

Functional characterization of *Ralstonia insidiosa*, a bona fide resident at the maternal-fetal interface

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Probe name	Sequence 5' → 3'	Label (5' end)	Specificity	Source
EUB338	GCTGCCTCCCGTAGGAGT	CY3	Conserved domain of the eubacterial 16S rRNA gene	Amann et. al 1990 ⁶⁷
NON-EUB338	ACTCCTACGGGAGGCAGC	CY3	Nonsense control complementary to EUB338	Wallner et. al 1993 ⁶⁸
<i>R. insidiosa</i>	TTAGTAAGTGCGATTTCTTT CCGGA	FITC	Species-specific sequence of <i>R. insidiosa</i> 16S rRNA gene	This study

Table S1. **Overview of the 16S rRNA fluorescent in situ hybridization probes applied to basal plate specimens in this study.**

<i>R. insidiosa</i> isolate	Source	Growth conditions
MJ602 (WT)	Johnson Space Center as <i>R. insidiosa</i> 130770013-1 ⁷³	Static; 32 °C Reasoner's 2B Medium
GGW102 (YFP strain)	attTn7 integration of <i>yfp</i> into strain MJ602	Static; 32 °C Reasoner's 2B Medium

Table S2. **Overview of the isolates used.**

Primer Name	Forward (5'-3')	Reverse Forward (5'-3')	Product Length (bp)
YFP 1	GCTACCCCGACCATGAAG	AAGAAGATGGTGCGCTCCTG	82
YFP 2	ACGTAAACGGCCACAAGTTC	CTTCATGTGGTCGGGGTAGC	176
YFP 3	AGCAAAGACCCCAACGAGAA	TCGTCCATGCCGAGAGTGAT	83
YFP 4	GAACCGCATCGAGCTGAA	TGCTTGTCGGCCATGATATAG	111

Table S3. **Primer pairs targeting yellow-fluorescent protein.**

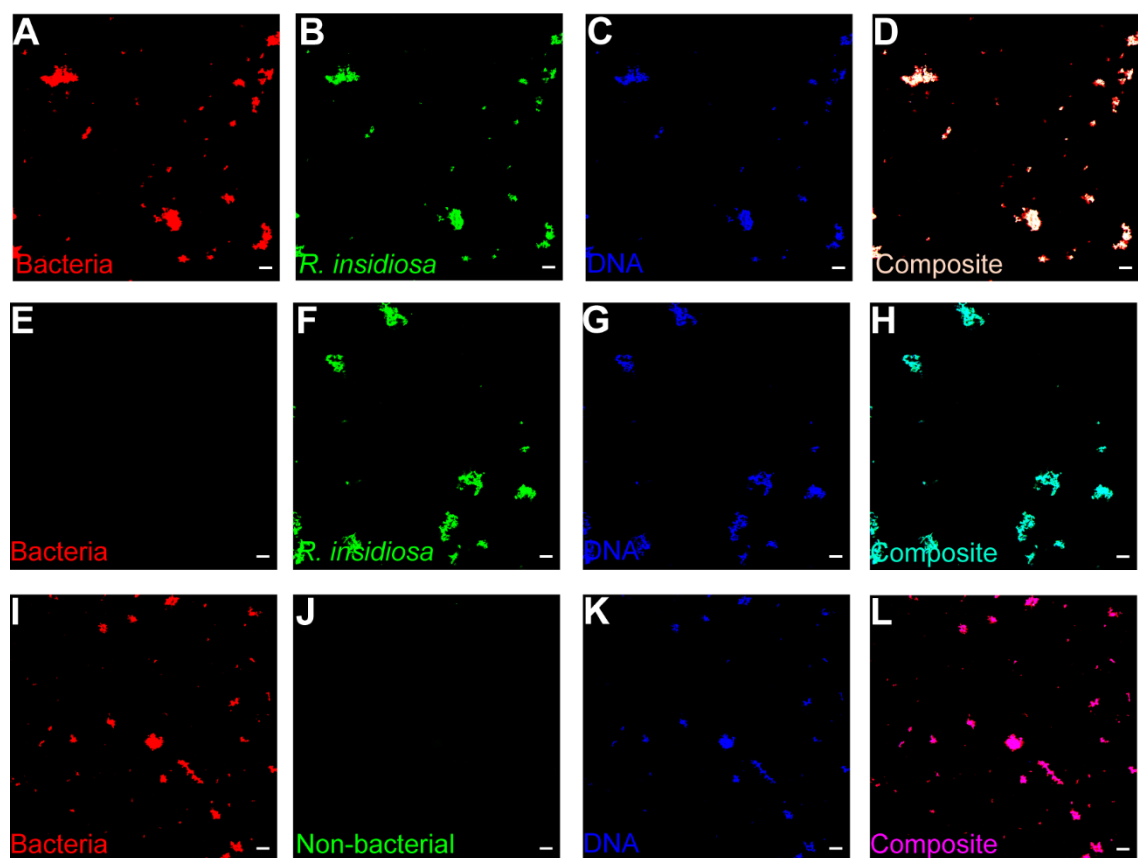


Figure S1. **Evaluation of probe functionality by whole bacterial cell fluorescent in situ hybridization using a *R. insidiosa* WT strain (MJ602).** EUB338-CY3 was used as a universal bacterial probe (bacteria-red), *R. insidiosa*-FITC was used as a species-specific probe (*R. insidiosa*-green), NON-EU338-FITC represents the complement to the universal eubacterial probe and was used as a negative control probe (non-bacteria-green), and DAPI was used for host nuclear and bacterial DNA staining (DNA-blue). Whole bacterial cells were simultaneously hybridized with (A-D) bacterial probe, *R. insidiosa* probe (*R. insidiosa*-green) and DAPI (DNA-blue); (E-H) *R. insidiosa* and DAPI; or with the bacterial and non-bacterial probe. Fluorescent images were acquired separately using three filter sets (CY3, FITC, DAPI) and are displayed as a final image (composite) (Magnification 40x oil, scale bar: 20 μ m).

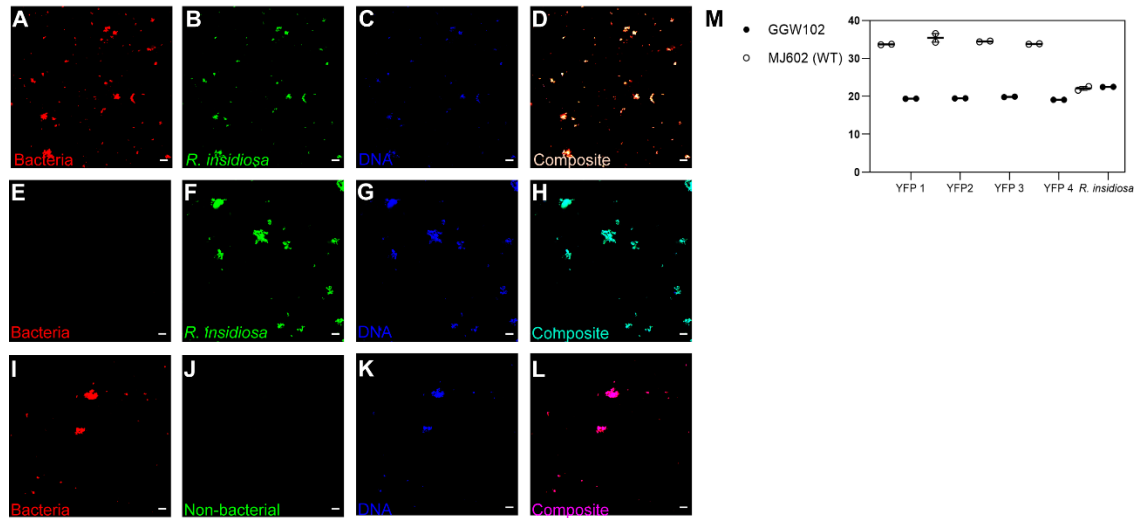


Figure S2. **Evaluation of FISH probe functionality and yfp-expression using GGW102.** Whole bacterial cells were simultaneously hybridized with the universal bacterial probe (bacteria-red), *R. insidiosa* probe (*R. insidiosa*-green) and DAPI (DNA-blue); (E-H) the *R. insidiosa* probe and DAPI; or with the bacterial probe (red) and non-bacterial probe (green). Fluorescent images were acquired separately using three filter sets (CY3, FITC, DAPI) are displayed as a final image (composite) (Magnification 40x oil, scale bar: 20 μ m). (M) YFP primers (YFP 1-4) were used to verify the presence of YFP. YFP is expressed in GGW102, but not in MJ602. The *R. insidiosa*-specific product is amplified in both isolates. Cq values are reported.

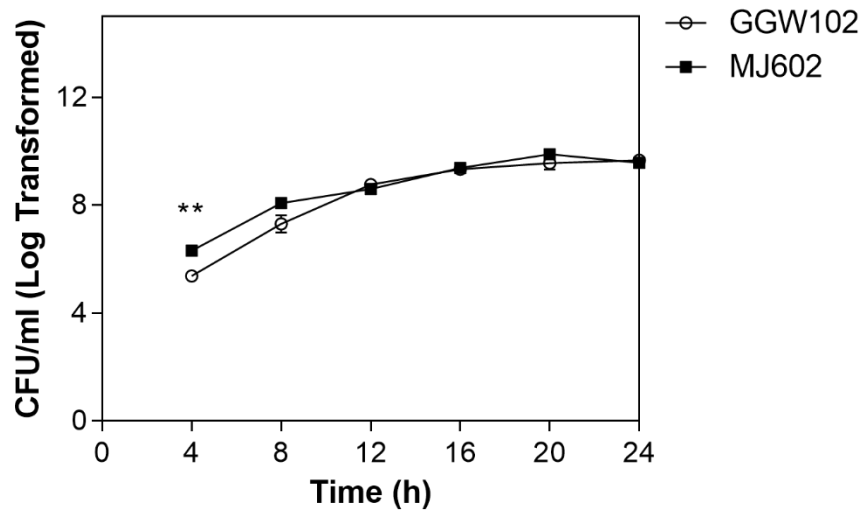


Figure S3. **Growth profiles *R. insidiosa* strains MJ602 and GGW102 in monocultures.** The data is the average CFUs over the course of 24 hours for MJ602 and GGW102 (n = 6 colonies each). Single colonies were inoculated in 20 mLs of R2B. Although there is a significant difference in the log CFUs in MJ602 compared to GGW102 at 4 h ($p = 0.00220$), there is no significant difference between the growth of isolates at 8h and onward (8h, $p = 0.180$, 12h, $p = > 0.999$; 16h, $p = > 0.999$, 20h, $p = 0.240$, 24h, $p = 0.818$) suggesting that they can be used interchangeably for replication analysis ex vivo, in vitro, and in vivo studies. P-values were calculated using the two-tailed Mann-Whitney U test at each time point. ** indicates a $p < 0.005$.

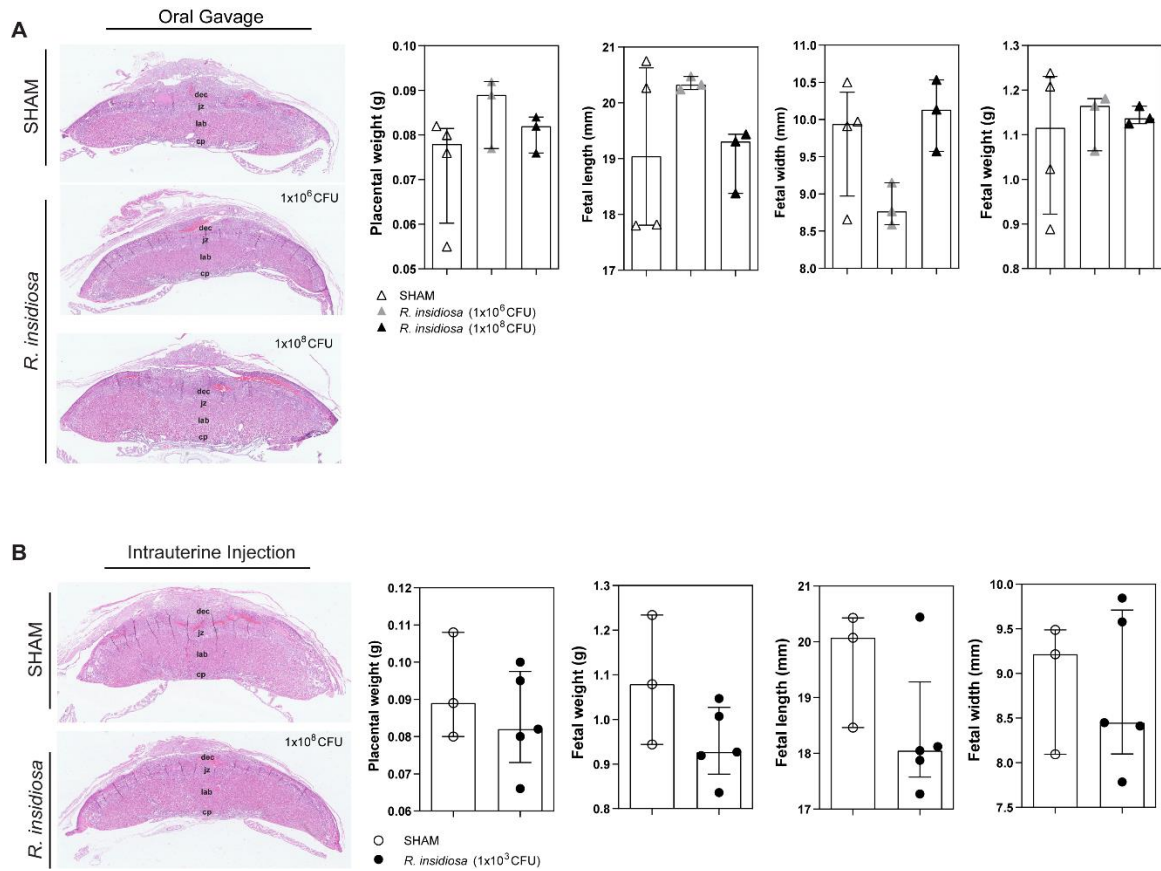


Figure S4. Placentas and fetuses from mice challenged with *R. insidiosus* do not exhibit significant anatomical anomalies. (A) Representative hematoxylin and eosin staining of mouse placentas (gd 18.5) derived from SHAM (n= 4) or *R. insidiosus* challenged dams following oral gavage of 1x10⁶ (n=3) and 1x10⁸ (n=3) CFUs at gd 15.5. Fetoplacental units of *R. insidiosus*-challenged dams compared to SHAM controls exhibit no differences in the median placental weight (n=10, Kruskal-Wallis statistic = 2.92; p = 0.260), fetal length by oral gavage (n=10, Kruskal-Wallis statistic = 2.38; p = 0.326), fetal width (n=10, Kruskal-Wallis statistic = 4.27; p = 0.123), or fetal weight (n=10, Kruskal-Wallis statistic = 0.164; p = 0.941). (B) Representative hematoxylin and eosin staining mouse placentas (gd 18.5) from (H) SHAM or (I) *R. insidiosus*-challenged dams following intrauterine injection of 1x10³ CFUs at gd 15.5. Fetoplacental units of *R. insidiosus*-challenged dams compared to SHAM controls exhibit no differences in the median placental weight (Mann-Whitney U = 5.50; p = 0.643), fetal length by oral gavage (Mann-Whitney U=3; p =0.250), fetal width (Mann-Whitney U=7; p = >0.999), or fetal weight (Mann-Whitney U = 2; p = 0.143). P-values were calculated using the two-tailed Mann-Whitney U test.