

Supplementary material

Analysis of the Metabolic Response of Planktonic Cells and Biofilms of *Klebsiella pneumoniae* to Sublethal Disinfection with Sodium Hypochlorite Measured by NMR

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Table S1. Growth of *K. pneumoniae* measured by OD after the exposure to NaOCl and reactivation of the metabolism by adding minimal media

| Time (h) | OD ₆₀₀ | | | | | |
|----------|---------------------|---------------------|--------------------------------------|------------------------------------|--------------------------------------|------------------------------------|
| | Control [10 min] | Control [30 min] | [3.5 mgL ⁻¹] [10 min] | [7 mgL ⁻¹] [10 min] | [3.5 mgL ⁻¹] [30 min] | [7 mgL ⁻¹] [30 min] |
| 0 | 2.49 | 2.49 | 2.49 | 2.49 | 2.49 | 2.49 |
| 1 | 2.82 | 2.82 | 2.79 | 2.49 | 2.43 | 2.49 |
| 2 | 3.48 | 3.3 | 3.54 | 2.64 | 3.36 | 3.15 |
| 3 | 3.12 | 3.03 | 3.21 | 3.18 | 3.18 | 2.76 |
| 4 | 3 | 2.94 | 3 | 2.82 | 2.7 | 2.94 |

Table S2. Intake of glucose and production of different metabolites in the minimal mineral medium after recovery from stress with sodium hypochlorite.

| | Metabolite concentration (mM) | | | | | |
|----------------------|-------------------------------|---------|---------|-----------|---------|---------|
| | Ethanol | Lactate | Acetate | Succinate | Formate | Glucose |
| Control[10 min] | 0.22 | 0 | 0.11 | 0 | 0 | 0.98 |
| Control[30 min] | 4.23 | 3.66 | 2.89 | 0.79 | 4.43 | 0.49 |
| [7 ppm][10 min][1 h] | 1.72 | 0.63 | 1.21 | 0.27 | 1.39 | 0.70 |
| [7 ppm][30 min][2 h] | 8.94 | 4.53 | 7.11 | 1.54 | 11.06 | 0.29 |
| [7 ppm][10 min][0 h] | 0.26 | 0 | 0.10 | 0 | 0 | 1.19 |
| Control[10 min][2h] | 6.22 | 4.61 | 4.61 | 1.06 | 5.36 | 0 |
| [7 ppm][30 min][1 h] | 2.32 | 1.06 | 1.71 | 0.42 | 2.52 | 0.72 |
| Control[10 min][1 h] | 3.73 | 2.91 | 2.30 | 0.66 | 3.68 | 0.47 |
| [7 ppm][30 min][0 h] | 0.12 | 0 | 0.14 | 0 | 0 | 1.45 |
| Control[30 min][2 h] | 6.96 | 6.11 | 5.89 | 1.26 | 7.99 | 0 |
| [7 ppm][10 min][2 h] | 4.71 | 1.83 | 3.67 | 0.92 | 5.64 | 0.44 |
| Contro[30 min][0 h] | 0.11 | 0.16 | 0.13 | 0 | 0 | 0.88 |

Table S3. Total and free chlorine concentrations during stress.

| Type | Time (min) | Concentration (mgL ⁻¹) |
|-------------------|---------------|---------------------------------------|
| Total chlorine | 0 | 8.6 |
| Total chlorine | 0 | 7.8 |
| Total chlorine | 0 | 6.7 |
| Total chlorine | 10 | 5.7 |
| Total chlorine | 10 | 5.8 |
| Total chlorine | 10 | 5.1 |
| Total chlorine | 15 | 2.9 |
| Total chlorine | 15 | 2.2 |
| Total chlorine | 15 | 1.7 |
| Free chlorine | 0 | 0.7 |
| Free chlorine | 0 | 2.55 |
| Free chlorine | 0 | 0.5 |
| Free chlorine | 10 | 0.25 |
| Free chlorine | 10 | 0.1 |
| Free chlorine | 10 | 0.55 |
| Free chlorine | 15 | 0.02 |
| Free chlorine | 15 | 0.02 |
| Free chlorine | 15 | 0.02 |
| combined chlorine | 0 | 7.9 |
| combined chlorine | 0 | 5.25 |
| combined chlorine | 0 | 6.2 |
| combined chlorine | 10 | 5.45 |
| combined chlorine | 10 | 5.7 |
| combined chlorine | 10 | 4.55 |
| combined chlorine | 15 | 2.9 |
| combined chlorine | 15 | 2.2 |
| combined chlorine | 15 | 1.7 |

Table S4. pH during stress

| Type | Time | Concentration |
|------|------|---------------|
| pH | 0 | 6.6 |
| pH | 0 | 6.2 |
| pH | 10 | 6.4 |
| pH | 10 | 6.0 |
| pH | 30 | 6.3 |
| pH | 30 | 6.7 |

Table S5. Effect of the sublethal concentration of sodium hypochlorite on the metabolic profile.

| Cell type | Group | Ethanol | Lactate | Acetate | Succinate | Formate | Glucose |
|----------------------|---------|---------|---------|---------|-----------|---------|---------|
| Biofilm_control | Control | 1.66 | 0.68 | 1.40 | 0.01 | 1.05 | 1.09 |
| Biofilm_control | Control | 0.43 | 0.64 | 1.39 | 0.00 | 0.96 | 1.08 |
| Biofilm_control | Control | 1.15 | 1.25 | 2.29 | 0.06 | 2.14 | 1.19 |
| Biofilm_control | Control | 0.70 | 2.19 | 1.81 | 0.03 | 1.77 | 1.02 |
| Biofilm_control | Control | 0.86 | 2.30 | 1.88 | 0.04 | 1.98 | 1.10 |
| Biofilm_control | Control | 2.60 | 1.19 | 2.02 | 0.06 | 1.80 | 1.21 |
| Biofilm_treatment | Treat | 1.82 | 1.09 | 2.05 | 0.04 | 1.89 | 1.06 |
| Biofilm_treatment | Treat | 4.04 | 1.35 | 1.72 | 0.00 | 1.58 | 1.40 |
| Biofilm_treatment | Treat | 1.74 | 1.55 | 2.47 | 0.05 | 2.48 | 1.07 |
| Biofilm_treatment | Treat | 0.98 | 1.27 | 1.62 | 0.02 | 1.56 | 1.18 |
| Biofilm_treatment | Treat | 2.16 | 1.21 | 2.00 | 0.04 | 1.78 | 1.39 |
| Planktonic_treatment | Treat | 2.77 | 0.74 | 2.96 | 0.68 | 3.90 | 1.00 |
| Planktonic_treatment | Treat | 3.60 | 0.64 | 3.71 | 0.85 | 4.80 | 0.92 |
| Planktonic_treatment | Treat | 2.50 | 0.59 | 2.63 | 0.61 | 3.53 | 0.92 |
| Planktonic_treatment | Treat | 3.01 | 0.46 | 3.08 | 0.68 | 4.24 | 1.03 |
| Planktonic_treatment | Treat | 3.59 | 0.69 | 3.88 | 0.93 | 5.33 | 0.97 |
| Planktonic_treatment | Treat | 3.11 | 0.58 | 3.56 | 0.87 | 5.11 | 0.84 |
| Planktonic_Control | Control | 3.99 | 1.57 | 4.00 | 0.91 | 5.79 | 0.85 |
| Planktonic_Control | Control | 3.89 | 1.38 | 3.77 | 0.83 | 5.10 | 0.87 |
| Planktonic_Control | Control | 3.53 | 1.31 | 3.49 | 0.81 | 4.96 | 0.82 |
| Planktonic_Control | Control | 4.04 | 1.52 | 4.25 | 0.97 | 5.86 | 0.72 |
| Planktonic_Control | Control | 4.14 | 1.56 | 4.01 | 0.93 | 5.77 | 0.83 |
| Planktonic_Control | Control | 4.06 | 1.57 | 3.98 | 0.92 | 5.68 | 0.84 |

Table S6. Shapiro-Wilk normality test.

| Planktonic cells | | | | |
|------------------|---------|---------|-----------|---------|
| | Control | | Treatment | |
| Metabolite | W | p-value | W | p-value |
| Ethanol | 0.89137 | 0.1104 | 0.92336 | 0.5299 |
| Lactate | 0.80267 | 0.06211 | 0.96821 | 0.8802 |
| Acetate | 0.93012 | 0.5811 | 0.94313 | 0.6845 |
| Succinate | 0.93373 | 0.3964 | 0.89304 | 0.3344 |
| Formate | 0.79778 | 0.05612 | 0.94826 | 0.7262 |
| Glucose | 0.82885 | 0.1051 | 0.96081 | 0.826 |
| Biofilm cells | | | | |
| | Control | | Treatment | |
| Metabolite | W | p-value | W | p-value |
| Ethanol | 0.91529 | 0.4721 | 0.87488 | 0.2868 |
| Lactate | 0.8635 | 0.2015 | 0.9752 | 0.9074 |
| Acetate | 0.92652 | 0.5532 | 0.94226 | 0.6819 |
| Succinate | 0.93164 | 0.5928 | 0.93013 | 0.5972 |
| Formate | 0.85848 | 0.184 | 0.84372 | 0.1755 |
| Glucose | 0.93358 | 0.608 | 0.82118 | 0.1192 |

Table S7. Levene's test for homogeneity of variances.

| Planktonic cells | | | | |
|------------------|---------|--------|----------|---------|
| Metabolite | F | Num df | Denom df | p-value |
| Ethanol | 0.24319 | 5 | 5 | 0.1469 |
| Lactate | 1.3 | 5 | 5 | 0.7804 |
| Acetate | 0.28226 | 5 | 5 | 0.1913 |
| Succinate | 0.22645 | 5 | 5 | 0.1288 |
| Formate | 0.30412 | 5 | 5 | 0.2174 |
| Glucose | 0.64594 | 5 | 5 | 0.6432 |
| Biofilm cells | | | | |
| Metabolite | F | Num df | Denom df | p-value |
| Ethanol | 0.47984 | 5 | 4 | 0.4411 |
| Lactate | 17.157 | 5 | 4 | 0.0166 |
| Acetate | 1.1294 | 5 | 4 | 0.9322 |
| Succinate | 1.5751 | 5 | 4 | 0.6805 |
| Formate | 1.7276 | 5 | 4 | 0.6164 |
| Glucose | 0.1927 | 5 | 4 | 0.1001 |

Table S8. T-test of means differences.

| Planktonic cells | | | |
|------------------|----------|----|-----------|
| Metabolite | t | df | p-value |
| Ethanol | 4.2075 | 10 | 0.001807 |
| Lactate | 14.216 | 10 | 5.849E-08 |
| Acetate | 2.7095 | 10 | 0.02195 |
| Succinate | 2.1824 | 10 | 0.05403 |
| Formate | 3.1467 | 10 | 0.01039 |
| Glucose | -3.5128 | 10 | 0.005605 |
| Biofilm cells | | | |
| Metabolite | t | df | p-value |
| Ethanol | -1.5706 | 9 | 0.1507 |
| Lactate | 0.24471 | 9 | 0.8122 |
| Acetate | -0.82539 | 9 | 0.4305 |
| Succinate | 0.17895 | 9 | 0.8619 |
| Formate | -0.8905 | 9 | 0.3964 |
| Glucose | -0.13892 | 9 | 0.1982 |