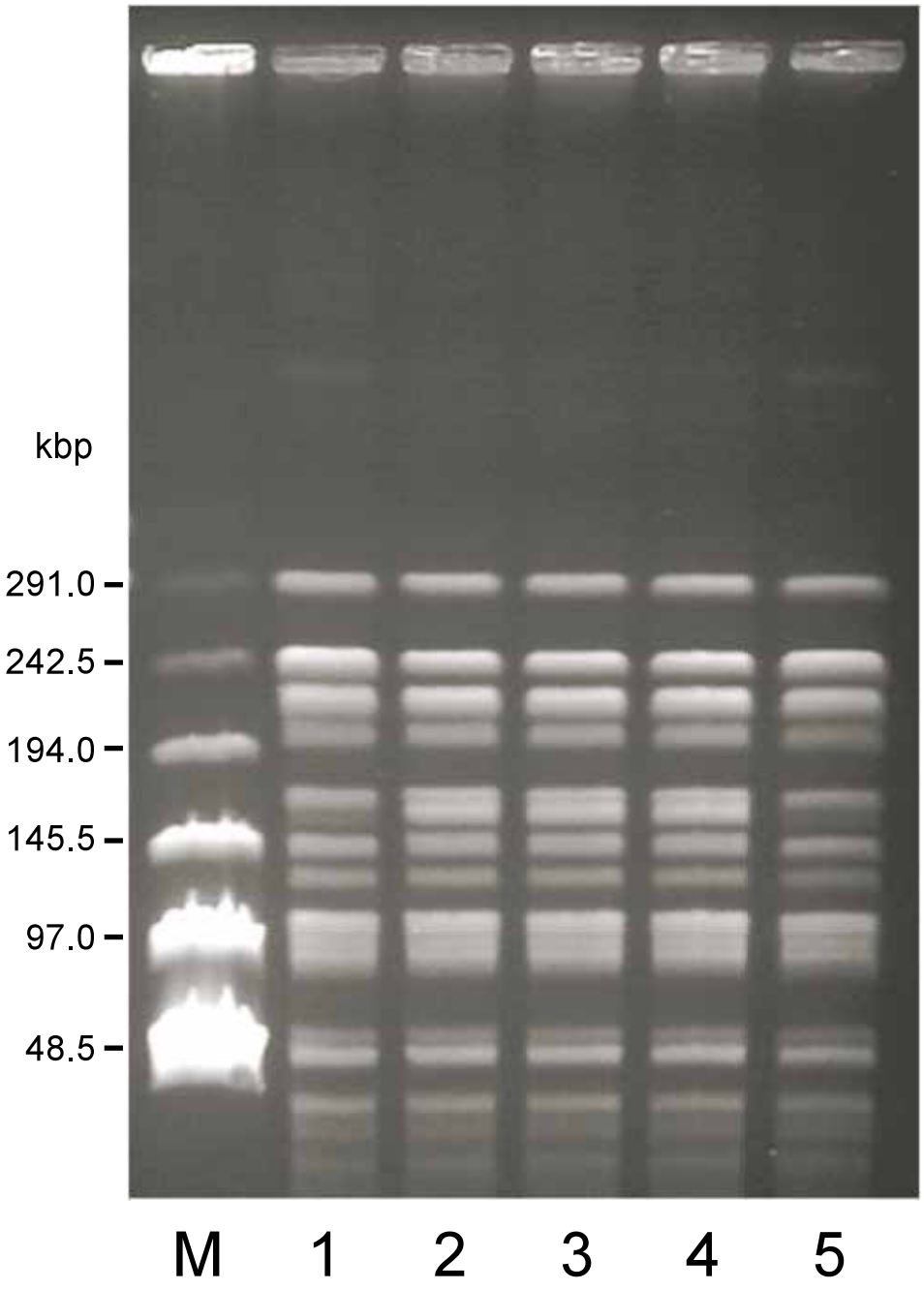
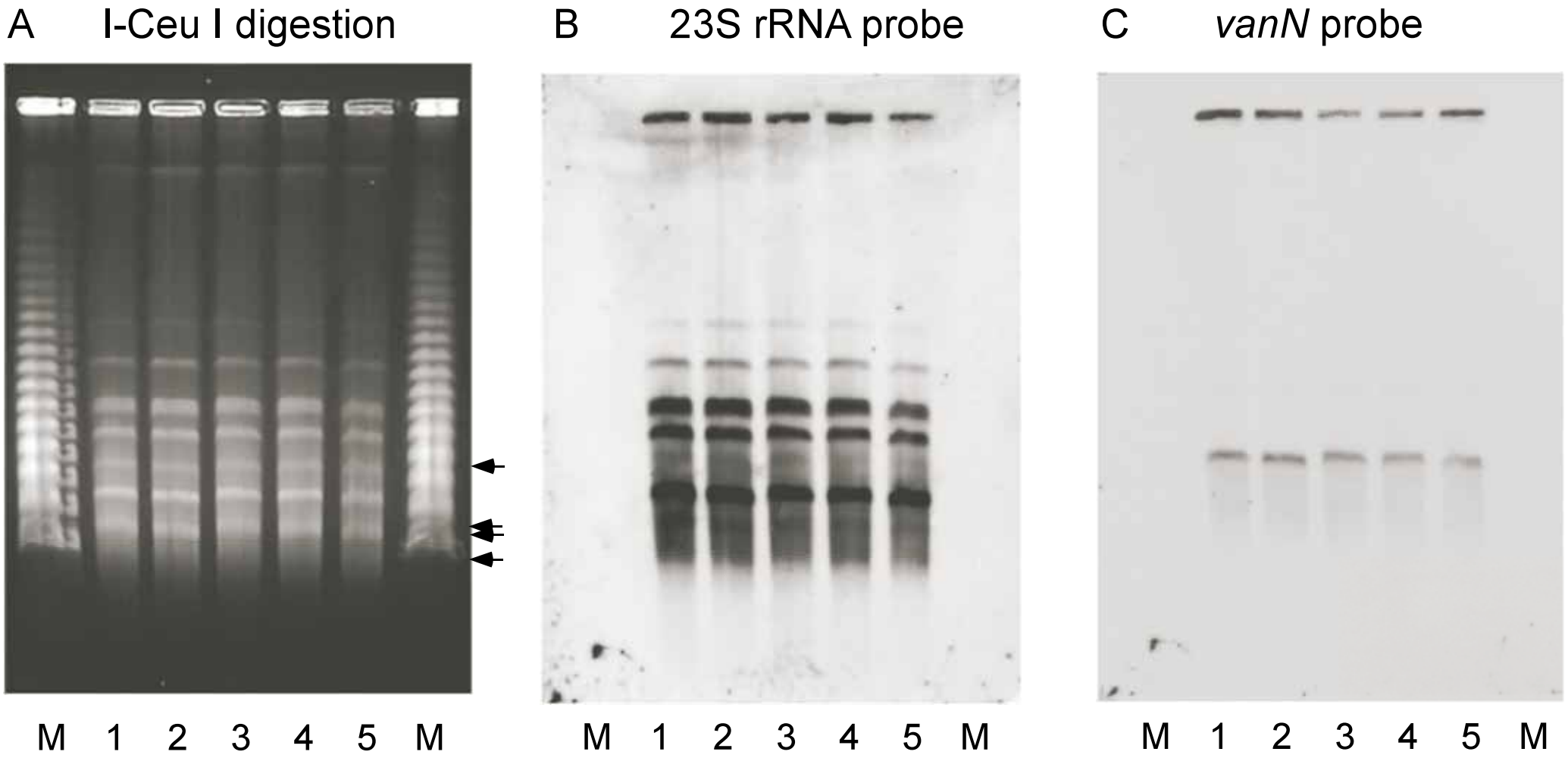
S1. PFGE analysis of the five VanN-type isolates



Pulsed Field Gel Electrophoresis (PFGE) analysis using *Sma*I restriction enzyme digestions of the five VanN-type *E. faecium* isolates. The PFGE method is described in reference 14.
Lane M; molecular weight marker, lane 1 to 5; *E. faecium* GU121-1, GU121-2, GU121-3, GU121-4, GU121-5, respectively.

S2 PFGE analysis using I-Ceu I enzyme and Southern hybridization

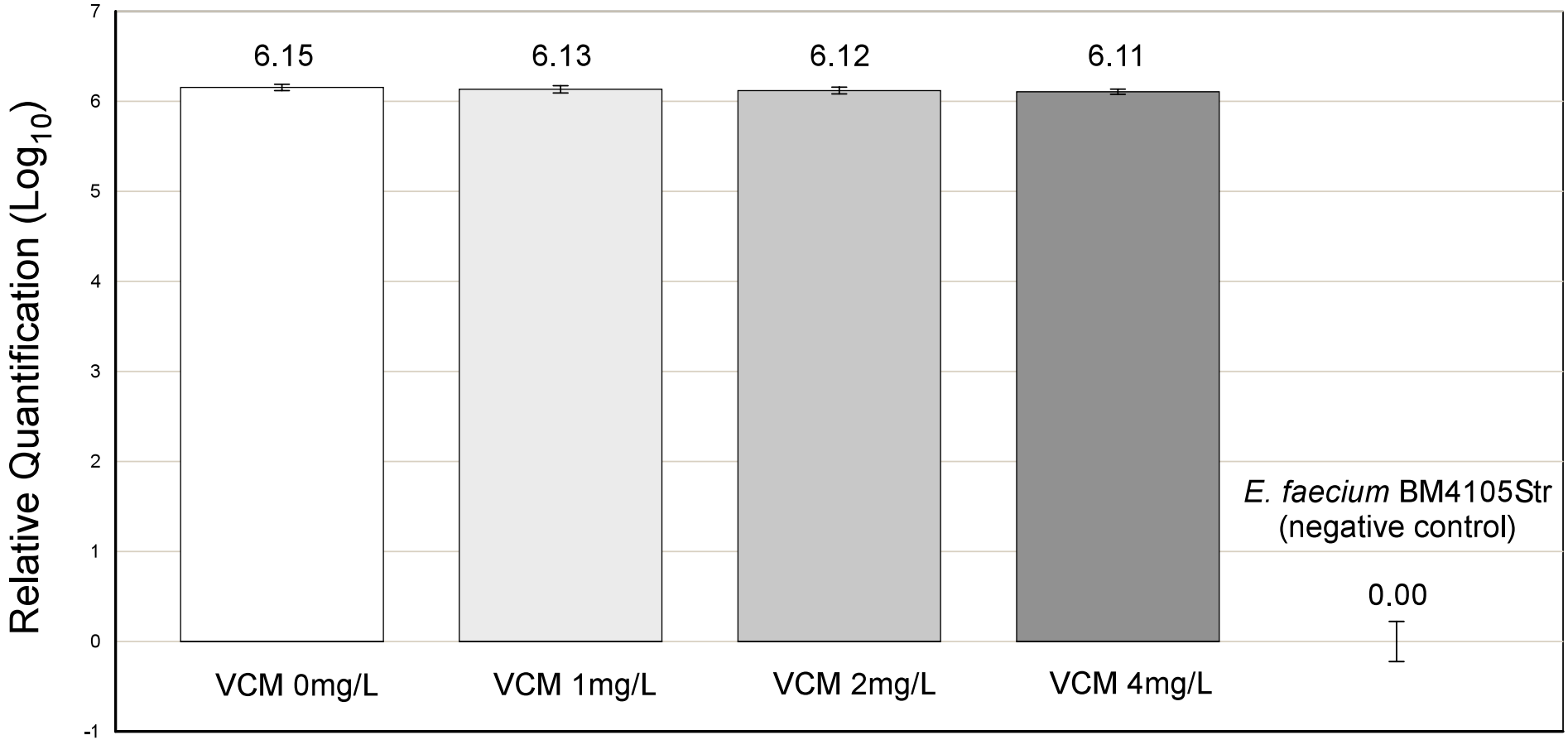


PFGE analysis of VanN-type GRE isolates using I-Ceu I enzyme and Southern hybridizations with the specific probes for 23S rRNA and the *vanN* gene. A; PFGE profiles of I-Ceu I-digested DNAs, B; Southern hybridization with rRNA probe, C; Southern hybridization with *vanN* probe. Lane M; lambda ladder PFG marker (NEB), lane 1; VanN-type isolate GU121-1, lane 2; GU121-2, lane 3; GU121-3, lane 4; GU121-4, lane 5; GU121-5.

The 2,890 bp length specific probe for 23S ribosomal RNA region was constructed by PCR amplification using the primers forward; GGTTAAGTGAATAAGGGCGC, reverse; GTATCAGTCCGCTCCATACA. The specific *vanN* probe was also constructed by PCR using the primers described in the text. The four arrows on panel A indicated the non-specific-broken linear plasmid DNA bands which were relatively-obscure comparing to other bands. The four bands were not detected by the chromosomal 23S rRNA probe (panel B). The bands were equivalent to those shown in Fig. 2, panel A. The *vanN* probe hybridized to the largest 160 kbp plasmid DNA band.

S3

Real-time PCR analysis of *vanN* gene of VanN-type *E. faecium* GU121-1



vanN gene expression levels of *E. faecium* GU121-1 with or without vancomycin

RT-PCR was performed using the 7500 Fast Real-Time PCR system (ABI). The endogenous controls were the rrsA(SYBR) and *ddl* genes. *E. faecium* BM4105Str was used as calibrator sample (negative control). The total RNAs were prepared from the culture samples after 4 hours incubation at each concentration of vancomycin in the medium. One nanogram of total RNA was used and divided into three sample wells for each RT-PCR amplification. The thermal cycle was 40 repeats of two steps of 95 degree/3 sec and 60 degree/30 sec. The primer sequences for *vanN* gene were as follows, forward; TTGCAAAAAGTGTCGGAGTGA, reverse; TGTTGTTCGTCCGTCGATGA. The result showed that *vanN* gene expression was vancomycin-independent and constitutive.