

Effect of manuka honey on biofilm-associated genes expression during methicillin-resistant *Staphylococcus aureus* biofilm formation

Barbara Kot*, Hubert Sytykiewicz, Iwona Sprawka, Małgorzata Witeska

Supplementary materials

Table S1 Three-factorial ANOVA analysis of the examined indicators (strains, treatment, growth period) and interactions on relative expression of the tested genes (*cna*, *ebps*, *eno*, *fib*) of MRSA strains

Indicators	<i>cna</i>	<i>ebps</i>	<i>eno</i>	<i>fib</i>
Strains (S)	$F_{1, 48} = 615$ (***)	$F_{1, 48} = 3,862$ (***)	$F_{1, 48} = 1,320$ (***)	$F_{1, 48} = 2,812$ (***)
Treatment (T)	$F_{2, 48} = 2,040$ (***)	$F_{2, 48} = 1,250$ (***)	$F_{2, 48} = 4,505$ (***)	$F_{2, 48} = 1,565$ (***)
Growth period (G)	$F_{3, 48} = 1,358$ (***)	$F_{3, 48} = 812$ (***)	$F_{3, 48} = 618$ (***)	$F_{3, 48} = 952$ (***)
S × T	$F_{2, 48} = 835$ (***)	$F_{2, 48} = 486$ (***)	$F_{2, 48} = 435$ (**)	$F_{2, 48} = 240$ (**)
T × G	$F_{6, 48} = 425$ (**)	$F_{6, 48} = 364$ (***)	$F_{6, 48} = 206$ (**)	$F_{6, 48} = 137$ (*)
S × G	$F_{3, 48} = 115$ (**)	$F_{3, 48} = 208$ (**)	$F_{3, 48} = 140$ (**)	$F_{3, 48} = 69$ (*)
S × T × G	$F_{6, 48} = 62$ (*)	$F_{6, 48} = 90$ (*)	$F_{6, 48} = 25$ (*)	$F_{6, 48} = 44$ (*)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Tested variables: i) strains – weak and strong biofilm formation; ii) treatment: control, ¼ MIC and ½ MIC; iii) growth period – 4, 8, 12 and 24 h.

Table S2 Three-factorial ANOVA analysis of the examined indicators (strains, treatment, growth period) and interactions on relative expression of the tested genes (*icaA*, *icaD*, *map/eap*) of MRSA strains

Indicators	<i>icaA</i>	<i>icaD</i>	<i>map/eap</i>
Strains (S)	$F_{1, 48} = 1,450$ (***)	$F_{1, 48} = 257$ (**)	$F_{1, 48} = 4,854$ (***)
Treatment (T)	$F_{2, 48} = 1,220$ (***)	$F_{2, 48} = 162$ (**)	$F_{2, 48} = 2,690$ (***)
Growth period (G)	$F_{3, 48} = 955$ (***)	$F_{3, 48} = 98$ (*)	$F_{3, 48} = 2,215$ (***)
S × T	$F_{2, 48} = 664$ (***)	$F_{2, 48} = 50$ (*)	$F_{2, 48} = 1,107$ (***)
T × G	$F_{6, 48} = 273$ (**)	$F_{6, 48} = 26$ (*)	$F_{6, 48} = 814$ (***)
S × G	$F_{3, 48} = 82$ (*)	$F_{3, 48} = 12$ (*)	$F_{3, 48} = 658$ (***)
S × T × G	$F_{6, 48} = 29$ (*)	$F_{6, 48} = 0.5$ (ns)	$F_{6, 48} = 116$ (**)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns – non-significant. Tested variables: i) strains – weak and strong biofilm formation; ii) treatment: control, ¼ MIC and ½ MIC; iii) growth period – 4, 8, 12 and 24 h.

Table S3 Three-factorial ANOVA analysis of the studied variables (strains, treatment, growth period) and interactions on the bacteria viability in biofilm

Indicators	Bacteria viability
Strains (S)	$F_{1, 48} = 390$ (***)
Treatment (T)	$F_{2, 48} = 255$ (***)
Growth period (G)	$F_{3, 48} = 127$ (**)
S × T	$F_{2, 48} = 104$ (**)
T × G	$F_{6, 48} = 38$ (*)
S × G	$F_{3, 48} = 20$ (*)
S × T × G	$F_{6, 48} = 14$ (*)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Tested variables: i) strains – weak and strong biofilm formation; ii) treatment – control, $\frac{1}{4}$ MIC and $\frac{1}{2}$ MIC; iii) growth period – 4, 8, 12 and 24 h.