**Supplementary data**

**Part I: Microcalorimetry of planktonic** ***S. agalactiae* (ATCC 13813), *S. pyogenes* (ATCC 19615) and *S. oralis* (ATCC 35037).**



**Figure S1.** Microcalorimetry of planktonic *S. agalactiae* (ATCC 13813). Numbers represent concentrations (in mg/L) of fosfomycin (a), rifampicin (b), benzylpenicillin (c), daptomycin (d), gentamicin (e) and levofloxacin (f). Circled values represent the MHIC, defined as the lowest antimicrobial concentration inhibiting growth-related heat production after 24 h. GC, growth control; NC, negative control.



**Figure S2.** Microcalorimetry of planktonic *S. pyogenes* (ATCC 19615). Numbers represent concentrations (in mg/L) of fosfomycin (a), rifampicin (b), benzylpenicillin (c), daptomycin (d), gentamicin (e) and levofloxacin (f). Circled values represent the MHIC, defined as the lowest antimicrobial concentration inhibiting growth-related heat production after 24 h. GC, growth control; NC, negative control.



**Figure S3.** Microcalorimetry of planktonic *S. oralis* (ATCC 35037). Numbers represent concentrations (in mg/L) of fosfomycin (a), rifampicin (b), benzylpenicillin (c), daptomycin (d), gentamicin (e) and levofloxacin (f). Circled values represent the MHIC, defined as the lowest antimicrobial concentration inhibiting growth-related heat production after 24 h. GC, growth control; NC, negative control.

**Part II: Microcalorimetry of biofilm S. pyogenes (ATCC 19615) and S. oralis (ATCC 35037).**

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**Figure S4.** Microcalorimetry analysis of *S. pyogenes* (ATCC 19615) biofilm treated with different antibiotic concentrations. Each curve shows the heat produced by viable bacteria present in the biofilm after 24h of antibiotic treatment or no treatment. Numbers represent concentrations (in mg/L) of fosfomycin (a), rifampicin (b), benzylpenicillin (c), daptomycin (d), gentamicin (e) and levofloxacin (f). Circled values represent the MBBC, defined as the lowest antimicrobial concentration leading to absence of bacterial regrowth after 48 h. GC, growth control; NC, negative control.

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**Figure S5.** Microcalorimetry analysis of *S. oralis* (ATCC 35037) biofilm treated with different antibiotic concentrations. Each curve shows the heat produced by viable bacteria present in the biofilm after 24h of antibiotic treatment or no treatment. Numbers represent concentrations (in mg/L) of fosfomycin (a), rifampicin (b), benzylpenicillin (c), daptomycin (d), gentamicin (e) and levofloxacin (f). Circled values represent the MBBC, defined as the lowest antimicrobial concentration leading to absence of bacterial regrowth after 48 h. GC, growth control; NC, negative control.

**Part III:** **Microcalorimetry of *S. agalactiae* (ATCC 13813) and *S. pyogenes* (ATCC 19615) biofilms treated with proteinase K and antibiotics.**



**Figure S6.** Evaluation by microcalorimetry of antimicrobial activity of fosfomycin (a), rifampicin (b), benzylpenicillin (c) and levofloxacin (d) on *S. agalactiae* and *S. pyogenes* biofilms pretreated with proteinase K. Numbers represent concentrations (in mg/L). ProtK, proteinase K; GC, growth control; FOF, fosfomycin; RIF, rifampicin; PEN, benzylpenicillin; LEV, levofloxacin. Bacterial growth-related heat was suppressed by sub-inhibitory concentrations of tested antibiotics when combined with treated biofilms with proteinase K at different concentrations (25 mg/L, 50 mg/L and 100 mg/L).

**Microcalorimetry of biofilm *S. oralis* (ATCC 35037) and proteinase K.**

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**Figure S7.** Evaluation of enzymatic activity of proteinase K by microcalorimetry on *S. oralis* (ATCC 35037) biofilm after 12 h incubation. Numbers represent concentrations (in mg/L). ProtK, proteinase K; GC, growth control.

**Part IV:** **Microcalorimetry of combination of antibiotics on *S. oralis* (ATCC 35037).**



**Figure S8.** Evaluation of synergistic activity of benzylpenicillin and gentamicin by microcalorimetry on planktonic (A) and adherent (B) *S. oralis* (ATCC 35037). Numbers represent concentrations (in mg/L). Circled values represent the MHIC/MBEC. GC, growth control; PEN, benzylpenicillin; GEN, gentamicin.



**Figure S9.** Evaluation of synergistic activity of rifampicin and gentamicin by microcalorimetry on planktonic (A) and adherent (B) *S. oralis* (ATCC 35037). Numbers represent concentrations (in mg/L). Circled values represent the MHIC/MBEC. GC, growth control; RIF, rifampicin; GEN, gentamicin.



**Figure S10.** Evaluation of synergistic activity of benzylpenicillin and rifampicin by microcalorimetry on planktonic (A) and adherent (B) *S. oralis* (ATCC 35037). Numbers represent concentrations (in mg/L). Circled values represent the MHIC/MBEC. GC, growth control; PEN, benzylpenicillin; RIF, rifampicin.



**Figure S11.** Evaluation of synergistic activity of rifampicin and fosfomycin by microcalorimetry on planktonic (A) and adherent (B) *S. oralis* (ATCC 35037). Numbers represent concentrations (in mg/L). Circled values represent the MHIC/MBEC. GC, growth control; RIF, rifampicin; FOF, fosfomycin.