

SUPPLEMENTAL MATERIAL

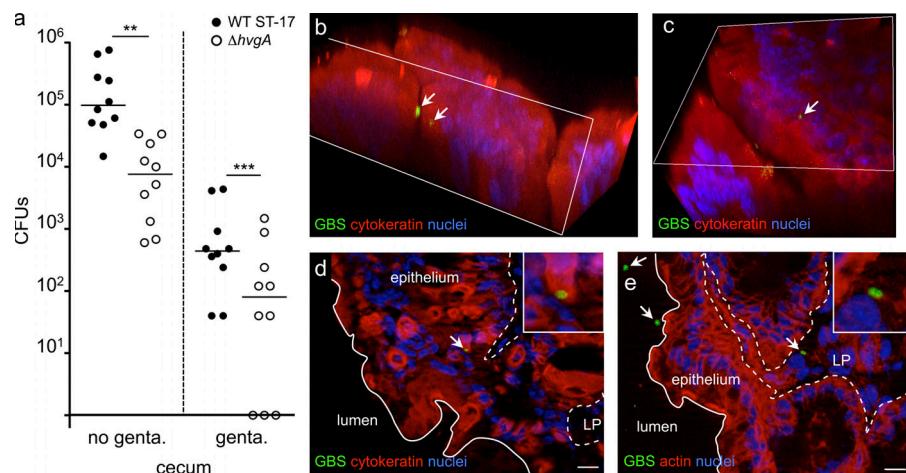
Tazi et al., <http://www.jem.org/cgi/content/full/jem.20092594/DC1>

Figure S1. HvgA promotes the crossing of the intestinal barrier. (a) Groups of 3-wk-old BALB/c female mice ($n = 10$) were inoculated orally with 5×10^8 CFUs WT ST-17 or $\Delta hvgA$ mutant strain. 8 h after infection, animals were sacrificed and bacteria were enumerated in the cecum. This experiment was repeated two times, and groups of mice contained at least five animals. Asterisks indicate significant differences as assessed by the Mann Whitney test (*, $P < 0.05$; **, $P < 0.01$). (b–e) 5-wk-old BALB/c germ-free mice were orally infected with 10^{10} CFUs WT ST-17. 24 h after infection, animals were sacrificed. (b and c) Whole tissue staining. Colons were stained with an anti-GBS antibody (green), and nuclei were labeled with DAPI (blue) and cytokeratin with a pan antibody (red). A three-dimensional reconstruction was realized from a stack of confocal images of an infected colon. The three-dimensional reconstruction was numerically cut (white square) to show an intraepithelial bacterium (arrow) in a transversal (b) or longitudinal (c) view. Arrowhead, luminal bacterium. (d and e) Colon sections were stained with an anti-GBS antibody (green), and nuclei were labeled with DAPI (blue) and cytokeratin with a pan antibody (d, red) or F-actin with phalloidin (e, red). LP, lamina propria. Arrowheads, luminal bacteria. Arrows, bacteria in the lamina propria. Bars, 10 μ m.

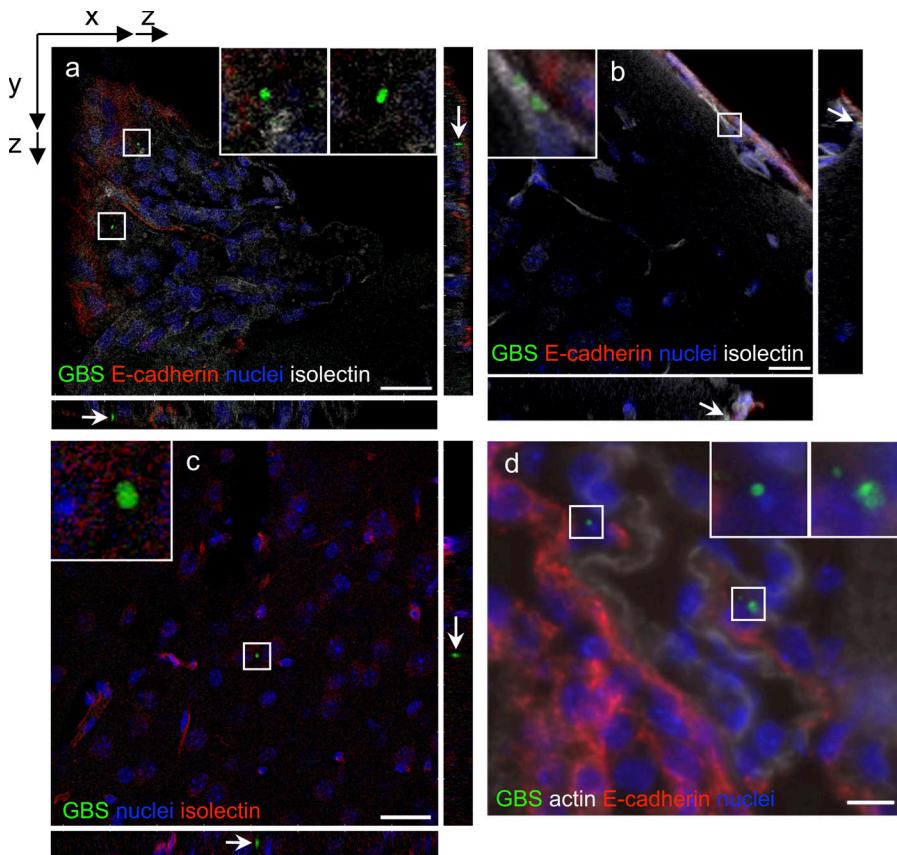


Figure S2. GBS ST-17 can cross the blood–brain barrier after an oral infection. 3-wk-old BALB/c mice (a–c) or 5-wk-old BALB/c germ-free (d) mice were infected orally with 10^9 or 10^{10} CFUs WT ST-17, respectively. Animals were sacrificed 12 (a–c) or 24 h (d) after infection. (a–c) 200- μ m brain sections were stained with an anti-GBS antibody (green), and nuclei were labeled with DAPI (blue), epithelial cells with an anti-E-cadherin antibody (red), and brain vessels with the isolectin (white, a and b; red, c). Confocal images were acquired as a z-stack, and an orthogonal view of each stack was constructed. Arrows indicate bacteria. (d) An 8- μ m brain section was stained with an anti-GBS antibody (green), and nuclei were labeled with DAPI (blue), epithelial cells with an anti-E-cadherin antibody (red), and F-actin with the phalloidin (white). Bars, 10 μ m.

Table S1. Bacterial strains and plasmids used in this study

| Strains or plasmid | Relevant properties | Source or reference |
|--|---|--|
| Strains | | |
| <i>Escherichia coli</i> | | |
| DH5α | recA1 gyrA (Nal), Δ(lacZYA-argF)[Φ80ΔlacΔ(lacZ)M15] | Invitrogen |
| BL21λDE3 | F- ompT gal (dcm) (lon) hsdSB(r _b - m _b -) endA1 hsdR17(r _K -m _K ') | (Studier and Moffatt, 1986) |
| <i>Streptococcus agalactiae</i> | | |
| NEM316 | Serotype III ST-23 isolated from neonate blood culture | (Glaser et al., 2002) |
| BM110 | Serotype III ST-17 isolated from neonate blood culture | (Musser et al., 1989; Stalhammar-Carlemalm et al., 1993) |
| CCH375 | NEM316ΔbibA | This study |
| CCH395 | BM110ΔhvgA | This study |
| CCH423 | NEM316ΔbibA, PbibA (pOri23ΩbibA) | This study |
| CCH571 | NEM316ΔbibA, PhvgA (pOri23Ω hvgA) | This study |
| CCH562 | BM110Δ hvgA, PhvgA (pOri23Ω hvgA) | This study |
| CCH678 | BM110, pTCVΩlux | This study |
| CCH692 | BM110Δ hvgA, pTCVΩlux | This study |
| <i>Lactococcus lactis</i> subsp. <i>cremoris</i> | | |
| MG1363 | Lac- Prt-; NCDO 712 derivative | (Wegmann et al., 2007) |
| CCH223 | MG1363, pOri23 | This study |
| CCH224 | MG1363, PbibA (pOri23ΩbibA) | This study |
| CCH561 | MG1363, PhvgA (pOri23Ω hvgA) | This study |
| Plasmids | | |
| pG+host5 | Em; ColE1 replicon, thermosensitive derivative of pGK12; MCS pBluescript | (Biswas et al., 1993) |
| pDIA17 | Cm; oriR pACYC184, Tet promoter ΔlacI | (Munier et al., 1991) |
| pET-26b(+) | Km; oriR pBR322, peLΒ coding sequence, T7 promoter, His-Tag coding sequence | Novagen |
| pET2817 | Amp; oriR pBR322, T7 promoter, His-Tag coding sequence, pET28/16 derivative. | Provided by S. Mesnage, INSERM 872, Paris, France |
| pOri23 | Em; ermAM ori ColE1, thermosensitive derivative of pIL253, P23 promoter of <i>L. lactis</i> MG1363 | (Braun et al., 2000) |
| pTCVlux | Em, Km; pTCV-erm derivative with an additional lux operon fused to PcyIΔ promoter. | This study |

Table S2. GBS strains isolated from neonatal invasive infections used in adhesion assays

| Strains | Capsular serotype | ST cluster | Type of neonatal infection ^a |
|-------------|-------------------|------------|---|
| CNRCCH0705 | II | 28 | EOD/bacteremia |
| CNRCCH0845 | Ia | 23 | EOD/meningitis |
| CNRCCH0851 | Ia | 23 | EOD/bacteremia |
| CNRCCH0889 | Ia | 23 | EOD/bacteremia |
| CNRCCH08141 | III | 17 | LOD/meningitis |
| CNRCCH08147 | III | 17 | LOD/meningitis |
| CNRCCH08198 | III | 17 | LOD/meningitis |
| CNRCCH08201 | Ia | 24 | EOD/bacteremia |
| CNRCCH08214 | III | 17 | EOD/bacteremia |
| CNRCCH08240 | III | 17 | LOD/meningitis |
| CNRCCH08271 | III | 17 | LOD/meningitis |
| CNRCCH08274 | Ia | 23 | EOD/bacteremia |
| CNRCCH08380 | III | 17 | EOD/meningitis |
| CNRCCH08400 | III | 17 | LOD/meningitis |
| CNRCCH08472 | V | 453 | EOD/bacteremia |
| CNRCCH08523 | V | 1 | LOD/bacteremia |
| CNRCCH08557 | III | 17 | EOD/meningitis |
| CNRCCH08725 | Ib | 10 | EOD/meningitis |
| CNRCCH08726 | Ia | 23 | LOD/bacteremia |
| CNRCCH08738 | III | 17 | LOD/meningitis |
| CNRCCH08914 | IV | 1 | EOD/bacteremia |
| 2603V/R | V | 110 | ND |
| COH1 | III | 17 | ND |
| NEM316 | III | 23 | neonatal infection |
| BM110 | III | 17 | neonatal infection |

Table S3. Primers used in this study

| Primer | Sequence (5' to 3') |
|--|--|
| Construction of <i>bibA/hvgA</i> in-frame deletion mutants | |
| 01 | ACAAAG <u>AATT</u> CGCCGCAGGAGTCATGGAC |
| 02 | GGCACGCCGGGTGCTGCCGCCGTGCTGACATTGAGGCCAAACC |
| 03 | GCGGCAGCACCCGGCGTGCACGGGTGAAGCCGCAAGTCCACTC |
| 04 | CATTAGG <u>ATCC</u> GAGTAGGGATTCCACACGC |
| Complementation experiments and expression of <i>bibA/hvgA</i> in <i>L. lactis</i> | |
| 05 | <u>CGCGGATCCC</u> GTGGTCTATCTAATAAAATTAG |
| 06 | GGCA <u>ACTG</u> CAGGATGAGGTTGCCTTAAGATTG |
| Cloning in pIVEX2.4b | |
| <i>bibA-Nco</i> | CACGCC <u>ATGG</u> ATAGTTCAAGGAATATCGGCTCA |
| <i>bibA-Xho</i> | TTTAT <u>CTCG</u> GAGAGTACCTCTGGTAAGGTCTG |
| <i>hvgA-Nco</i> | AAAC <u>CCATGGGTGGTATTG</u> TAAACTCCTACAGTG |
| <i>hvgA-Xho</i> | AAAGG <u>ATCCTAG</u> CTCAAGTAATAATTTTAACATCTC |
| qRT-PCR analysis | |
| <i>hvgA-R</i> | ATACAAATTCTGCTGACTACCG |
| <i>hvgA-F</i> | TAAATCCTCCTGACCATTCC |
| <i>rpoB-F</i> | AATTAGTCCGTTCTCCTGGTGT |
| <i>rpoB-R</i> | ACGTGTGCGGTCGATACGT |
| Specific primers (competitive index assay) | |
| <i>dltR-F</i> | GCAGAGGCTATT <u>CAGG</u> CTT |
| <i>dltR-R</i> | CCAAGCC <u>GAATTG</u> ATTGCTC |
| <i>bibA-F</i> | TGTA <u>ACTCAAGCCACG</u> CACTG |
| <i>bibA-R</i> | TTCTGG <u>CAACTCC</u> TGTGCT |
| <i>hvgA-F</i> | GGTG <u>CTAAAGAGCAAG</u> CACT |
| <i>hvgA-R</i> | TCC <u>TGGACTTCG</u> TCTCCTCA |
| Primers for pTCVlux construction | |
| <i>PcyΔ-Eco</i> | <u>CGGAATT</u> CGAGAGTGC <u>GGGTTCTG</u> A |
| <i>PcyΔ-Bam</i> | <u>CGGGATCCC</u> ACAA <u>ATCATGAGTAAACAC</u> |
| <i>lux-Bam</i> | GAT <u>CTAGTGGATCCGGCAGATGAAGCAAGAGG</u> |
| <i>lux-Pst</i> | TATT <u>ACTG</u> CAGT <u>CCTCG</u> ACTTA <u>ACTATCAAACGC</u> |

The restriction sites included in the oligonucleotides for subsequent cloning of the amplified fragments are underlined.

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