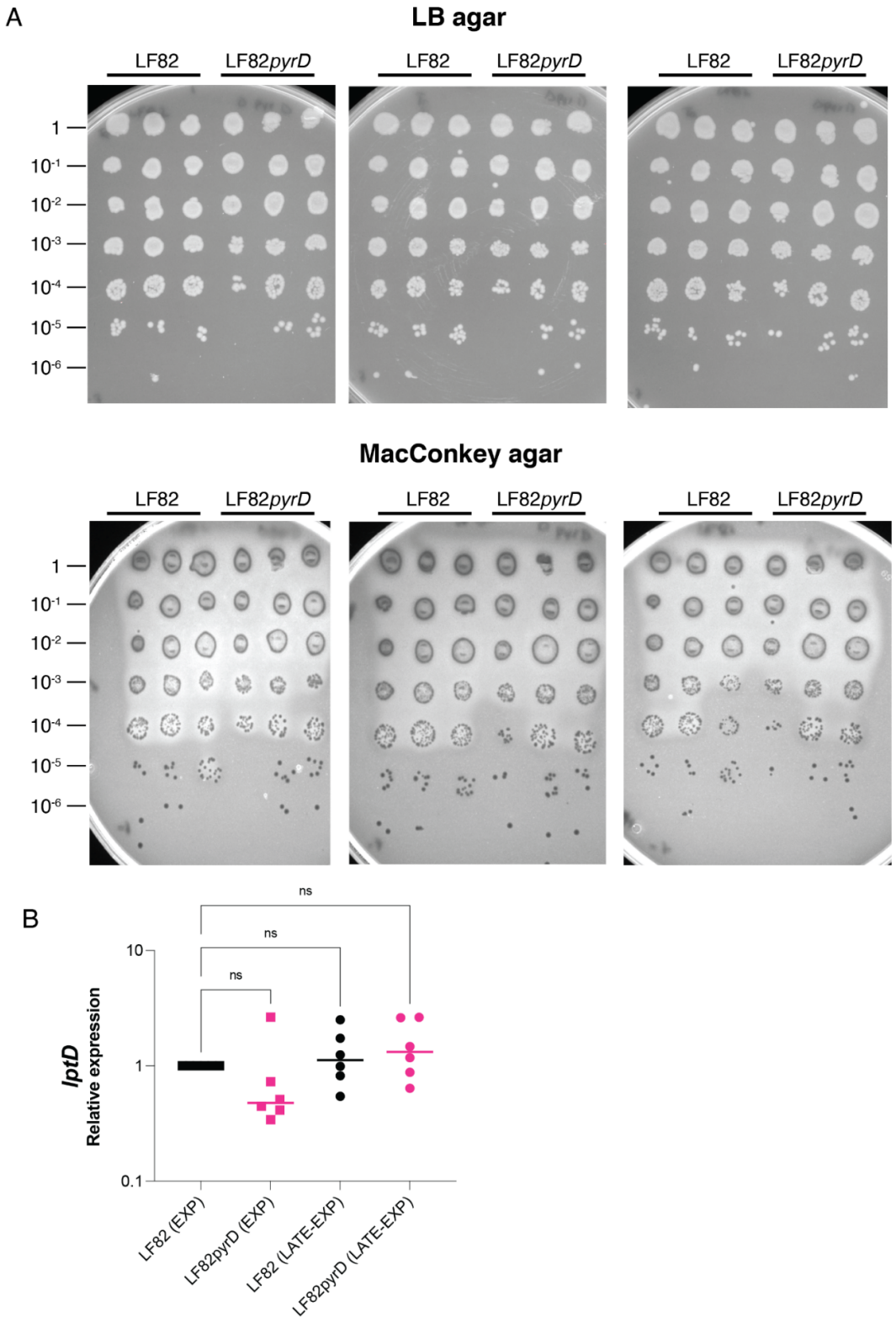

Figure S3. (**A**) Plating efficiency of LF82 and its LF82*pyrD*::Tn5 mutant derivative on Luria Agar (LA) and MacConkey media. Overnight cultures grown in LB medium were adjusted to OD600nm=1.0 and serially diluted 1:10 in microtiter plates to a final dilution of 10^{-6}. Dilutions were replicated on both LA and MacConkey. (**B**) Determination of gene expression levels in the LF82 vs the LF82*pyrD*::Tn5 (LF82*pyrD*) strain by qRT-PCR. Results of six independent experiments are shown. ns, not significant, one-way ANOVA with Tukey's test for multiple comparisons.

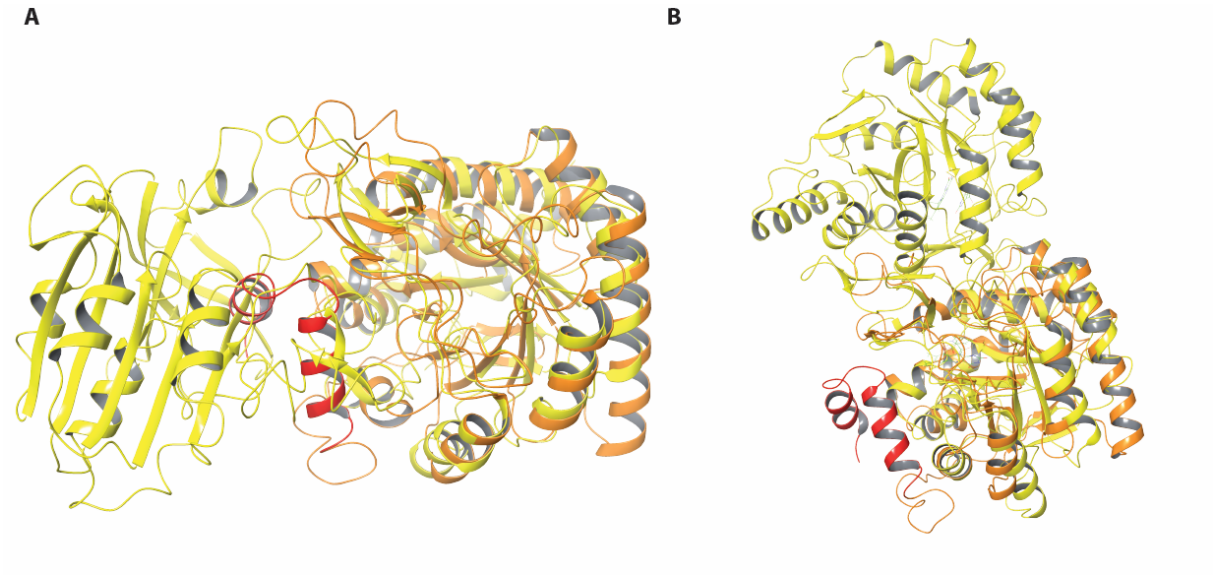

Figure S4. (A) Crystal structure of *Lactococcus lactis* DHOD type B (pdb 1EP2) where PyrD domain is superimposed to the crystal structure of EcDHOD (pdb 1F76). (**B**) Crystal structure of *Lactococcus lactis* DHOD type A (pdb 2DOR) where one of the PyrD domains is superimposed to with the crystal structure of EcDHOD (pdb 1F76).