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## FTIR metabolomic fingerprint reveals different modes of action exerted by active pharmaceutical ingredient based Ionic Liquids (API-ILs) on *Salmonella* Typhimurium

P. Mester,<sup>a</sup> A. K. Jehle,<sup>a</sup> C. Leeb,<sup>a</sup> R. Kalb,<sup>b</sup> T. Grunert<sup>c,†</sup> and P. Rossmanith<sup>a,†</sup>

### Supplement

**Table S1** MICs of all 36 ILs against eight different *Salmonella* serovars.

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<sup>a</sup> Christian Doppler Laboratory for Monitoring of Microbial Contaminants, Department of Veterinary Public Health and Food Science, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna (Austria).

<sup>b</sup> Proionic GmbH Parkring 18, 8074 Grambach (Austria).

<sup>c</sup> Functional Microbiology, Institute of Microbiology, Department of Pathobiology, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna (Austria).

† These authors have contributed equally to this work.

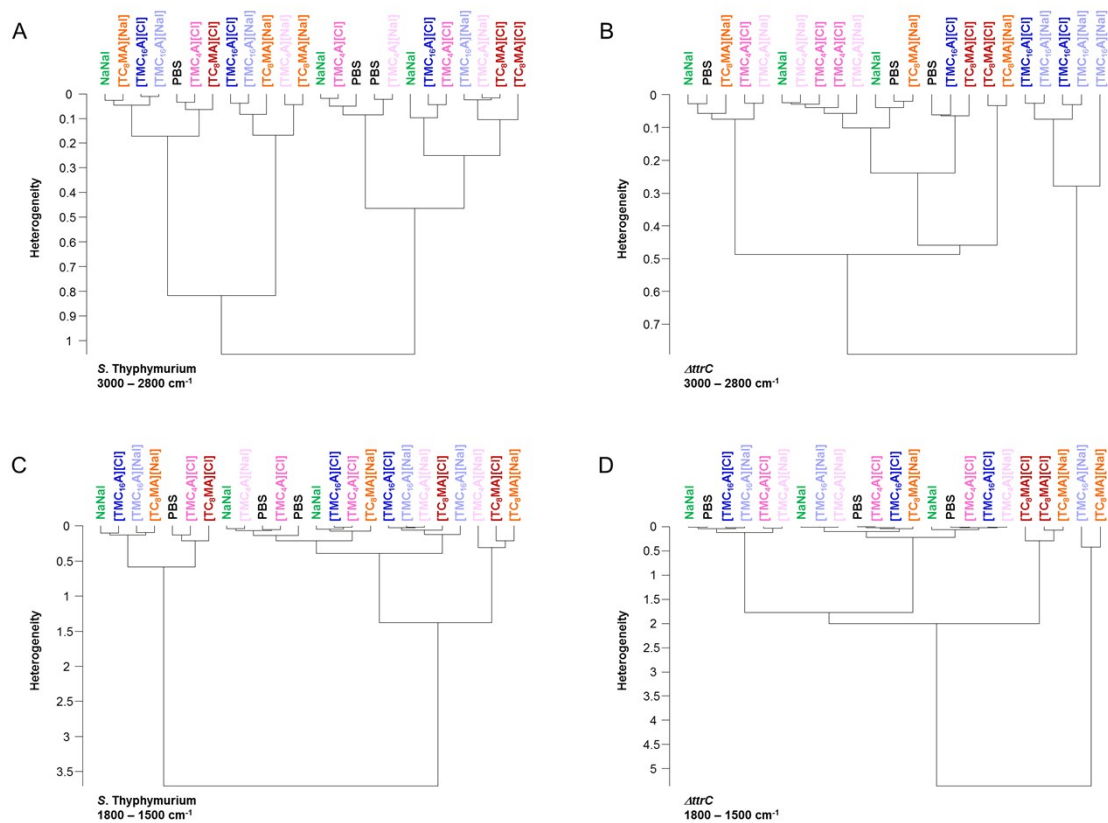
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

MIC ( $\mu\text{M}$ ) 24h									
S. Typhimurium			$\Delta\text{invA}$		$\Delta\text{trc}$		S. Bongori		
IL cation	IL anion								
	chloride	nalidixate	chloride	nalidixate	chloride	nalidixate	chloride	nalidixate	nalidixate
[C <sub>1</sub> mim] <sup>+</sup>	> 1000	79.3 $\pm$ 27.5	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	23.8 $\pm$ 0.0
[C <sub>2</sub> mim] <sup>+</sup>	> 1000	91.3 $\pm$ 0.0	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	60.8 $\pm$ 26.3
[C <sub>3</sub> C <sub>4</sub> mim] <sup>+</sup>	> 1000	38.4 $\pm$ 9.5	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	21.9 $\pm$ 0.0
[C <sub>4</sub> mim] <sup>+</sup>	> 1000	98.4 $\pm$ 64.4	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	35.1 $\pm$ 12.2
[C <sub>5</sub> mim] <sup>+</sup>	> 1000	94.8 $\pm$ 62.1	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	20.3 $\pm$ 0.0
[C <sub>6</sub> mim] <sup>+</sup>	> 1000	161.8 $\pm$ 124.3	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	48.1 $\pm$ 35.6
[TMC <sub>1</sub> A] <sup>+</sup>	> 1000	50.5 $\pm$ 32.5	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	23.2 $\pm$ 4.8
[TMC <sub>2</sub> A] <sup>+</sup>	> 1000	87.7 $\pm$ 20.1	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	92.7 $\pm$ 61.1
[TMC <sub>3</sub> A] <sup>+</sup>	> 1000	50.0 $\pm$ 26.7	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	21.8 $\pm$ 3.4
[TMC <sub>4</sub> A] <sup>+</sup>	> 1000	72.4 $\pm$ 62.7	> 1000	579.2	> 1000	579.2	> 1000	> 1000	30.2 $\pm$ 10.5
[TMC <sub>5</sub> A] <sup>+</sup>	> 1000	74.4 $\pm$ 67.0	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	68.0 $\pm$ 88.3
[TMC <sub>10</sub> A] <sup>+</sup>	109.9 $\pm$ 14.1	14.7 $\pm$ 3.9	140.4 $\pm$ 70.1	87.1 $\pm$ 43.5	94.6 $\pm$ 25.2	87.1 $\pm$ 43.5	42.7 $\pm$ 10.6	13.3 $\pm$ 3.3	13.3 $\pm$ 3.3
[TC <sub>1</sub> MA] <sup>+</sup>	> 1000	40.0 $\pm$ 30.7	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	15.8 $\pm$ 3.9
[TC <sub>2</sub> MA] <sup>+</sup>	38.7 $\pm$ 0.0	34.7 $\pm$ 15.0	51.6 $\pm$ 22.3	52.1 $\pm$ 0.0	38.7 $\pm$ 0.0	52.1 $\pm$ 0.0	38.7 $\pm$ 0.0	26.0 $\pm$ 0.0	26.0 $\pm$ 0.0
[Emmor] <sup>+</sup>	> 1000	104.5 $\pm$ 61.5	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	30.6 $\pm$ 11.3
[Bmpyr] <sup>+</sup>	> 1000	50.7 $\pm$ 33.2	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	43.5 $\pm$ 37.6
[Bmpyr] <sup>+</sup>	> 1000	177.5 $\pm$ 143.8	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	34.1 $\pm$ 11.8
[TC <sub>1</sub> MP] <sup>+</sup>	> 1000	87.1 $\pm$ 34.8	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	30.5 $\pm$ 8.7
[TC <sub>2</sub> MP] <sup>+</sup>	37.1 $\pm$ 0.0	22.2 $\pm$ 5.5	37.1 $\pm$ 0.0	22.2 $\pm$ 5.5	37.1 $\pm$ 0.0	22.2 $\pm$ 5.5	37.1 $\pm$ 0.0	25.4 $\pm$ 0.0	25.4 $\pm$ 0.0
Na <sup>+</sup>	n. t.	38.9 $\pm$ 19.9	n. t.	> 1000	n. t.	> 1000	n. t.	28.1 $\pm$ 9.7	28.1 $\pm$ 9.7

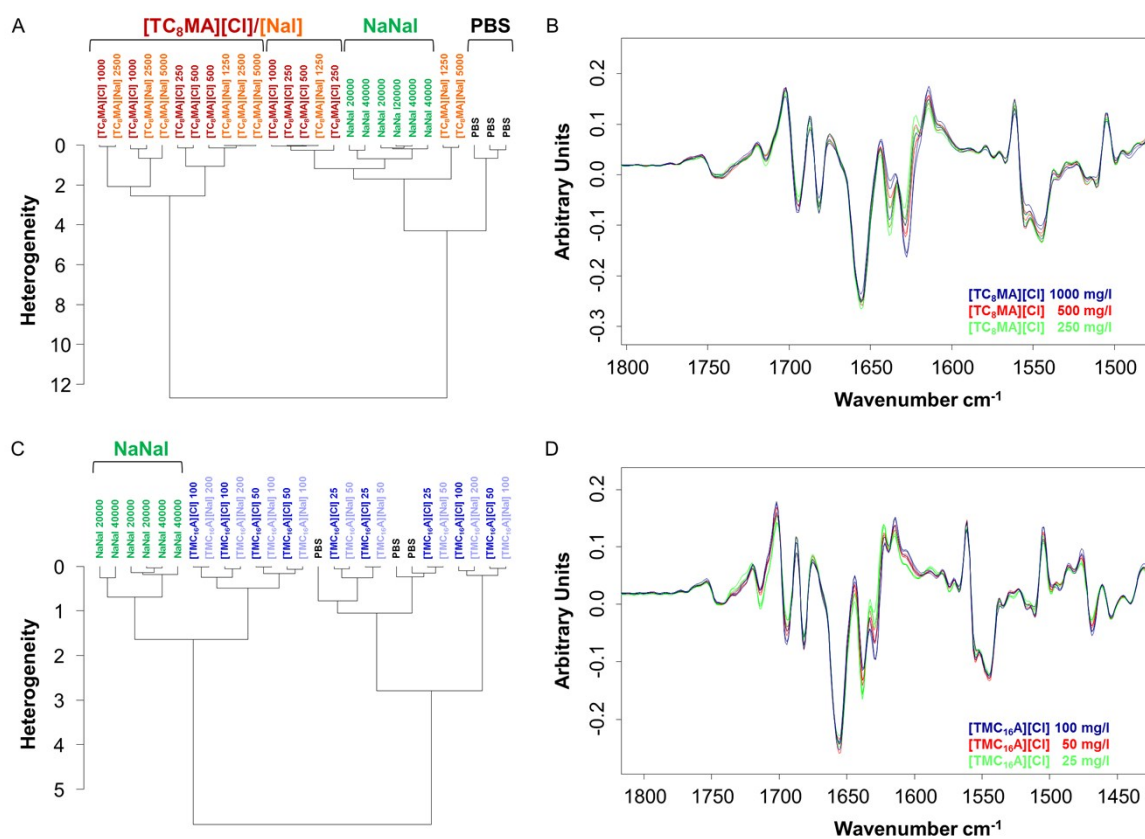
  

MIC ( $\mu\text{M}$ ) 24h									
S. Arizonae		S. Indica		S. Houtenae		S. Salamae			
IL cation	IL anion								
	chloride	nalidixate	chloride	nalidixate	chloride	nalidixate	chloride	nalidixate	nalidixate
[C <sub>1</sub> mim] <sup>+</sup>	> 1000	23.8 $\pm$ 0.0	> 1000	63.4 $\pm$ 27.5	> 1000	47.6 $\pm$ 0.0	> 1000	31.7 $\pm$ 13.7	31.7 $\pm$ 13.7
[C <sub>2</sub> mim] <sup>+</sup>	> 1000	45.6 $\pm$ 0.0	> 1000	121.7 $\pm$ 52.7	> 1000	45.6 $\pm$ 0.0	> 1000	30.4 $\pm$ 13.2	30.4 $\pm$ 13.2
[C <sub>3</sub> C <sub>4</sub> mim] <sup>+</sup>	> 1000	21.9 $\pm$ 0.0	> 1000	96.8 $\pm$ 74.4	> 1000	67.6 $\pm$ 34.8	> 1000	21.9 $\pm$ 0.0	21.9 $\pm$ 0.0
[C <sub>4</sub> mim] <sup>+</sup>	> 1000	35.1 $\pm$ 12.2	> 1000	112.5 $\pm$ 48.7	> 1000	98.4 $\pm$ 64.4	> 1000	42.2 $\pm$ 36.5	42.2 $\pm$ 36.5
[C <sub>5</sub> mim] <sup>+</sup>	> 1000	20.3 $\pm$ 0.0	> 1000	81.3 $\pm$ 0.0	> 1000	54.2 $\pm$ 23.5	> 1000	33.9 $\pm$ 11.7	33.9 $\pm$ 11.7
[C <sub>6</sub> mim] <sup>+</sup>	> 1000	56.5 $\pm$ 29.1	> 1000	225.9 $\pm$ 116.3	> 1000	88.5 $\pm$ 52.1	> 1000	44.3 $\pm$ 26.0	44.3 $\pm$ 26.0
[TMC <sub>1</sub> A] <sup>+</sup>	> 1000	26.4 $\pm$ 17.4	> 1000	59.2 $\pm$ 30.2	> 1000	42.4 $\pm$ 17.6	> 1000	26.4 $\pm$ 17.4	26.4 $\pm$ 17.4
[TMC <sub>2</sub> A] <sup>+</sup>	> 1000	85.7 $\pm$ 68.0	> 1000	140.5 $\pm$ 46.3	> 1000	74.5 $\pm$ 30.9	> 1000	48.5 $\pm$ 31.2	48.5 $\pm$ 31.2
[TMC <sub>3</sub> A] <sup>+</sup>	> 1000	31.5 $\pm$ 10.3	> 1000	62.9 $\pm$ 20.5	> 1000	62.9 $\pm$ 20.5	> 1000	83.5 $\pm$ 101.0	83.5 $\pm$ 101.0
[TMC <sub>4</sub> A] <sup>+</sup>	> 1000	24.1 $\pm$ 10.5	> 1000	72.4 $\pm$ 0.0	> 1000	60.3 $\pm$ 20.9	> 1000	30.2 $\pm$ 10.5	30.2 $\pm$ 10.5
[TMC <sub>12</sub> A] <sup>+</sup>	> 1000	68.0 $\pm$ 88.3	> 1000	102.0 $\pm$ 58.9	> 1000	79.3 $\pm$ 78.5	> 1000	68.0 $\pm$ 88.3	68.0 $\pm$ 88.3
[TMC <sub>10</sub> A] <sup>+</sup>	85.4 $\pm$ 21.1	13.3 $\pm$ 3.3	170.9 $\pm$ 42.3	26.5 $\pm$ 6.6	75.3 $\pm$ 38.8	13.3 $\pm$ 3.3	59.0 $\pm$ 34.7	13.3 $\pm$ 3.3	13.3 $\pm$ 3.3
[TC <sub>1</sub> MA] <sup>+</sup>	> 1000	15.8 $\pm$ 3.9	> 1000	63.4 $\pm$ 15.7	> 1000	55.8 $\pm$ 28.7	> 1000	15.8 $\pm$ 3.9	15.8 $\pm$ 3.9
[TC <sub>2</sub> MA] <sup>+</sup>	64.4 $\pm$ 22.3	26.0 $\pm$ 0.0	64.4 $\pm$ 22.3	26.0 $\pm$ 0.0	51.6 $\pm$ 22.3	26.0 $\pm$ 0.0	38.7 $\pm$ 0.0	34.7 $\pm$ 15.0	34.7 $\pm$ 15.0
[Emmor] <sup>+</sup>	> 1000	37.8 $\pm$ 10.8	> 1000	75.7 $\pm$ 18.7	> 1000	75.7 $\pm$ 18.7	> 1000	37.8 $\pm$ 22.9	37.8 $\pm$ 22.9
[Bmpyr] <sup>+</sup>	> 1000	29.0 $\pm$ 12.5	> 1000	72.4 $\pm$ 25.1	> 1000	43.5 $\pm$ 0.0	> 1000	18.1 $\pm$ 6.3	18.1 $\pm$ 6.3
[Bmpyr] <sup>+</sup>	> 1000	27.3 $\pm$ 11.8	> 1000	54.6 $\pm$ 23.6	> 1000	54.6 $\pm$ 23.6	> 1000	17.1 $\pm$ 5.9	17.1 $\pm$ 5.9
[TC <sub>1</sub> MP] <sup>+</sup>	> 1000	30.5 $\pm$ 8.7	> 1000	121.9 $\pm$ 104.5	> 1000	81.3 $\pm$ 53.2	> 1000	21.8 $\pm$ 8.7	21.8 $\pm$ 8.7
[TC <sub>2</sub> MP] <sup>+</sup>	49.5 $\pm$ 21.4	22.2 $\pm$ 5.5	37.1 $\pm$ 0.0	22.2 $\pm$ 5.5	30.9 $\pm$ 10.7	22.2 $\pm$ 5.5	27.8 $\pm$ 13.1	22.2 $\pm$ 5.5	22.2 $\pm$ 5.5
Na <sup>+</sup>	n. t.	22.4 $\pm$ 9.7	n. t.	67.3 $\pm$ 0.0	n. t.	56.1 $\pm$ 19.4	n. t.	22.4 $\pm$ 9.7	22.4 $\pm$ 9.7

n.t. – not tested



**Fig. S1** FTIR spectroscopy-based dendrograms of *S. Typhimurium* after long-term treatments. The dendrograms show no cluster formation caused by the long-term treatments with the ionic liquids based on [TC<sub>8</sub>MA], [TMC<sub>16</sub>A] and [TMC<sub>4</sub>A] and the controls, PBS and sodium nalidixate. No distinct effect is obvious in the region of fatty acids (A, B) or in the protein region (C, D), neither in *S. Typhimurium* (A, C) or the nalidixine insensitive mutant  $\Delta trrC$  (B, D).



**Fig. S2** Clustering and concentration-dependent effects of [TC<sub>8</sub>MA][Cl] and [TMC<sub>16</sub>A][Cl] treatment on *S. Typhimurium* in the spectral region of proteins. (A, C) FTIR spectroscopy-based dendrogram of *S. Typhimurium* after short-term treatments with [TC<sub>8</sub>MA][Cl] / [NaI] (A) and [TMC<sub>16</sub>A][Cl] / NaI (C) with sodium nalidixate and PBS as controls show no cluster formation based on treatments with the ionic liquids. (B, D) FTIR spectra of *S. Typhimurium* treated with three different concentrations of [TC<sub>8</sub>MA][Cl] (B) and [TMC<sub>16</sub>A][Cl] (D) in the spectral range 1,800 to 1,500 cm<sup>-1</sup>, showing the concentration dependency of spectral changes.

**Table S2** Reduction of *S. Typhimurium* CFUs after 1 hour exposure to ILs.

		Log reduction of <i>S. Typhimurium</i> CFU after 1h exposure to ILs						
		10000 mg/L		20000 mg/L		40000 mg/L		
<b>[TMC<sub>4</sub>A]<sup>+</sup></b>	NaI	n.t.		-0.66	±0.25	<b>-3.97</b>	±1.72	
	nalidixate chloride	-1.21	±0.39	-0.30	±0.24	-0.66	±0.08	
		0.00	±0.04	-0.04	±0.06	-0.07	±0.08	
<b>[TMC<sub>16</sub>A]<sup>+</sup></b>			25 mg/L		50 mg/L		100 mg/L	
	nalidixate chloride	n.t.		-0.23	±0.04	-0.94	±0.11	-2.55 ±1.08
		-0.14	±0.02	-0.87	±0.32	-2.42	±1.62	n.t.
<b>[TC<sub>8</sub>MA]<sup>+</sup></b>			250 mg/L		500 mg/L		1000 mg/L	
	chloride	-1.87 ±0.75		<b>-4.04</b> ±1.61		<b>-6.94</b> ±3.86		
				1250 mg/L		2500 mg/L		5000 mg/L
<b>[TC<sub>8</sub>MA]<sup>+</sup></b>	nalidixate	<b>-4.17</b> ±5.26		<b>-8.52</b> ±2.96		<b>-6.81</b> ±4.41		

n.t. – not tested

Bold numbers indicate a > 3 log CFU reduction (99.9%)

