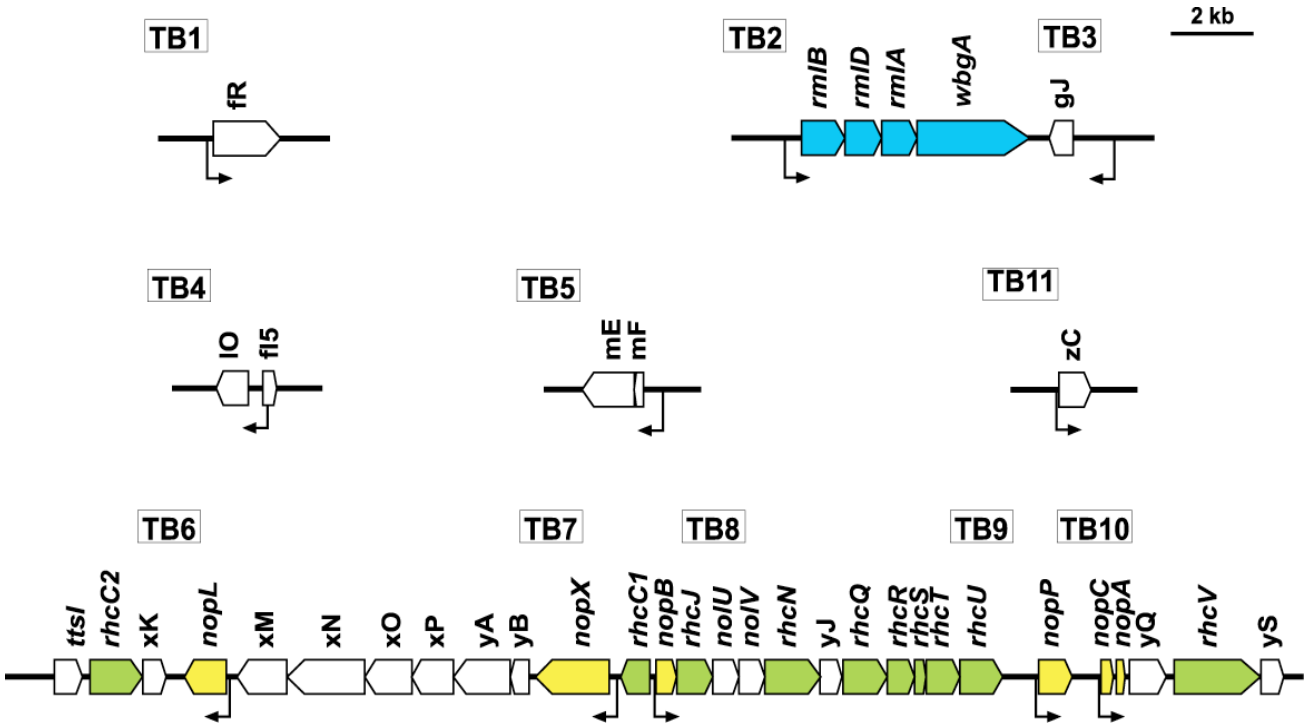
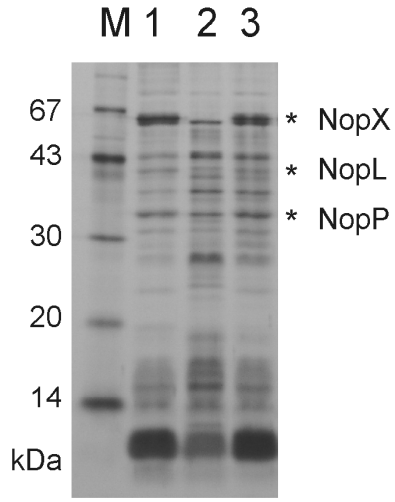
SUPP. FIG. 1. Genetic organisation of loci controlled by *tts*-boxes in pNGR234*a*



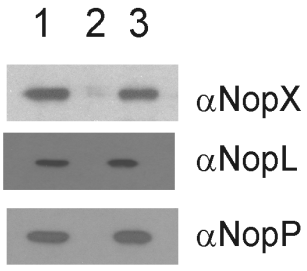
Supp. Fig. 2. Verification of NGRΔ*ttsI* as a non-polar mutant

(S2A) Nop secretion by NGR234 strains in the presence of apigenin



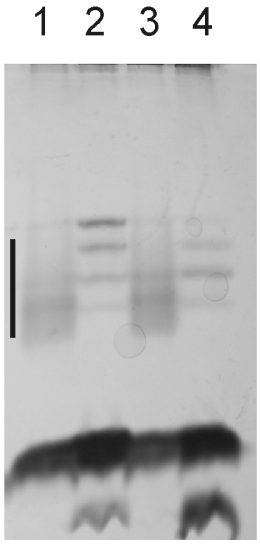
M = markers
1 = NGR234
2 = NGRΔ*ttsI*
3 = NGRΔ*ttsI* p*ttsI-2*

(S2B) Verification of Nop secretion

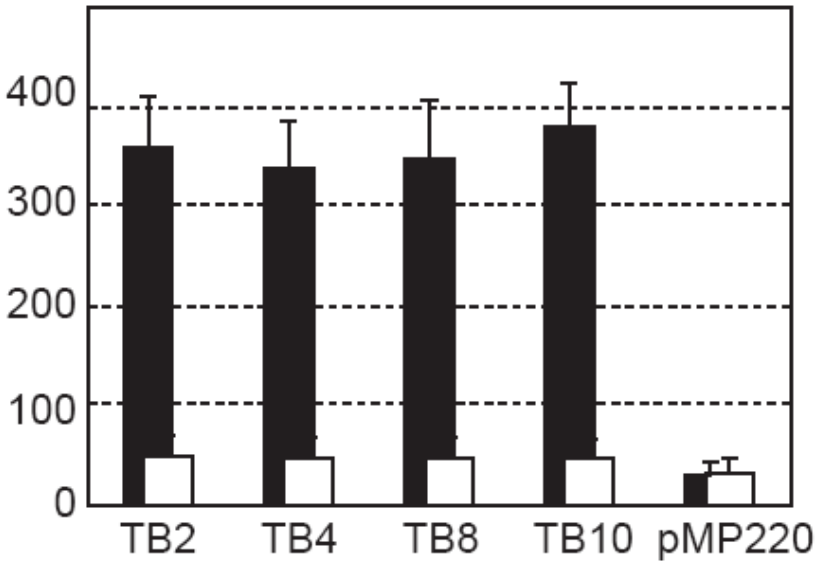


(S2C) LPS profiles of NGR234 strains

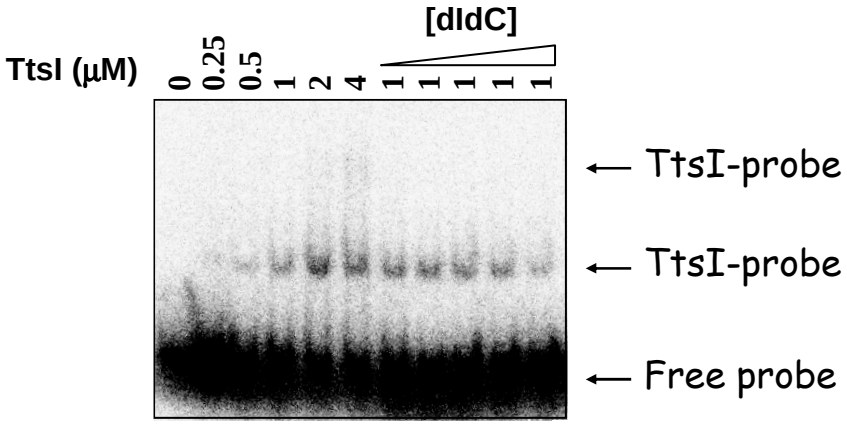
1 = NGR234
2 = NGRΔ*ttsI*
3 = NGRΔ*ttsI* p*ttsI-2*
4 = NGRΔ*rmlB-wbgA*



SUPP. FIG. 3. Transcriptional analysis of complemented NGRΔ*ttsI*.



SUPP. FIG. 4. The effect of poly(dI-dC) on TtsI binding to the *nopB* (TB8) promoter.



SUPP. FIG. 1. Genetic organisation of loci controlled by *tts*-boxes in pNGR234*a*. Genes and gene-fragments are represented by box arrows showing the direction of transcription and marked according to their predicted function: green, the secretion machinery of the type III secretion system (*rhc*); yellow, *nop* genes; blue, synthesis of rhamnose-rich LPS; and open, other or unknown function. The positions and orientations of the *tts*-boxes (TB1 to TB11) are marked with black arrows.

SUPP. FIG. 2. Confirmation of NGRΔ*ttsI* as a non-polar mutant. **(A)** Nop secretion by NGR34 strains in the presence of apigenin. Proteins secreted into the supernatant of NGR234 strains were isolated and separated by 12% SDS-PAGE. The positions of the Nops detected by the specific antibodies in (B) are labelled with asterisks. **(B)** Verification of Nop secretion. Specific antibodies were used against NopL, NopP & NopX, and protein-primary antibody complexes were revealed using anti-rabbit antibodies labelled with horseradish peroxidase and ECL detection reagents (GE Healthcare, Fairfield, Connecticut). Nops are visible in the supernatants of NGR234, the complemented *ttsI* mutant, but not in NGRΔ*ttsI*. Loading order for (A) and (B): M = markers with molecular masses in kDa; 1 = NGR234; 2 = NGRΔ*ttsI*; 3 = NGRΔ*ttsI* p*ttsI*. **(C)** LPS profiles of NGR234 strains. LPS were extracted from apigenin-induced cultures of various NGR234 strains, migrated on 16% DOC-PAGE and stained for LPS. As discussed in the text, NGR234 has been shown to synthesise a flavonoid inducible rhamnose-rich LPS (termed rhamnan) in a TtsI/TB2 dependent manner. This rhamnan is visible in the NGR234 and NGRΔ*ttsI* p*ttsI*-2 lanes as a black smear (labelled with black vertical lines). For comparison, LPS extracted from NGRΔ*rmlB-wbgA* was also migrated, this mutant has been shown to be incapable of synthesising rhamnan (Marie, 2004) and no smear is evident – as is the case with NGRΔ*ttsI*. Loading order for (C): 1 = NGR234; 2 = NGRΔ*ttsI*; 3 = NGRΔ*ttsI* p*ttsI*; 4 = NGRΔ*rmlB-wbgA*.

SUPP. FIG. 3. Transcriptional analysis of complemented NGRΔ*ttsI*. *ttsI* on a transmissible plasmid restored flavonoid-inducibility of *tts*-boxes in NGRΔ*ttsI.* p*ttsI*-2 was introduced into NGRΔ*ttsI* harbouring TB-*lacZ* fusions (pMP-TB2, TB4, TB8 and TB10), and β-galactosidase activities were monitored 24 hpi in the presence (closed bars) or in the absence (open bars) of 2×10-7 M daidzein. The Y-axis represents the levels of β-galactosidase activity (Miller's units). The values reported represent the means of three independent experiments and error bars are shown on the top of each column. Note that we did not use p*ttsI* (Marie 2004) as it carries tetracycline resistance and thus is not compatible with the pMP220 derivatives used to verify TB transcription.

SUPP. FIG. 4. The effect of poly(dI-dC) on TtsI binding to the *nopB* (TB8) promoter. The mobility shift assay using a 32P-labelled 150bp PCR fragment containing the *nopB* promoter region was performed as in Fig. 3, except that purified TtsI-His was used at a fixed concentration of 1mM and increasing amounts (0-50 mg.ml-1) of poly(dI-dC) were added to the reactions, which were incubated for 20 min and then subject to electrophoresis under native conditions. The presence of poly(dI-dC) did not significantly disrupt the appearance of slower migrating bands, as described in text.