

Figure S1



Figure S2







Figure S4

| Primer Name    | sequence 5' - 3'                                                              | restriction site |
|----------------|-------------------------------------------------------------------------------|------------------|
| 1081 up for    | AATGACGTCGACCCGAGCGTTGCCAGTGCG                                                | SalI             |
| 1081 up rev    | TTACCGAAGCTTGCCGTCGGTGTTGCAGAC                                                | HindIII          |
| 1081 down for  | CGTTACAAGCTTTTCGGGCTTTACGGCCTG                                                | HindIII          |
| 1081_down_rev  | ATTACAGAGCTCAATCGACAGCGCATAGCC                                                | SacI             |
| 1081_seq_for   | CGCGGGTGTCTTGCAC                                                              | N/A              |
| 1081_seq_rev   | GCAAGGGTGGCAAGATT                                                             | N/A              |
| 1081_comp_for  | CGTTACAAGCTTTCAGCCCTGCGCCGCTTC                                                | NcoI             |
| 1081_comp_rev  | CGTTACAAGCTTTCAGCCCTGCGCCGCTTC                                                | HindIII          |
| 1081_lacZ_for  | CGACGGCGACCTGCAGGGGGGGGGGAA                                                   | PstI             |
| 1081_lacZ_rev  | TGGCAAGCGCGAGATCTGTGTTGCA                                                     | BglII            |
| 1932_up_for    | TAGCAGAGCTCTTGGGGTGATCGTGATGA                                                 | SacI             |
| 1932_up_rev    | GTACAAAGCTTAAGACTGACGACCAGAGA                                                 | HindIII          |
| 1932_down_for  | GTAGACAAGCTTGGGATCACCCTTGCGCTG                                                | HindIII          |
| 1932_down_rev  | GTACATCTAGACCATCAGCTTGCCGGTGGG                                                | XbaI             |
| 1932_comp_for  | TAGGTGGCCATGGTCCACTCGGCT                                                      | NcoI             |
| 1932_comp_rev  | TCAGGCTTCCGACATCGCAAGCTTGGGCAT                                                | HindIII          |
| tetAp_for      | GAATTCCAATTGATGATTCTCCGCCAGCAT                                                | ApalI            |
| tetAp_rev      | GAATTCGTGCACCCTCCGGACCAGC                                                     | MfeI             |
| sacB:accC1_for | GAATTCGTGCACATGAACATCAAAAAGTTTGC                                              | MfeI             |
| sacB:accC1_rev | GAATTCCAATTGTTAGGTGGCGGTACTTG                                                 | ApaLI            |
|                |                                                                               |                  |
| Plasmid Name   | Fragment/description                                                          | Reference        |
| pUC81UP        | upstream flank of <i>rcc01081</i> as SalI-HindIII frag; Amp <sup>R</sup>      | This Study       |
| pUC81DOWN      | downstream flank of <i>rcc01081</i> as HindIII-SacI; <b>Amp</b> <sup>R</sup>  | This Study       |
|                | rcc01081 upstream+downstream ligated into suicide                             |                  |
| pZDJ∆1081      | vector; <b>Gm<sup>K</sup></b>                                                 | This Study       |
| pIND1081       | <i>rcc01081</i> ORF as NcoI-HindIII frag; <b>Km<sup>K</sup></b>               | This Study       |
| pUC32UP        | upstream flank of <i>rcc01932</i> as SacI-HindIII frag; Amp <sup>k</sup>      | This Study       |
| pUC32DOWN      | downstream flank of <i>rcc01932</i> as HindIII-XbaI frag;<br>Amp <sup>R</sup> | This Study       |
| -              | <i>rcc01932</i> upstream+downstream ligated into suicide                      | ٠<br>۲           |
| pZDJΔ1932      | vector; Gm <sup>R</sup>                                                       | This Study       |

Table S1. Primers and plasmids used in this study

| pIND1932                       | <i>rcc01932</i> ORF as NcoI-HindIII frag; <b>Km</b> <sup>R</sup>                                     | This Study               |
|--------------------------------|------------------------------------------------------------------------------------------------------|--------------------------|
| pUC19                          | general cloning vector; $lacZ\alpha$ ; <b>Amp</b> <sup>R</sup>                                       | Invitrogen               |
| pZJD29a                        | suicide vector; contains <i>sacB</i> gene; $Gm^{R}$                                                  | Unpublished              |
| pZDJ                           | pZJD29a; <i>puc</i> promoter replaced with <i>tetA</i> promoter <b>Gm</b> <sup>R</sup>               | This Study               |
| pIND4                          | Inducible expression vector; <b>Km</b> <sup>R</sup>                                                  | Ind et al., 2009         |
| pXC01081                       | ~1500bp 5' of ORF <i>rcc01081</i> fused to lacZ;<br>translationally in frame; <b>Tc</b> <sup>R</sup> | This Study               |
| pXCA601                        | promoter probe vector; for construction of<br>promoter::lacZ fusions; <b>Tc</b> <sup>R</sup>         | (Adams et al.,<br>1989)  |
|                                |                                                                                                      |                          |
| <i>R. capsulatus</i><br>strain | Description                                                                                          | Reference                |
| B10                            | wild type isolate; encapsulated                                                                      | Weaver et al., 1975      |
| DE442                          | RcGTA overproducer; believed to originate from Y262                                                  | Yen et al., 1979         |
| 37b4                           | wild type isolate; non-encapsulated                                                                  | (Weckesser et al., 1972) |
| ∆gtaI                          | markerless deletion of <i>gtaI</i> gene ( <i>rcc00329</i> ); B10 background                          | Leung et al., 2012       |
| ∆gtaR                          | markerless deletion of <i>gtaR</i> gene ( <i>rcc00328</i> ); B10 background                          | Leung et al., 2012       |
| ∆gtaRI                         | double deletion of gtal and gtaR genes; B10 background                                               | Leung et al., 2012       |
| ⊿1081                          | markerless deletion of <i>rcc01081</i> ; B10 background                                              | This Study               |
| ∆gtaI/∆1081                    | double deletion of <i>gtal</i> and <i>rcc01081</i> ; B10 background                                  | This Study               |
| ⊿1932                          | markerless deletion of <i>rcc01932</i> ; B10 background                                              | This Study               |
| <i>∆1932/∆1081</i>             | markerless deletion of <i>rcc01932</i> and <i>rcc01081</i> ; B10<br>background                       | This Study               |

**Table S2.** Growth Curve Data: A statistical measure of correlation using a linear regression analysis.

| recipient capability assay |          |
|----------------------------|----------|
| Number of XY pairs         | 7        |
| Pearson r                  | 0.9501   |
| 95% conf. interval         | 0.693-   |
|                            | 0.992    |
| p-value                    | 0.00104  |
| Correlation significant?   | yes      |
| (α=0.05)                   |          |
| R squared                  | 0.9028   |
|                            |          |
| adsorption assay           |          |
| Number of XY pairs         | 7        |
| Pearson r                  | 0.9571   |
| 95% conf. interval         | 0.731-   |
|                            | 0.993    |
| p-value                    | 0.000715 |
| Correlation significant?   | yes      |
| (α=0.05)                   |          |
| R squared                  | 0.9162   |

| Table S3. | One-way | ANOVA | results for | comparison | of RcGTA | recepient ability |
|-----------|---------|-------|-------------|------------|----------|-------------------|
|           | 2       |       |             |            |          |                   |

| Strain 1 | Strain 2                        | p-value   |
|----------|---------------------------------|-----------|
| WT B10   | ∆gtaI                           | 0.0000558 |
| WT B10   | $\Delta gtaI + C16$             | 0.9993916 |
| WT B10   | ∆gtaR                           | 1         |
| WT B10   | ∆gtaRI                          | 1         |
| WT B10   | Δ1081                           | 0.0000936 |
| WT B10   | <i>∆1081</i> [p1081]            | 0.0324655 |
| WT B10   | ΔgtaI/Δ1081                     | 0.0001958 |
| WT B10   | $\Delta gtaI/\Delta 1081 + C16$ | 0.0000894 |
| WT B10   | A1932                           | 0.000061  |
| WT B10   | <i>∆1932</i> [p1932]            | 0.4535225 |
| WT B10   | Δ1081/Δ1932                     | 0.0000385 |
| WT B10   | 37b4                            | 0.0000333 |
| ⊿1081    | <i>∆1081</i> [p1081]            | 0.492496  |

| Strain 1 | Strain 2                 | p-value   |
|----------|--------------------------|-----------|
| No Cells | WT B10                   | 0         |
| No Cells | ∆gtaI                    | 0.3630966 |
| No Cells | $\Delta gtaI + C16$      | 0         |
| No Cells | ∆gtaR                    | 0         |
| No Cells | ∆gtaRI                   | 0         |
| No Cells | ⊿1081                    | 0.0332213 |
| No Cells | ⊿ <i>1081</i> [p1081]    | 0         |
| No Cells | ∆gtaI/∆1081              | 1E-07     |
| No Cells | <i>∆gtaI/∆1081</i> + C16 | 0         |
| No Cells | ⊿1932                    | 0.9930653 |
| No Cells | <i>∆1932</i> [p1932]     | 0         |
| No Cells | Δ1081/Δ1932              | 0.7869396 |
| No Cells | 37b4                     | 0.9982706 |
| ⊿1081    | ⊿ <i>1081</i> [p1081]    | 0.0000007 |
| ∆gtaI    | $\Delta gtaI + C16$      | 0.0000001 |

Table S4. One-way ANOVA results for comparison of RcGTA adsorption ability

| Strain 1 | Strain 2              | p-value  |
|----------|-----------------------|----------|
| WT B10   | ∆gtaI                 | 0        |
| WT B10   | $\Delta gtaI + C16$   | 2E-07    |
| WT B10   | ∆gtaR                 | 6.84E-05 |
| WT B10   | ∆gtaRI                | 0.165229 |
| WT B10   | Δ1081                 | 0        |
| WT B10   | <i>∆1081</i> [p1081]  | 0.005464 |
| WT B10   | ΔgtaI/Δ1081           | 0        |
| WT B10   | ΔgtaI/Δ1081 + C16     | 0        |
| WT B10   | Δ1932                 | 0        |
| WT B10   | Δ <i>1932</i> [p1932] | 0.00004  |
| WT B10   | Δ1081/Δ1932           | 0        |
| WT B10   | 37b4                  | 0        |
| Agtal    | $\Delta gtaI + C16$   | 0        |
| Δgiui    | Δ <i>1081</i> [p1081] | 0        |
| Δ1932    | Δ <i>1932</i> [p1932] | 0        |

Table S5. One-way ANOVA results for comparison of carbohydrate production

| Strain 1      | Strain 2            | p-value  |
|---------------|---------------------|----------|
| WT B10        | ∆gtaI               | 9.75E-05 |
| WT B10        | $\Delta gtaI + C16$ | 0.053569 |
| WT B10        | ∆gtaR               | 0.353396 |
| WT B10        | ∆gtaRI              | 0.266044 |
| $\Delta gtaI$ | $\Delta gtaI + C16$ | 3.27E-05 |

Table S6. One-way ANOVA results for comparison of  $\beta$ -galactosidase activities

Table S7. One-way ANOVA results for comparison of RcGTA adsorption inhibition ability

| Strain 1 | Strain 2              | p-value  |
|----------|-----------------------|----------|
| No Cells | WT                    | 0        |
| WT       | WT extracts           | 1.3E-06  |
| WT       | <i>∆gtaI</i> extracts | 0.881878 |
| WT       | △1932 extracts        | 0.999761 |