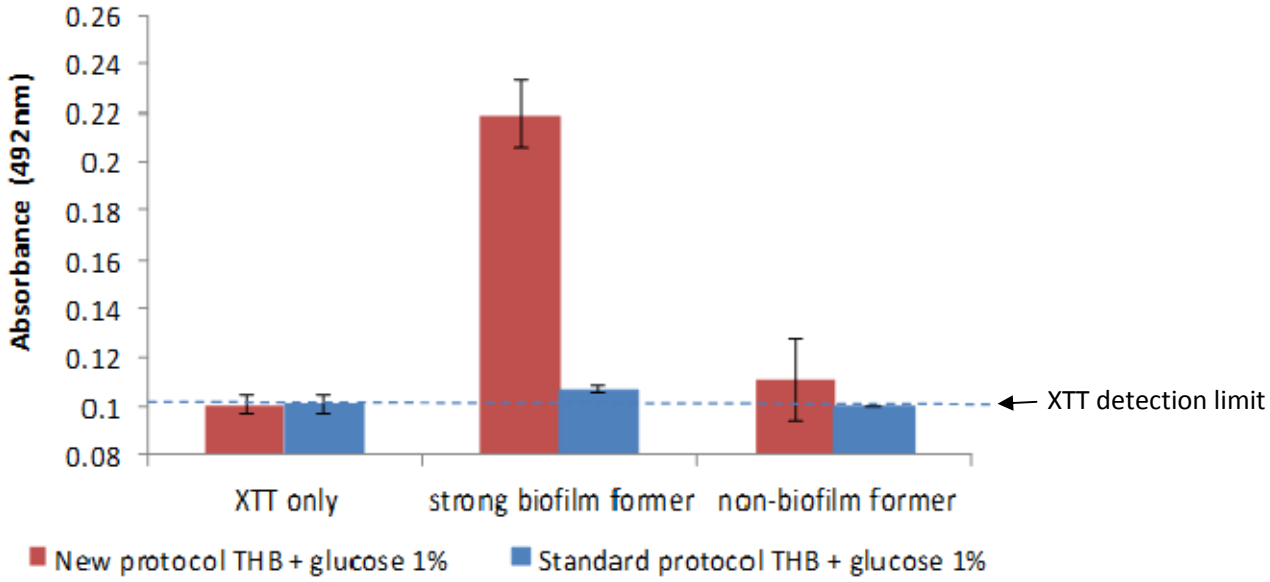



Figure S1. **Comparison of biofilm formation protocols**

Comparison of cell viability of a strong biofilm forming strains and a weak/non-biofilm forming strains growth using the standard and the new biofilm formation protocol. The GBS strains were grown in THB in presence of 1% glucose (blue, standard protocol and red, new protocol). Surface-attached cells were incubated 3h at 37°C with XTT-menadione solution and cell viability was monitored by measuring the absorbance at 492 nm. The mean values of three independent experiments and standard deviation are shown.

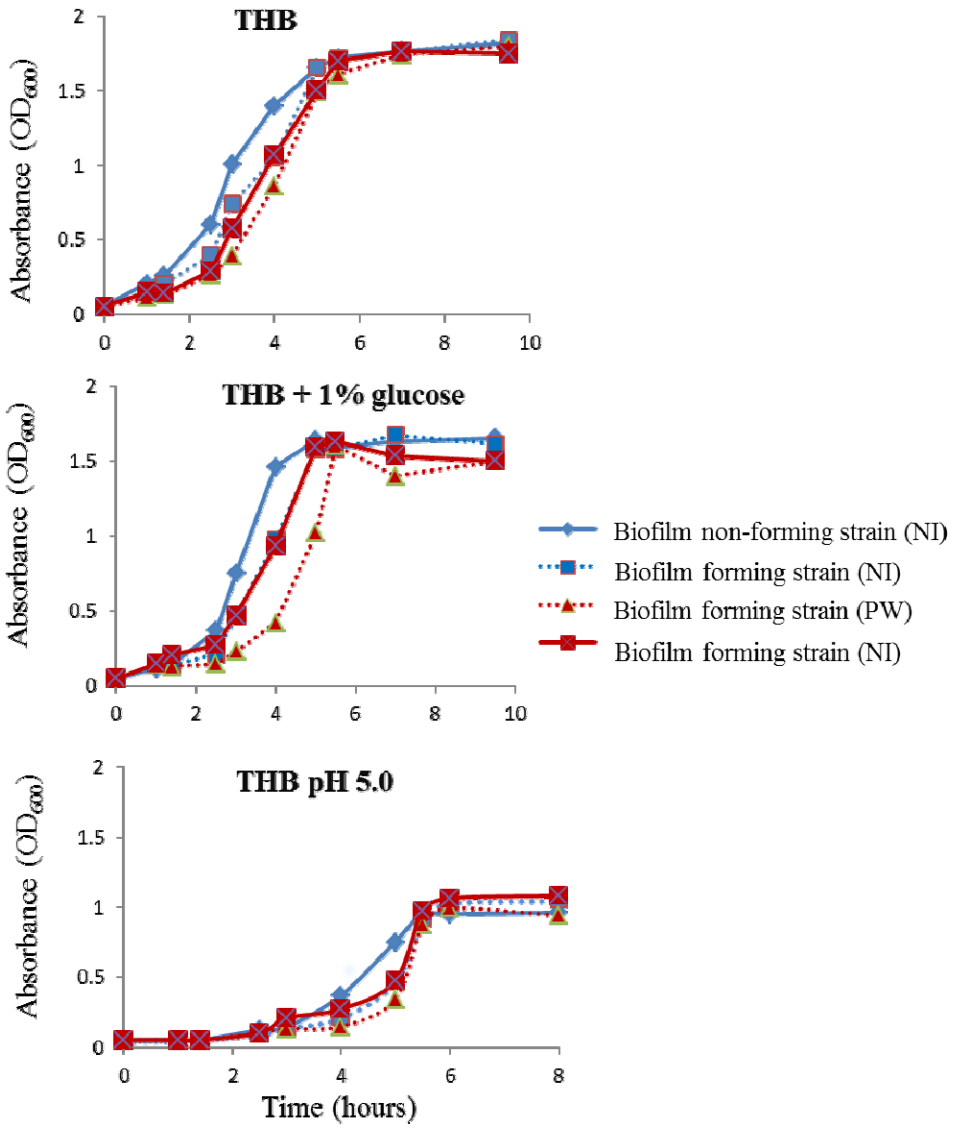



Figure S2**. Comparison of the Growth rate of GBS biofilm forming and non-forming strains**

3 GBS biofilm forming strains (clinical isolates, serotype III, ST-17) and a GBS non-biofilm forming strain (COH1, serotype III, ST-17) were compared for their growth rate at 37°C in THB, THB supplemented with 1% glucose and THB pH 5.0. (NI) indicates the strains isolates from infected neonates while (PW) indicates the colonizing strain isolate from pregnant women. The OD600 was monitored at different time point during the bacterial growth. The mean values of three independent experiments are shown

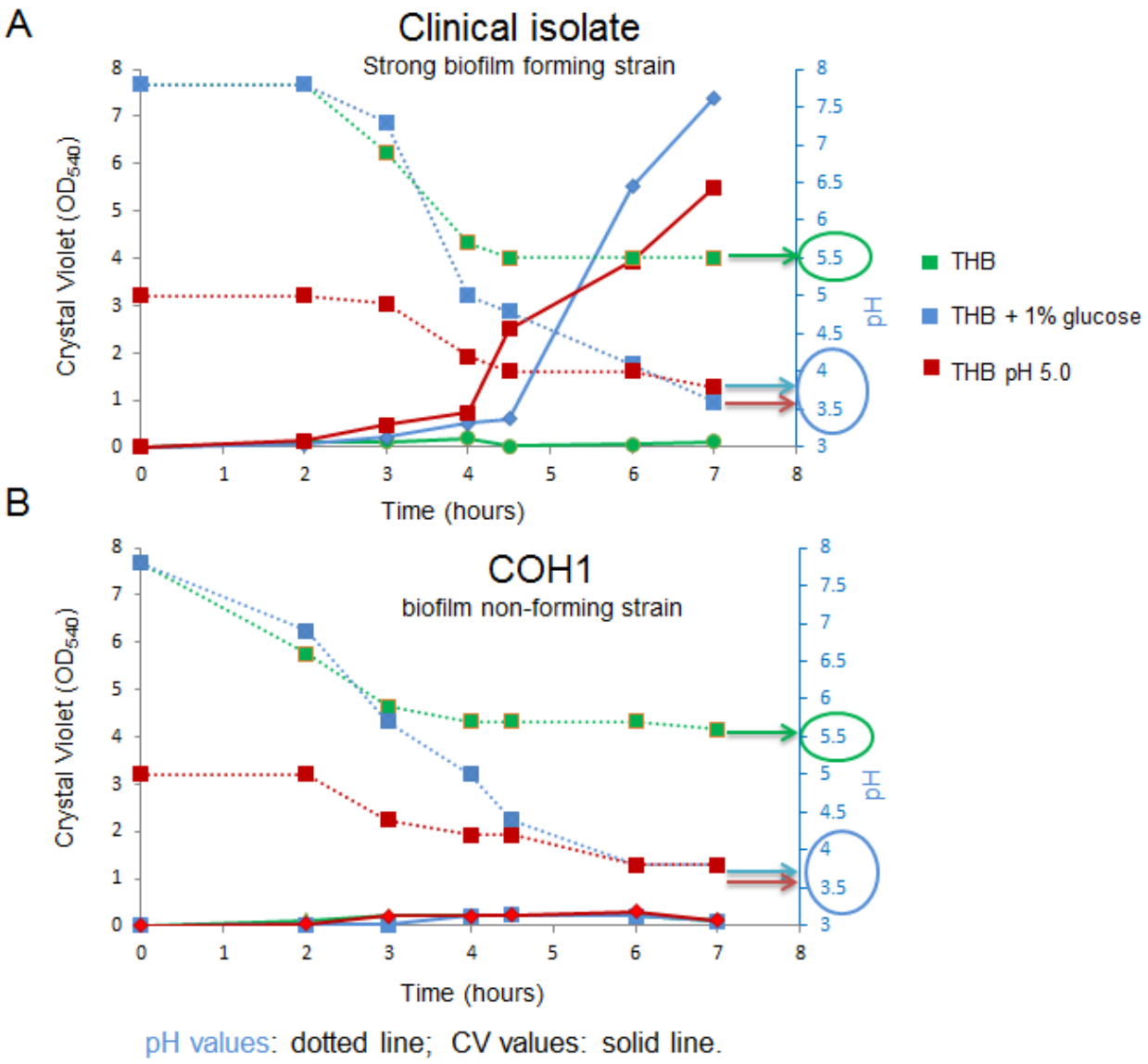
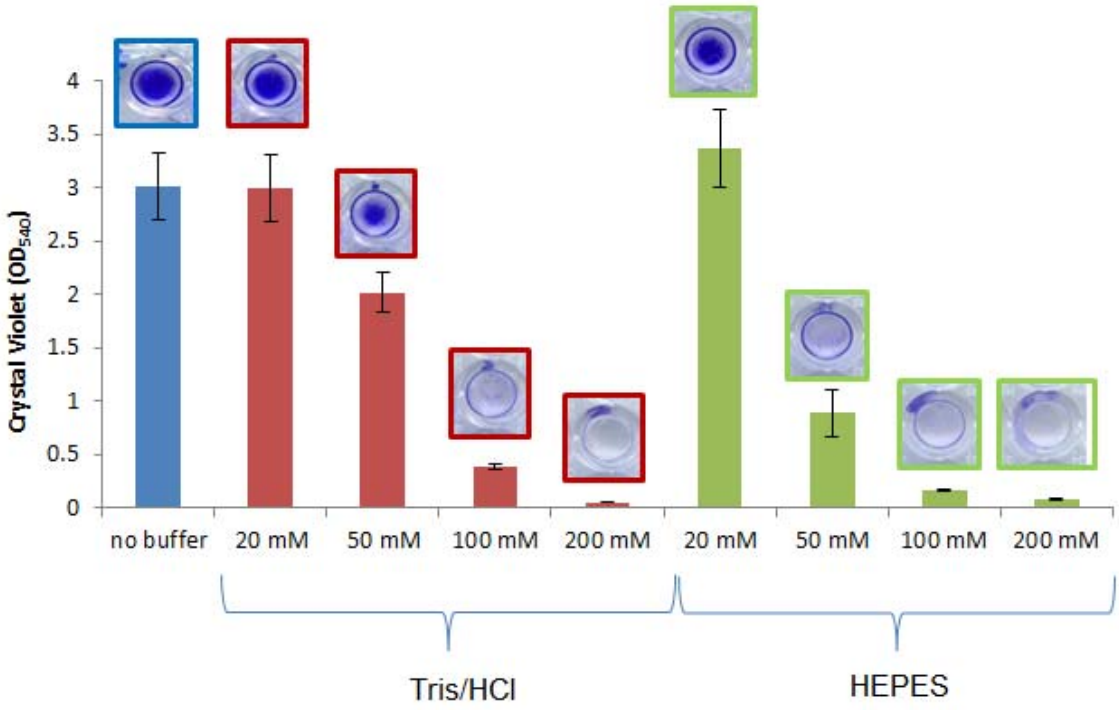



Figure S3. **Time-course of biofilm formation in correlation to pH.**

A) GBS biofilm forming strain (clinical isolate, serotype III, ST-17) and B) GBS non-biofilm forming strain (COH1, serotype III, ST-17) were compared for their ability to produce biofilm (solid lines) and induce acidification related to growth media (dotted lines). Three different growth media were tested: THB (green line), THB supplemented with 1% glucose (red line) and THB pH 5.0 (blue line). Biofilm formation was evaluated using Crystal Violet stain measuring the absorbance at 540 nm (solid line). pH values (round dots) were measured using pH-test-strips (pH increment: 0.2). The mean values of three independent experiments, performed for both the strains in each medium, are shown.




| | No buffer | Tris-HCl | | | | Hepes | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 20 mM | 50 mM | 100 mM | 200 mM | 20 mM | 50 mM | 100 mM | 200 mM |
| Final pH | 4.3 | 4.3 | 4.5 | 4.7 | 6.5 | 4.4 | 4.6 | 4.8 | 6.0 |
| Final $OD_{600}$ | 1.7 | 1.7 | 1.7 | 1.9 | 1.2 | 1.9 | 2.3 | 2.9 | 3.5 |

Figure S4. **Biofilm formation in buffered THB media.**

GBS strong biofilm forming strain (serotype III, ST-17) was compared for its ability to form biofilm in a THB medium supplemented with 1% of glucose and buffered with different concentration of Tris-HCl and HEPES to limit the pH-drop during the bacterial growth. Biofilm formation was evaluated 6h of incubation (37°C, 60 rpm) using Crystal Violet assay measuring the absorbance at 540 nm after. pH values (shown in the table) were measured using pH-test-strips (pH increment: 0.2). Bacterial growth was monitored by measuring the $OD_{600}$. The mean values of three independent experiments are shown.

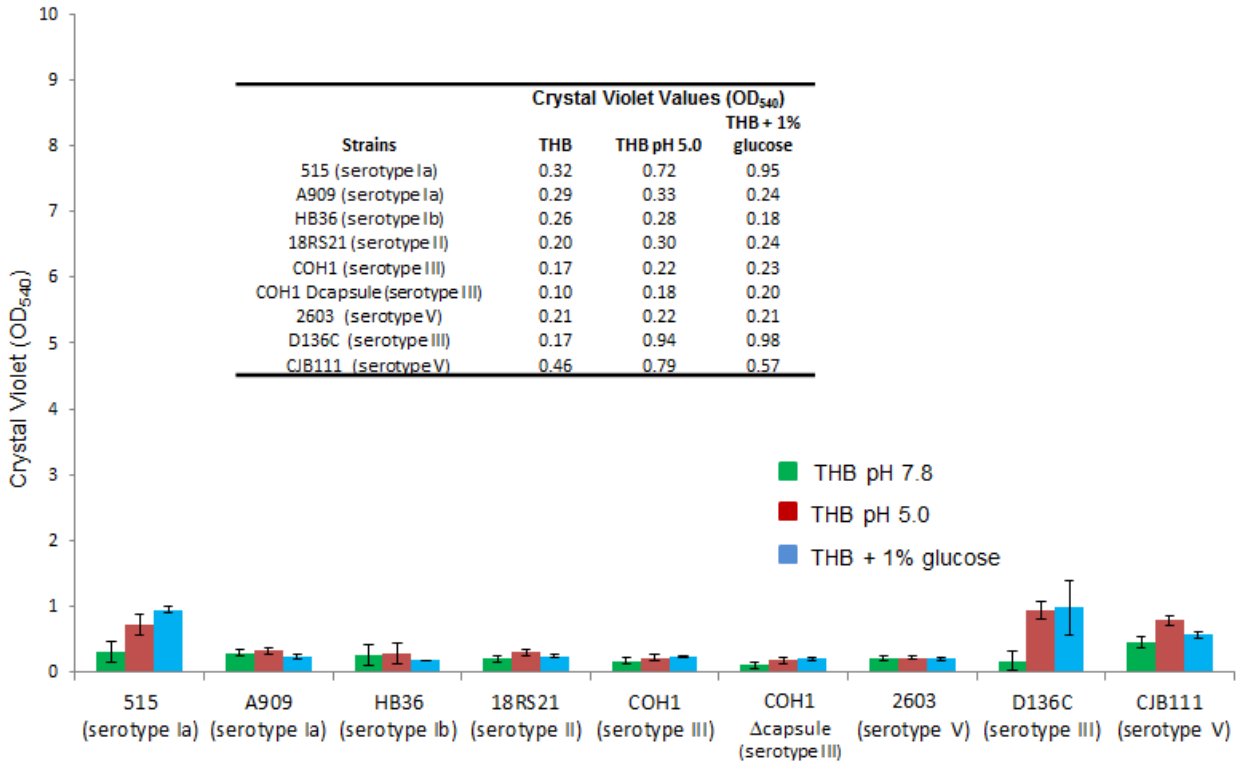



Figure S5. **Biofilm formation of sequence annotated GBS strains.**

Biofilm formation ability of GBS strains belonging from 5 different serotypes were grown in THB pH 7.8 (green bars) and THB supplemented with 1% of glucose (blue bars) and THB pH 5.0 (red bars) on 96-well polystyrene plates. Biofilm formation was evaluated using Crystal Violet stain by measuring the absorbance at 540 nm. The mean values of three independent experiments and standard deviation are shown.

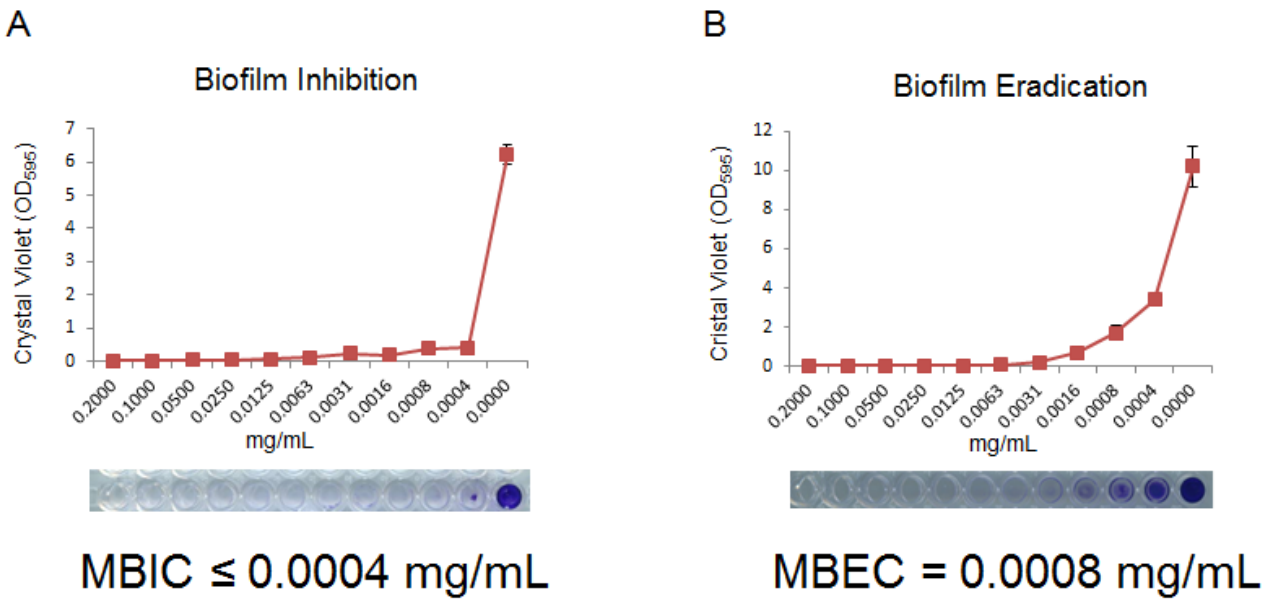



Figure S6. **Enzymatic biofilm inhibition and eradication using proteinase K.**

A) Minimal Biofilm Inhibition Concentration (MBIC) and B) Minimal Biofilm Eradication Concentration (MBEC) using proteinase K (range of concentration 0.2-0.0004 mg/mL). GBS biofilm forming strain (clinical isolate, serotype III, ST-17) was grown in THB pH 5.0. Biofilm formation was evaluated using Crystal Violet stain measuring the absorbance at 540 nm. The mean values of three independent experiments and standard deviation are shown. The plate stained with Crystal Violet is shown below the graph.