Figure S1. Samples for SEM.

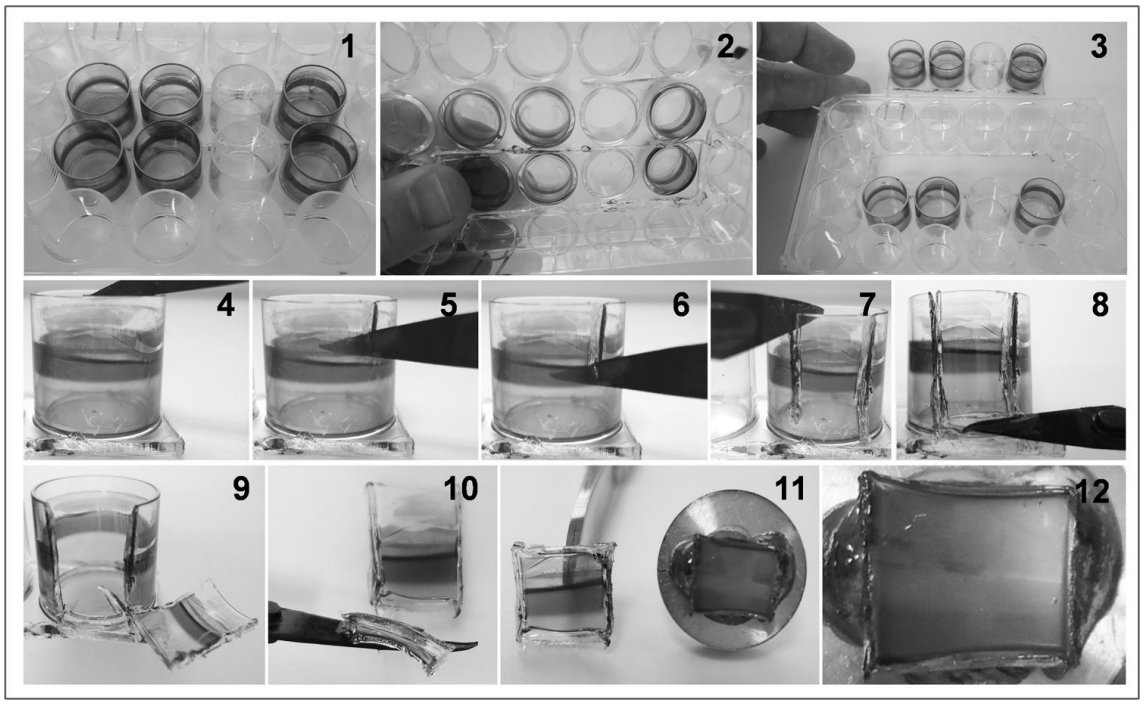

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Figure S1. Description of how biofilm samples were made for SEM. 1-3, Well separation; 4-10, cutting and selecting a fragment with a hot lancet; 10, Before and after coating with gold. 12, Result before SEM analysis.

Fig. S2. CLSM.

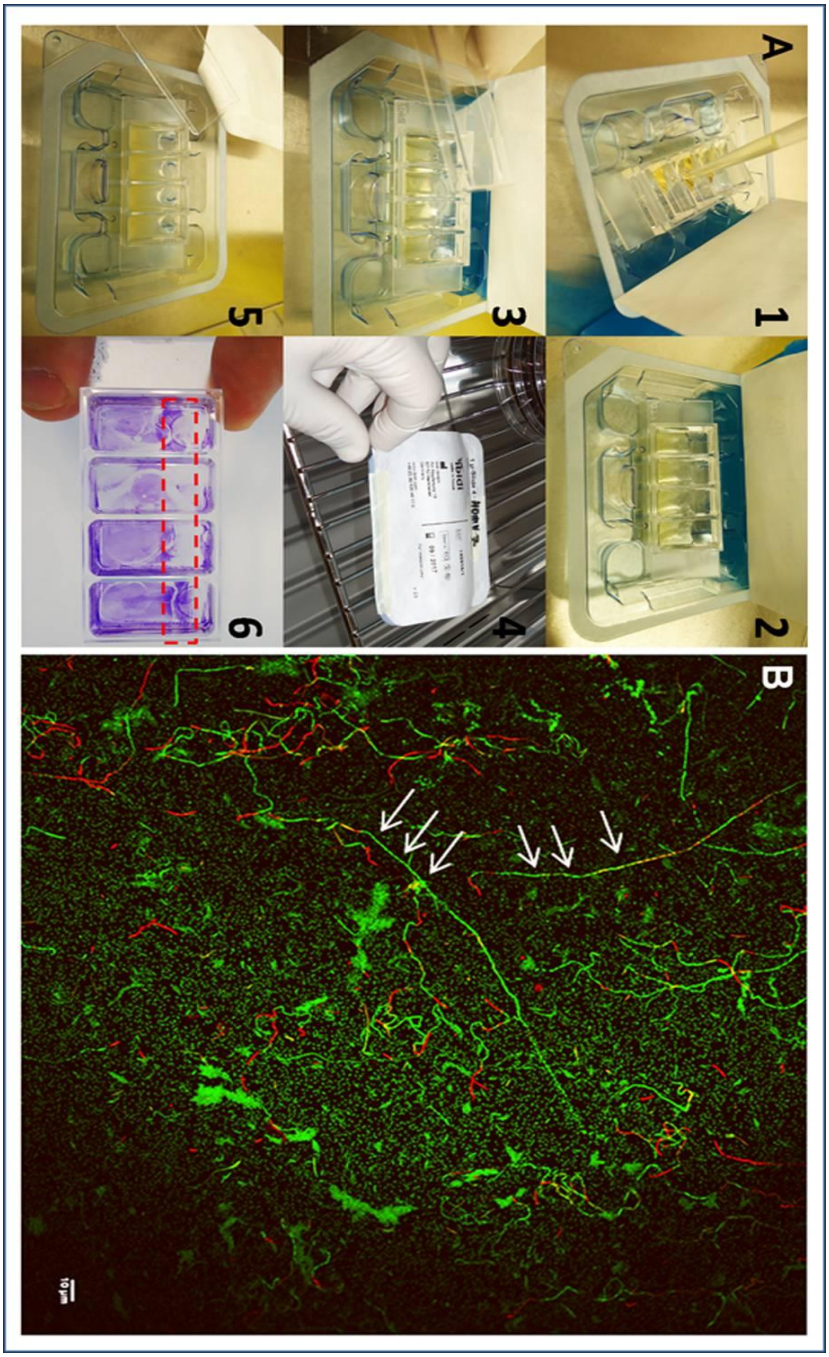

Figure S2. **A**, Description of how biofilm samples were made for confocal imaging. 1-3, Media inoculation; 4, Incubation, slides were placed inclined (~45º) into the incubator to form a liquid-air interface; 5, Washing; 6, Examples of staining with CV (same procedure were used for staining with LIVE/DEAD).
B, Maximum intensity projection CLSM images of unfixed biofilms formed by *S. liquefaciens* (nº1) and stained with LIVE/DEAD. Live bacteria are stained green and dead bacteria are stained red. Arrows indicate some dividing cells along a bacterial chain. Original magnification ×200. Scale bar: 10μm.

Fig. S3. Bioreporter based detection of AHL production using EPCs.

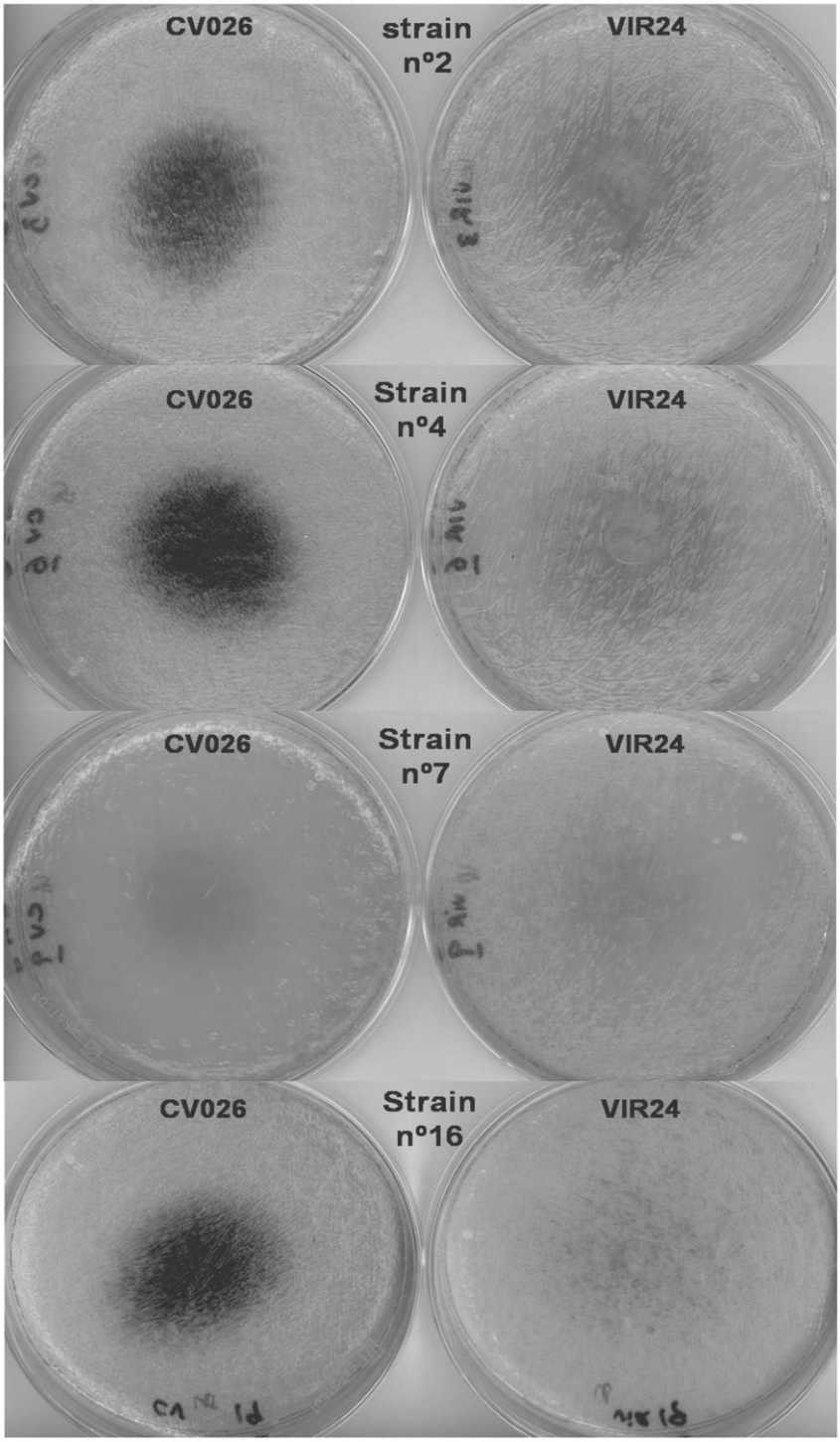

Figure S3. Bioreporter based detection of AHL production by *S. liquefaciens* strains. LB agar plates were seeded with 100 µl of a suspension of *Chromobacterium violaceum* CV026 or VIR24. The presence of AHLs in ECPs produced by *S. liquefaciens* strains cultured at 37ºC was detected after 48 h at 25ºC. Development of violet pigment in the reporter strains revealed the production of violacein.

Fig. S4. Production of AHLs in ECPs at low temperature.

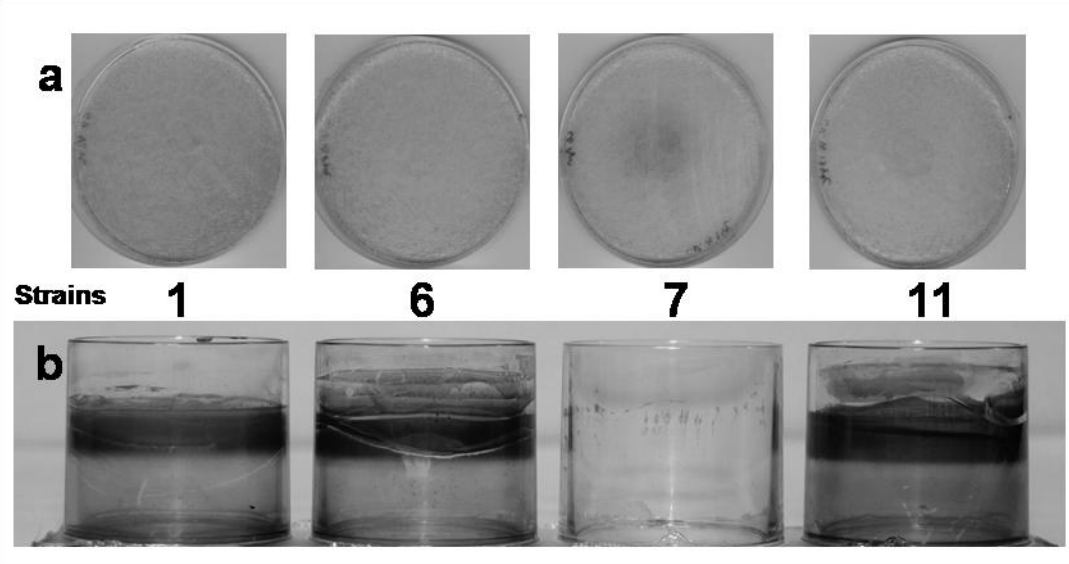

Figure S4. Production of AHLs in ECPs and biofilm formation by *S. liquefaciens* strains at low temperature. **a**: strains tested for induction of violacein in *C. violaceum* CV026 using 100µl of ECPs obtained from static cultures at 4ºC, during five days, in LB medium. Strain nº7 is positive and strains nº1, nº6 and nº11 are negative. **b**: biofilms formed by different strains in 24 well plates at 4ºC, after five days, in LB medium. Biofilms were stained with crystal violet and photographed.

Fig. S5. (HP)LC-MS.

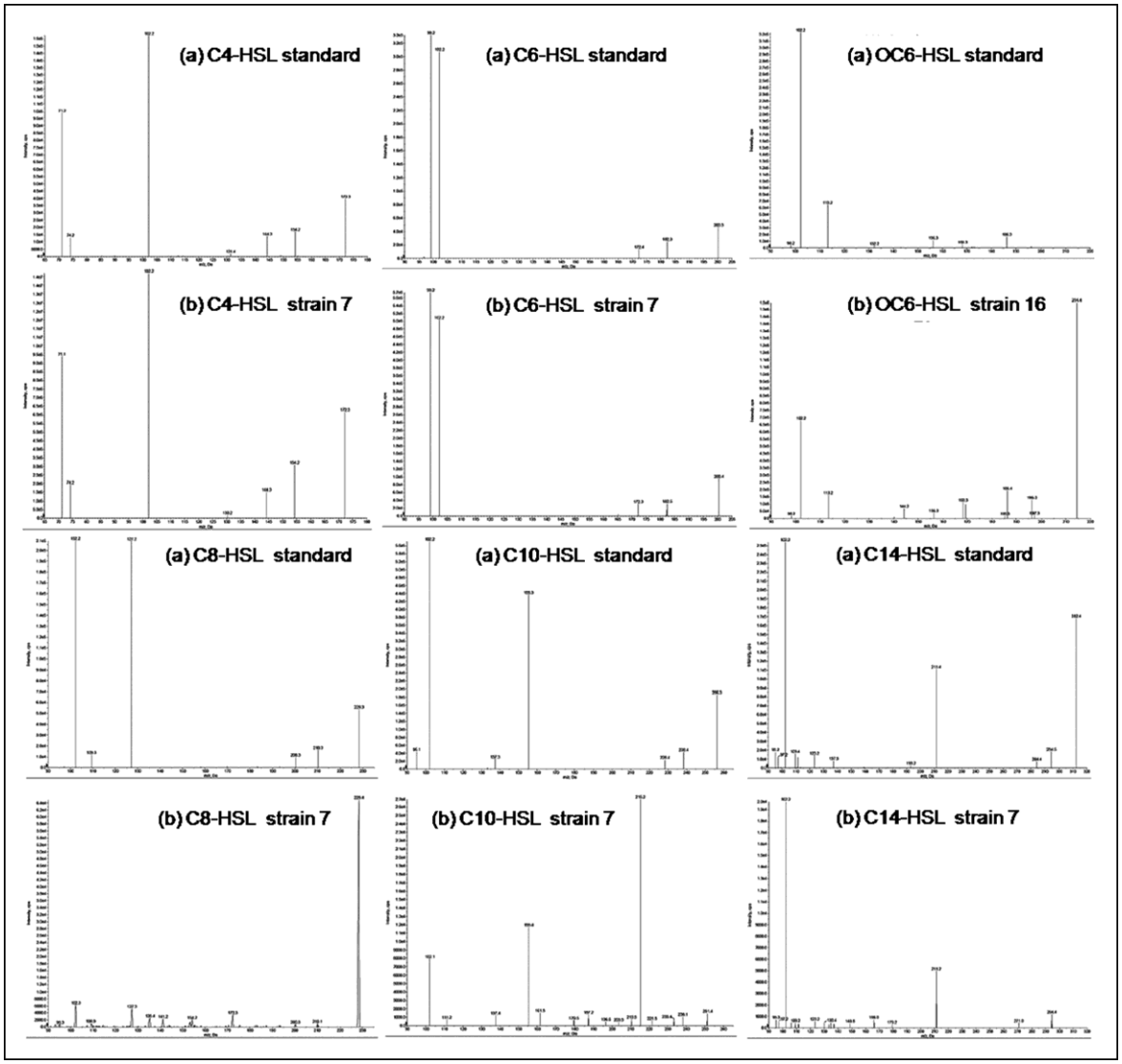

Figure S5. Examples of (HP)LC-MS chromatograms in extracts of cell-free supernatants of *S. liquefaciens* as discussed in the text and Table 2. Production spectrum of synthetic C4-HSL, C6-HSL, C8-HSL, C10-HSL and OC6-HSL, and the corresponding peak in the chromatograms of extracts of spent culture medium of strains nº7 and nº 16.

Fig. S6.

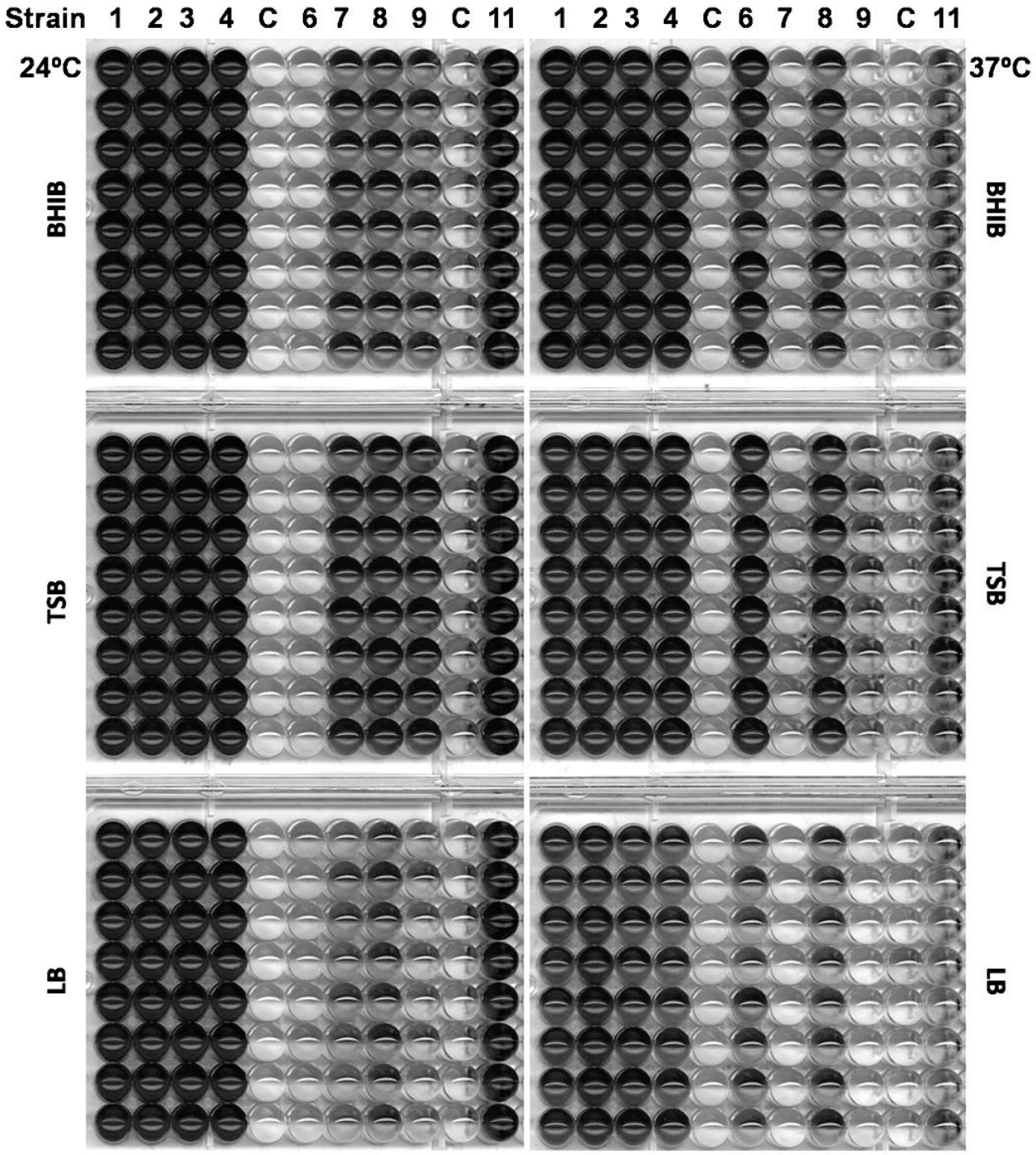

Figure S6. Examples of 96-well microtiter plates after CV solubilisation. Representative clinical isolates of *S. liquefaciens* at the indicated temperatures, 24ºC (left) and 37ºC (right), in three culture media, are shown. Strains are indicated as numbers, as presented in Table 1. **c**, controls (uninoculated wells).

Fig. S7. Planktonic growth by *S. liquefaciens*

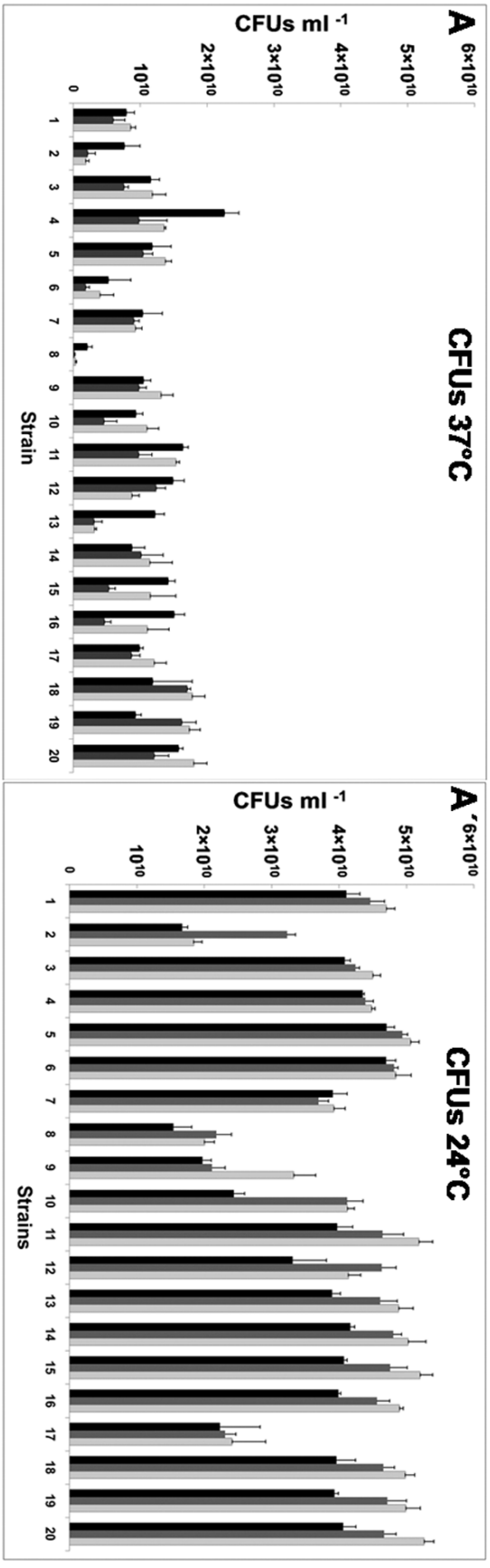

Figure S7 Planktonic growth in *S. liquefaciens* clinical isolates.

Quantification of planktonic growth (CFUs). Values are presented as mean ± standard deviation (SD) of three independent experiments. Each experiment was carried out in duplicate wells. A, 37ºC and B, 24ºC, in LB (black bars), TSB (grey bars), and BHIB (white bars). Strains are indicated as numbers, as presented in Table 1 values.

Figure S8. Mixing experiments using ECPs from AHLs producing strains.

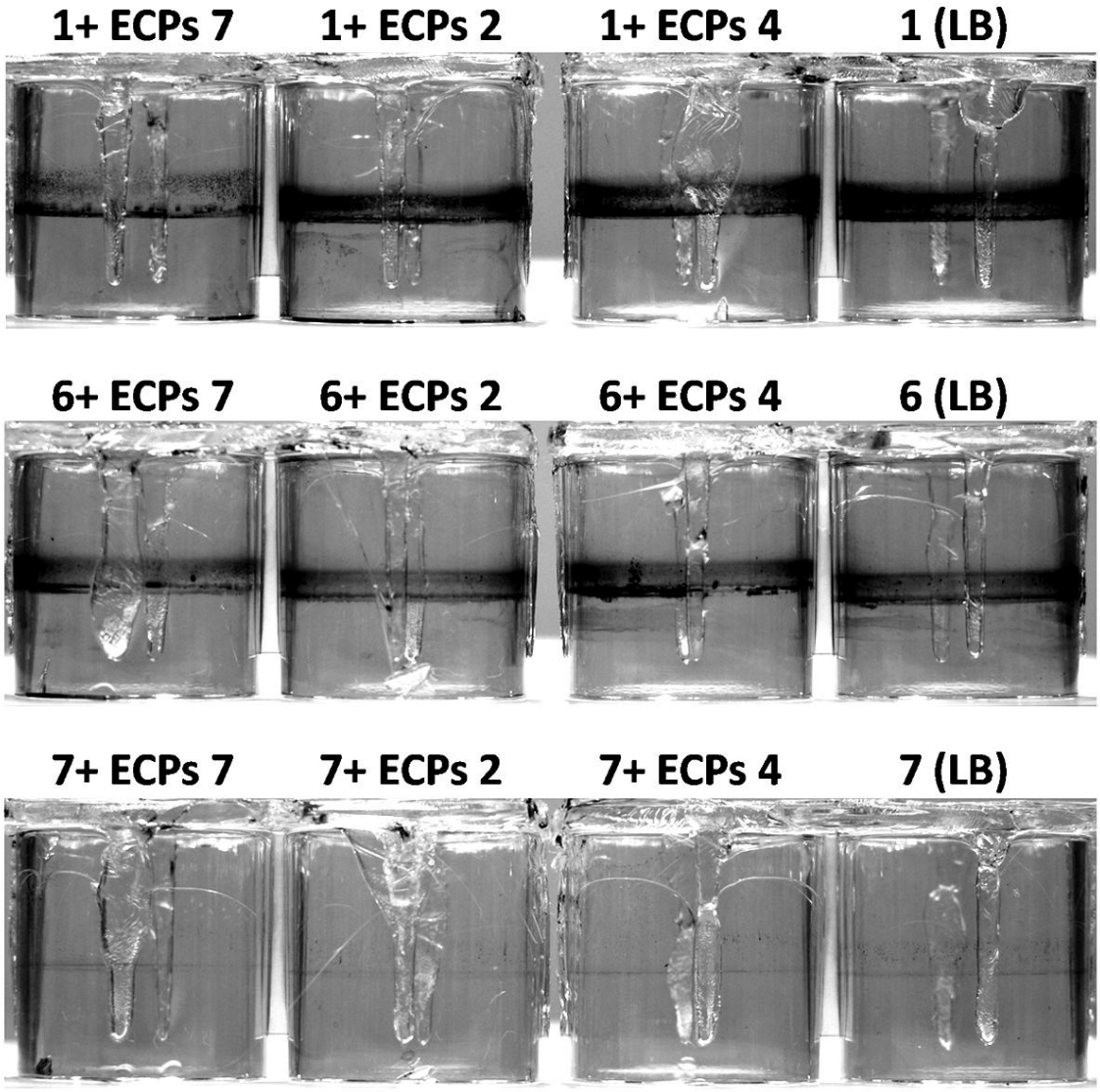

Figure S8. Examples of mixing experiments using spent culture media (ECPs) from AHLs-producing strains. Producing strains: nº2, nº4, and nº7. Non-producing strains nº1, and nº6. Example: 1+ ECPs 7 = Strain 1 + ECPs from strain 7. LB, inoculated control without ECPs.