

1 **Inhibitory role of acyl homoserine lactones in hemolytic activity and**
2 **viability of *Streptococcus pyogenes* M6 S165**

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16 **Running title:** AHLs interfere with GAS virulence

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18 **Keywords:** *Streptococcus pyogenes*, Acyl homoserine lactone, streptolysin,

19 microbiota, quorum sensing

20 **Supplementary Table 1. List of primers used for qPCR**

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Gene	Sequence
<i>sagA</i>	F 5'-GCTACTAGTGTAGCTGAAACAACTCAA-3'
	R 5'-AGCAACAAGTAGTACAGCAGCAA-3'
<i>luxS</i>	F 5'-AAGCTAATCCGCCAACGCAT-3'
	R 5'-TTTGGCGGCAAAGAGGCTAT-3'
<i>luxR</i>	F 5'-GCAGGCGTTAAAGGCACAAA-3'
	R 5'-CTTTGATGAGTCAGGGGCGT-3'
<i>gyrA</i>	F 5'-CGACTT GTCTGAACGCCAAAGT-3'
	R 5'-ATCACGTTCCAAACCAGTCAAAC-3'
<i>slo</i>	F 5'-TTGTTGAGGATAATGTAAGAATGTTTAG-3'
	R 5'-TCCTGGCTTGCAACTGATTG-3'
<i>covS</i>	F 5'-CATCTCCTGGCTTGCATGGT-3'
	R 5'-GGAAAACCCACGATACTGATCTTC-3'

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26 **Supplementary Table 2. List of primers used for construction of *S. pyogenes***

27 **M6 S165 mutant strains**

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Gene	Sequence
<i>slo</i>	F 5'-ATGAAAGGATCCCCGCCACTCTTTGTGAG-3'
	R 5'-AAGTTACTGCAGCGATCACTTTTCGCCAC-3'
<i>luxR</i>	F 5'-ATGAAAGGATCCCCGTTTGGCAATTTGTCCGT-3'
	R 5'-AAGTTACTGCAGCTTTGATGAGTCAGGGGCGT-3'
<i>FtsA</i>	F 5'-ATGAAAGGATCCGGCCGCTAAGCCAATTGATG-3'
	R 5'-AAGTTACTGCAGCTAGGAGGCGTTCTCTTGC-3'
<i>FtsB</i>	F 5'-ATGAAAGGATCCTGCCAGCCGATAACAGCATT-3'
	R 5'-AAGTTACTGCAGGCAAAACGGCCATAAAGCCA-3'
<i>FtsC</i>	F 5'-ATGAAAGGATCCCCACGTCCCCAGTCTTTACC-3'
	R 5'-AAGTTACTGCAG TGCCTCAAATTGCTGGTGTG-3'
<i>FtsD</i>	F 5'-ATGAAAGGATCCCAAACCCTTTGACGTTGCC-3'
	R 5'-AAGTTACTGCAGGCTGAAGACCTCACAATTGCC-3'
<i>luxR-P</i>	F 5'- ATTCCTTAAAATGACGAAGGGTATT-3'
	R 5'- CCTCATATGAGTTAGTTGTTAGAGGAGAA-3'
<i>gyrA-P</i>	F 5'- CCTGGATCCGCAATATTTTGTGTTACC-3'
	R 5'- CCTTCGTCATTTTAAGGAATGTCCTTTTC-3'

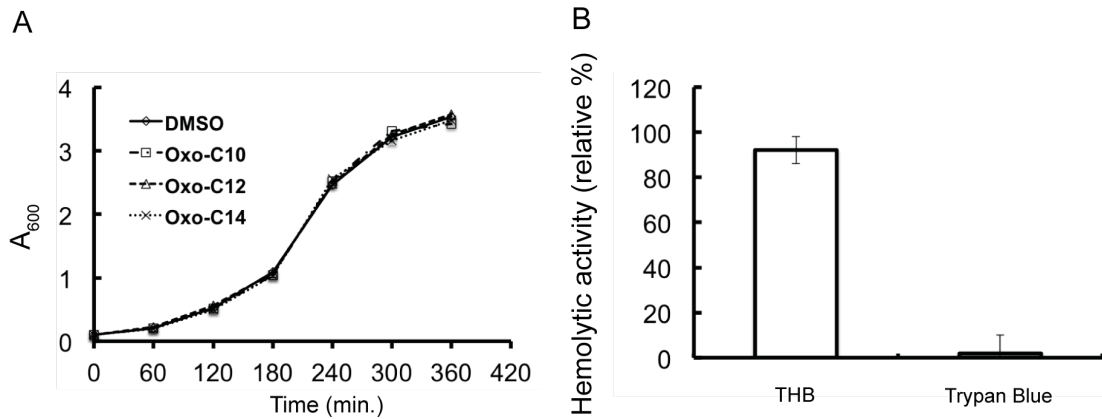
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31 **Supplementary Figure S1. Concentration of oxo-C12-HSL that inhibits**
32 **hemolytic activity, and an assay to determine the type of hemolysin**
33 **produced**

34 (A) Growth curve of *S. pyogenes* M6 S165 in the presence of different oxo-AHLs
35 (20 μ M) (B) *S. pyogenes* M6 S165 growth supernatant was incubated with trypan
36 blue and subjected to a hemolytic assay. The hemolytic activity was suppressed
37 in the presence of trypan blue, indicating that SLS is the major hemolysin
38 released into the growth media.

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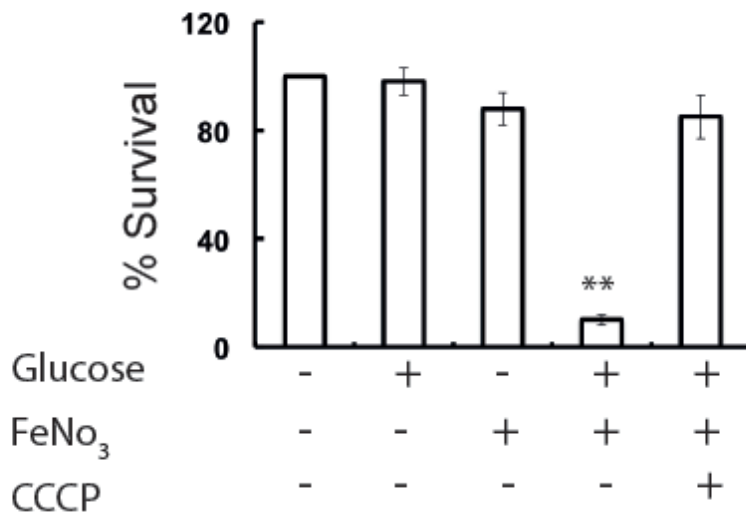
43 **Supplementary Figure S2. Bactericidal effect of oxo-C12-HSL on *S. pyogenes***

44 **M1**

45 Effect of glucose and iron on the *S. pyogenes* M1 growth inhibition by oxo-C12-

46 HSL was examined.

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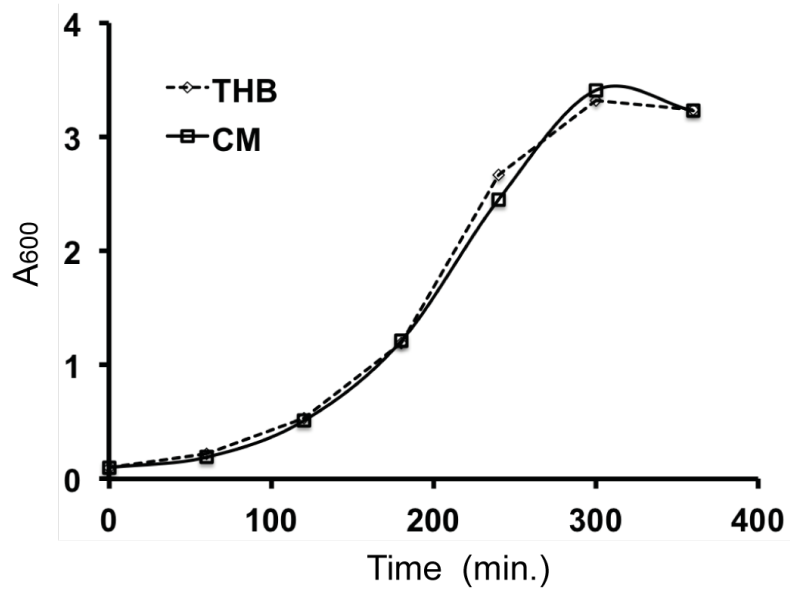
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51 **Supplementary Figure S3. Growth Curve of *S. pyogenes* M6 S165 in CM**
52 **prepared from *P. aeruginosa*.** The growth of *S. pyogenes* M6 S165 remains
53 unaffected in the CM.

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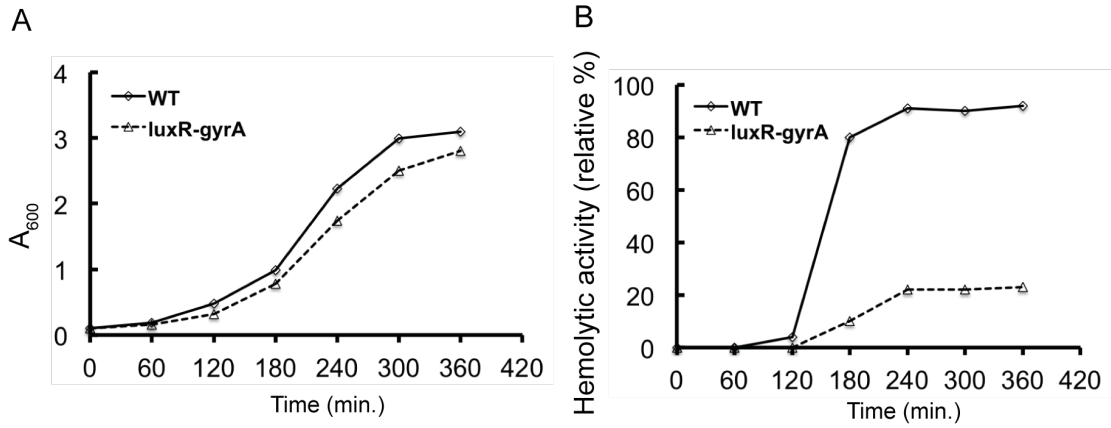
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57 **Supplementary Figure S4. Growth curve and hemolytic activity of *S.***
58 ***pyogenes* M6 S165 harboring luxR under gyrA promoter.**

59 (A) Growth curve (B) Hemolytic activity during different stages of growth

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