

Fig. S1 Phylogenetic neighbor-joining (NJ) tree based on cgMLST profiles using Ridom SeqSphere+ 3.5.0 software.

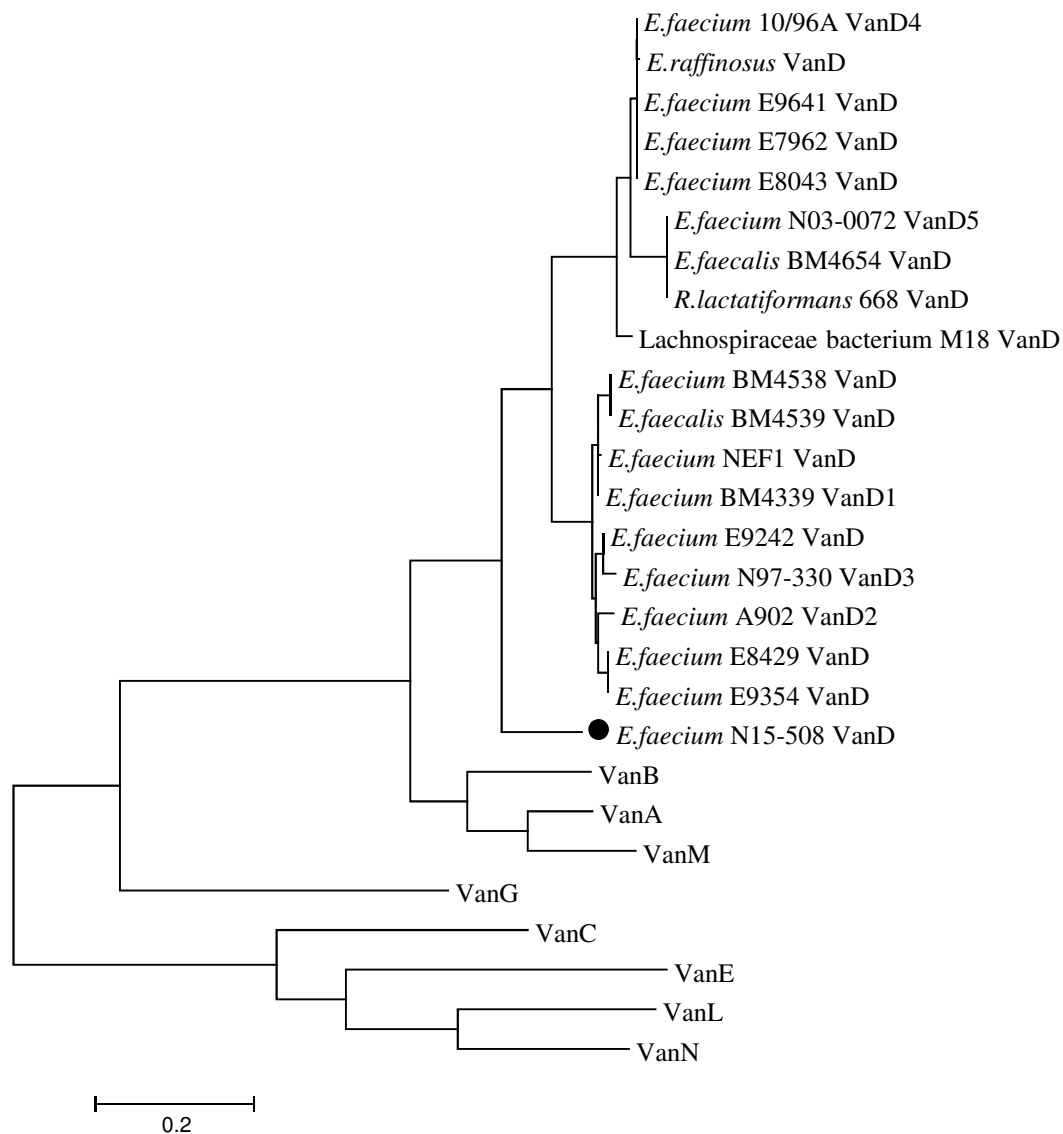
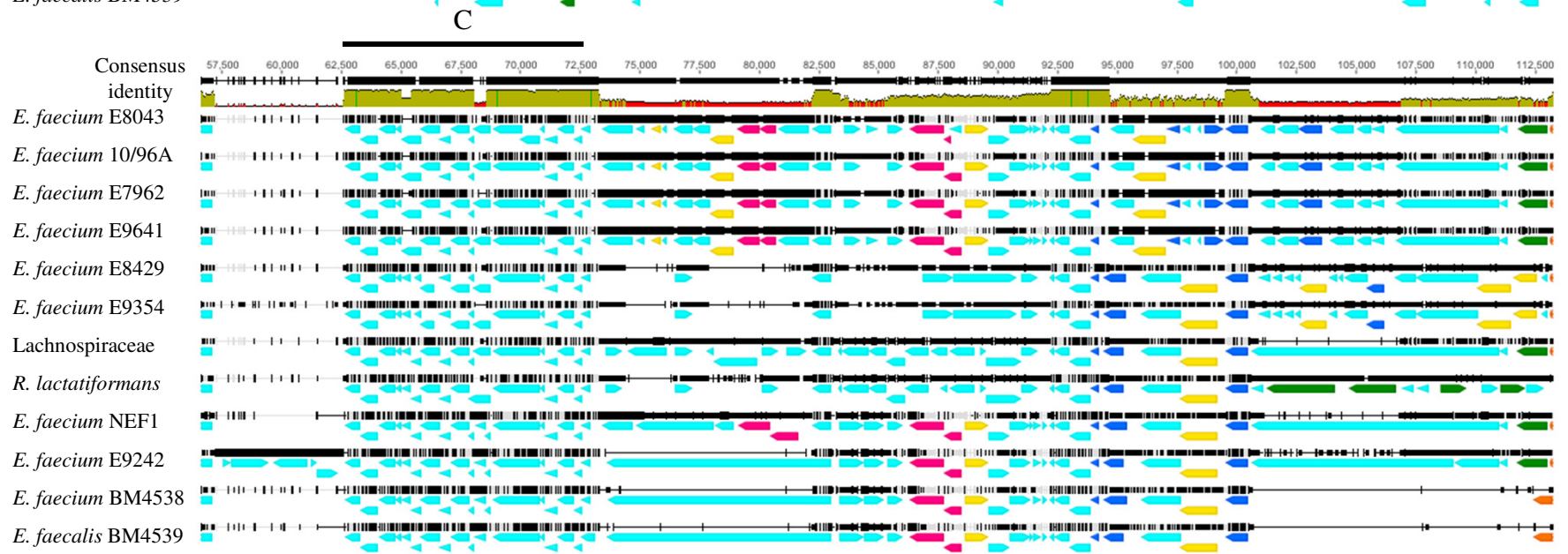
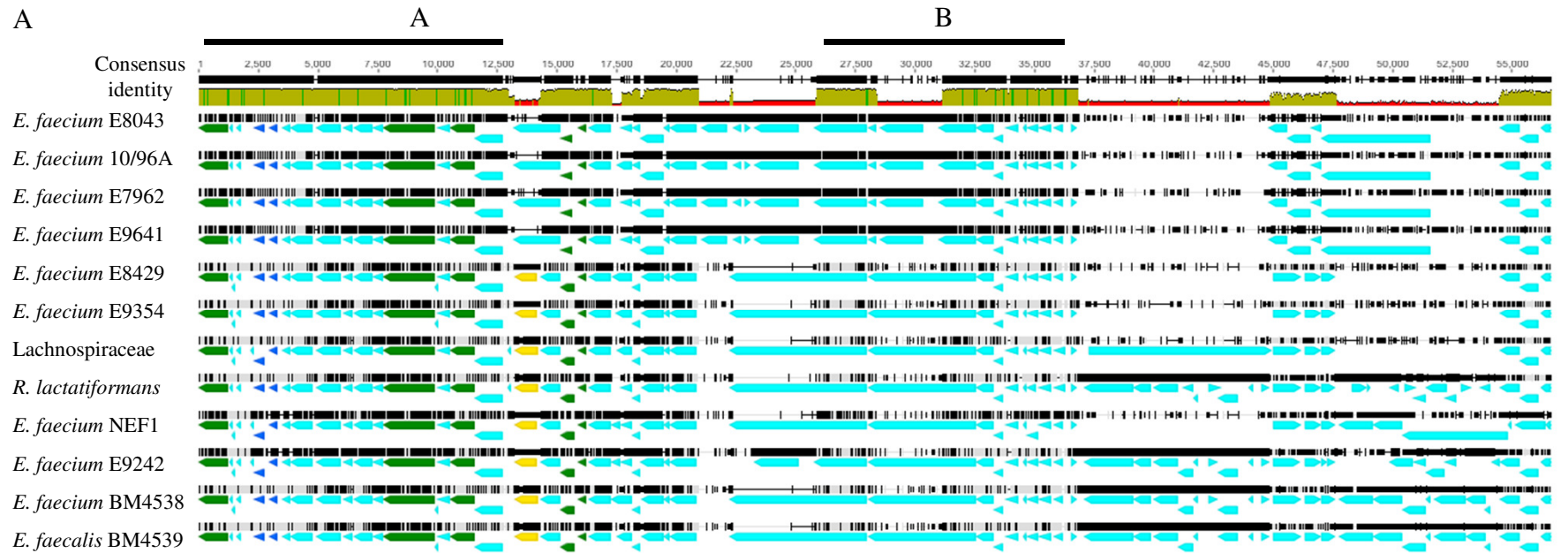
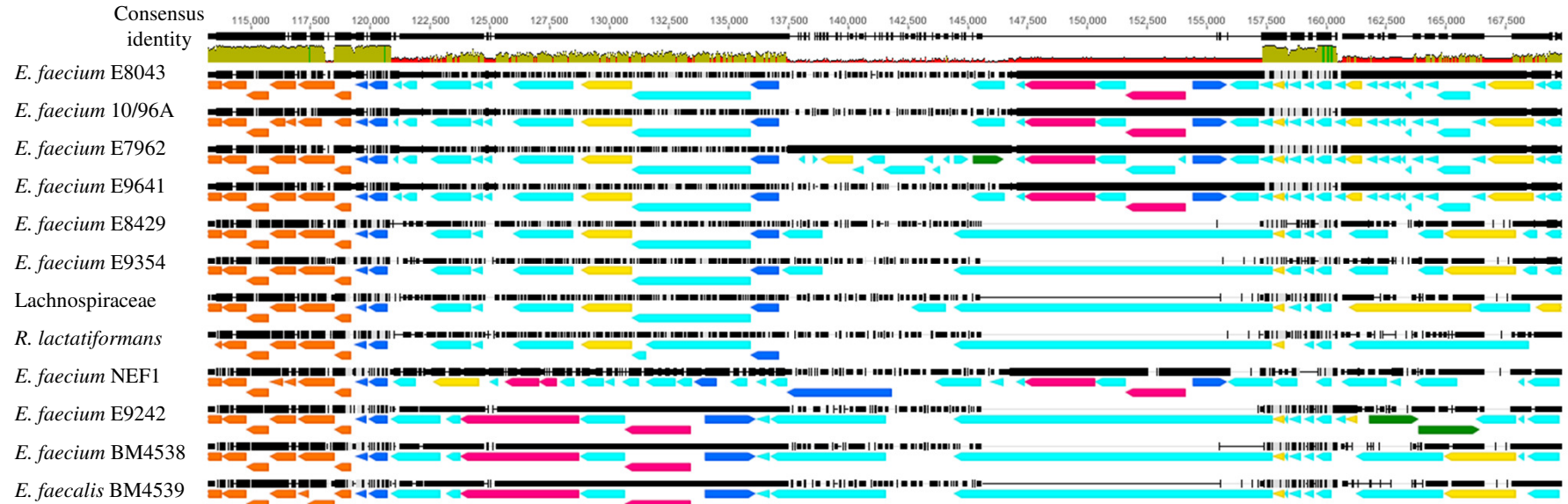


Fig. S2 Phylogenetic neighbor-joining (NJ) tree of d-Ala-D-XXX ligase proteins representing the currently in enterococci identified Van proteins. Corresponding reference sequences obtained in this study (all VanD) and sequences retrieved from GenBank, including VanA (accession no. M97297), VanB (accession no. NC_004668), VanC (accession no. AF162694), VanE (accession no. FJ872411), VanG (accession no. DQ212986), VanL (accession no. EU250284), VanM (accession no. FJ349556) and VanN (accession no. AB701345) were aligned using ClustalW and a neighbour-joining tree was constructed and validated with 1000 bootstrap analysis using MEGA 6.05 software. The *E. faecium* N15-508 with divergent VanD ligase is indicated with black dot.

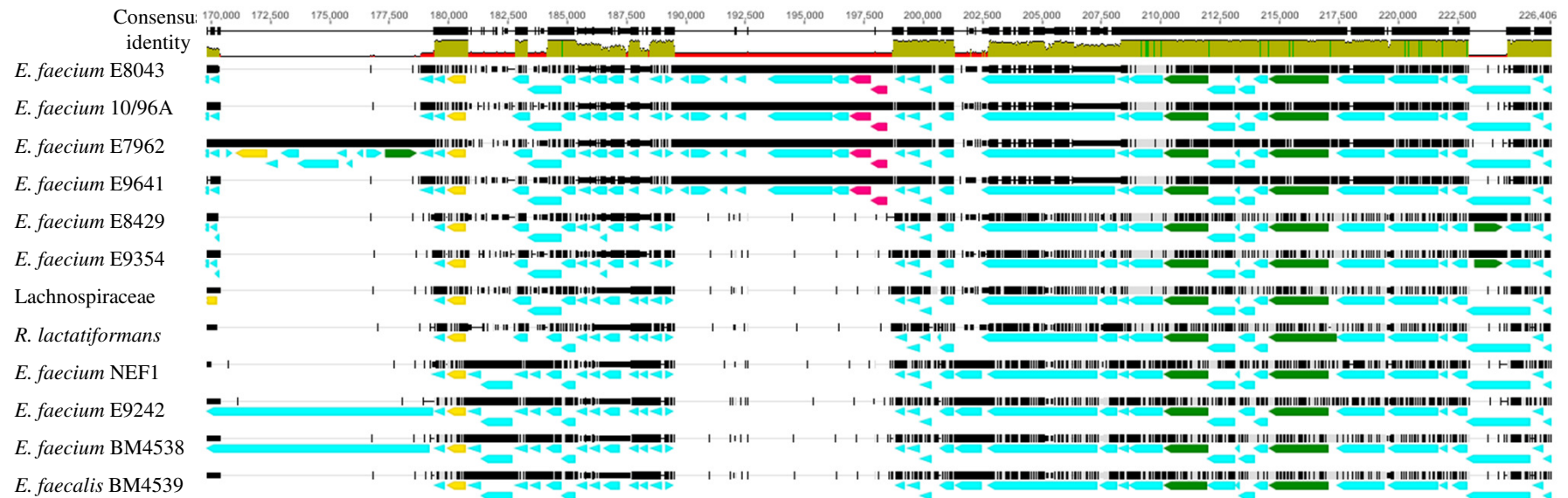


B

D



E



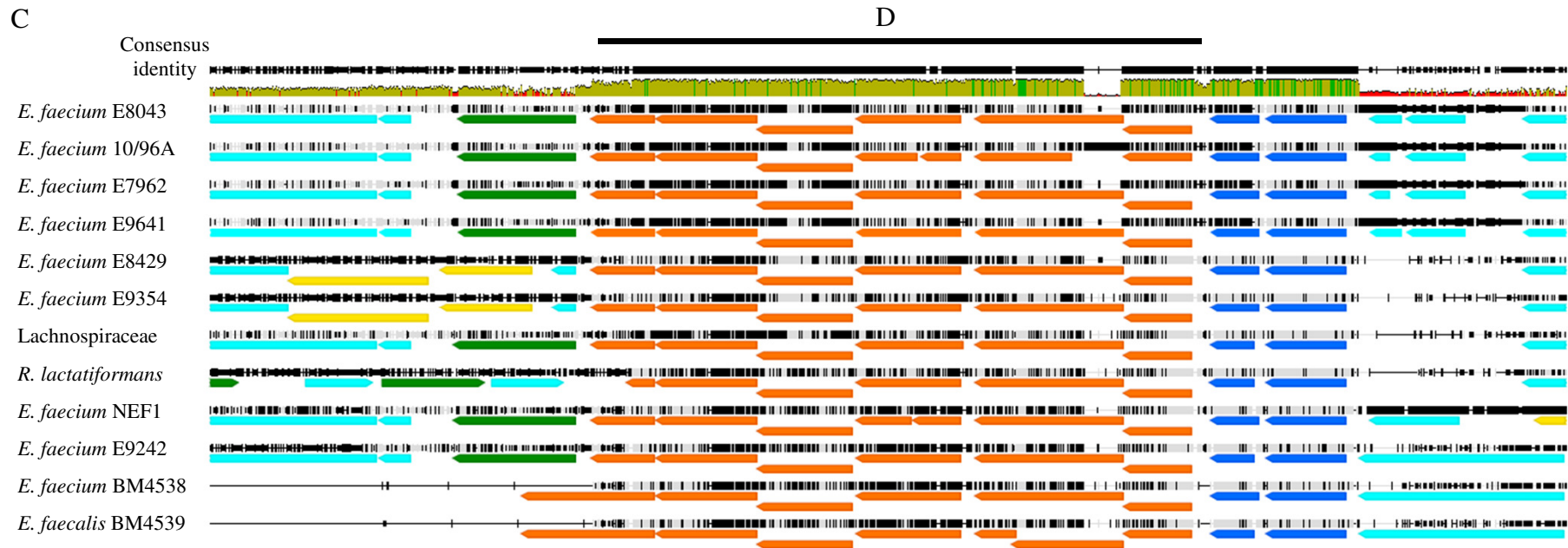


Fig. S3 Multiple alignment analysis using Geneious 8.1.2 software of the 120-190 kb genomic island is depicted in Panel A and B. The genetic environment of the *vanD*-gene cluster is enlarged depicted in panel C. Indicated colored genes in multiple alignment; green arrows: putatively involved in recombination/mobilization; dark blue arrows: putatively involved transcription regulation; yellow arrows: putative antibiotic resistance genes; orange arrows: *vanD*-gene cluster; pink arrows: putative two-component systems and light blue: various or hypothetical genes. Indicated with capital letters groups of conserved genes. The consensus identity is indicated at the top of the alignment, the height of the olive colored bars is representative of the % identity.

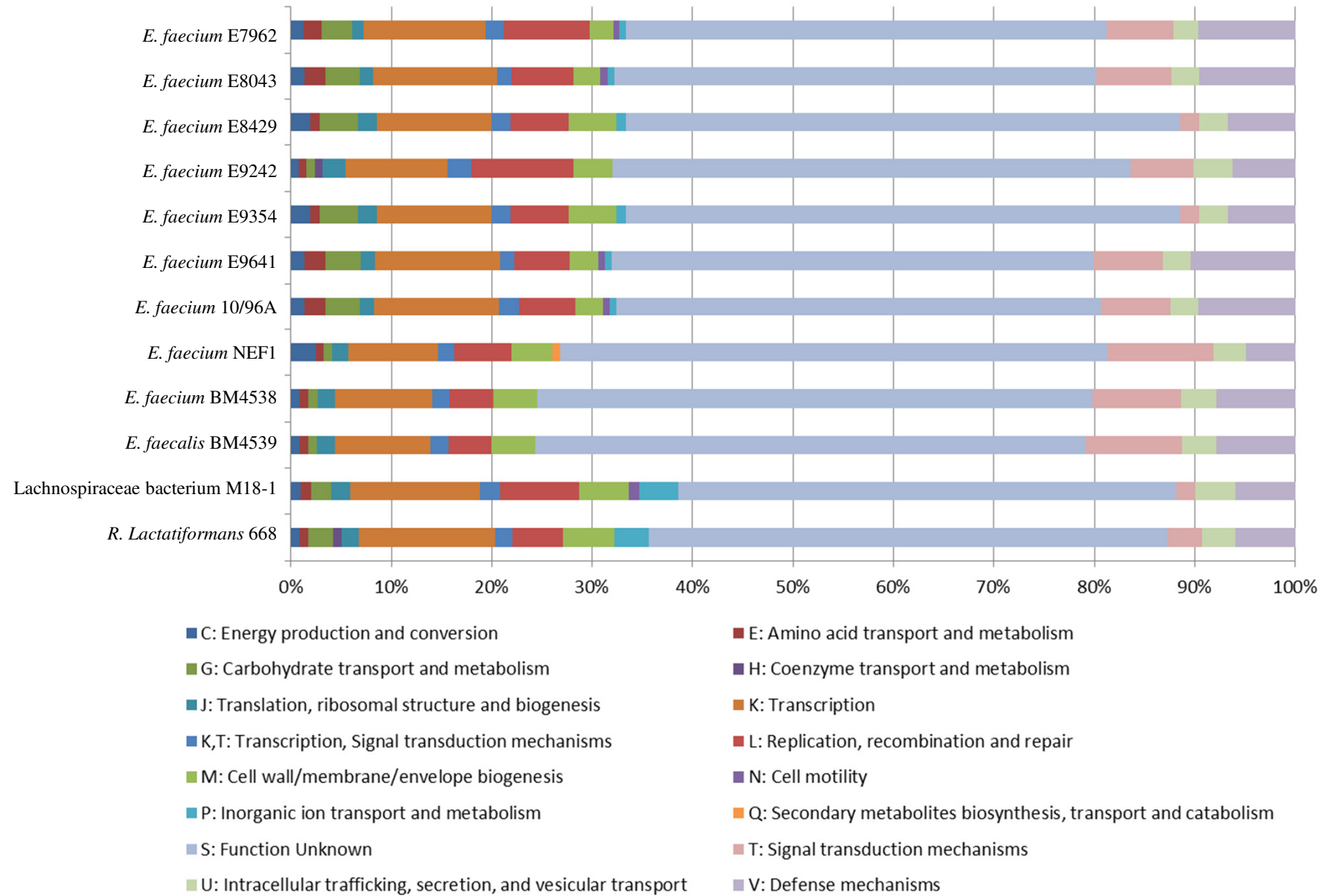


Fig. S4 Non-supervised Orthologous Groups (eggNOG) analysis. Distribution of the clusters of Orthologous Groups for the proteins encoded on the genomic island.

- 1 Casadewall B, Courvalin P. 1999. Characterization of the *vanD* glycopeptide resistance gene cluster from *Enterococcus faecium* BM4339. *J Bacteriol* 181:3644–3648.
- 2 Ostrowsky BE, Clark NC, Thauvin-Eliopoulos C, Venkataraman L, Samore MH, Tenover FC, Eliopoulos GM, Moellering RC, Gold HS. 1999. A cluster of VanD vancomycin-resistant *Enterococcus faecium*: molecular characterization and clinical epidemiology. *J Infect Dis* 180:1177–1185.
- 3 Boyd DA, Conly J, Dedier H, Peters G, Robertson L, Slater E, Mulvey MR. 2000. Molecular characterization of the *vanD* gene cluster and a novel insertion element in a vancomycin-resistant enterococcus isolated in Canada. *J Clin Microbiol* 38:2392–2394.
- 4 Depardieu F, Reynolds PE, Courvalin P. 2003. VanD-type vancomycin-resistant *Enterococcus faecium* 10/96A. *Antimicrob Agents Chemother* 47:7–18.
- 5 Boyd DA, Kibsey P, Roscoe D, Mulvey MR. 2004. *Enterococcus faecium* N03-0072 carries a new VanD-type vancomycin resistance determinant: characterization of the VanD5 operon. *J Antimicrob Chemother* 54:680–683.
- 6 Depardieu F, Foucault M-L, Bell J, Dubouix A, Guibert M, Lavigne J-P, Levast M, Courvalin P. 2009. New combinations of mutations in VanD-Type vancomycin-resistant *Enterococcus faecium*, *Enterococcus faecalis*, and *Enterococcus avium* strains. *Antimicrob Agents Chemother* 53:1952–1963.
- 7 Boyd DA, Lalancette C, Lévesque S, Golding GR. 2016. Characterization of a genomic island harbouring a new *vanD* allele from *Enterococcus faecium* N15-508 isolated in Canada. *J Antimicrob Chemother* 71:2052–2054.
- 8 Depardieu F, Kolbert M, Pruul H, Bell J, Courvalin P. 2004. VanD-type vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. *Antimicrob Agents Chemother* 48:3892–3904.
- 9 Tanimoto K, Nomura T, Maruyama H, Tomita H, Shibata N, Arakawa Y, Ike Y. 2006. First VanD-Type vancomycin-resistant *Enterococcus raffinosus* isolate. *Antimicrob Agents Chemother* 50:3966–3967.
- 10 Domingo M-C, Huletsky A, Giroux R, Picard FJ, Bergeron MG. 2007. *vanD* and *vanG*-Like Gene Clusters in a Ruminococcus Species Isolated from Human Bowel Flora. *ResearchGate* 51:4111–7.
- 11 Shkoporov AN, Chaplin AV, Shcherbakova VA, Suzina NE, Kafarskaia LI, Bozhenko VK, Efimov BA. 2016. *Ruthenibacterium lactatiformans* gen. nov., sp. nov., an anaerobic, lactate-producing member of the family Ruminococcaceae isolated from human faeces. *Int J Syst Evol Microbiol* 66:3041–3049.