



Susceptibility of several species of gram-negative and gram-positive bacteria to organic acids and their derivatives

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Introduction

- The use of antibiotics as growth promoters in livestock was banned in the U.S. in 2017 by the FDA.
- Potential non-drug alternatives such as organic acids are sought after for promoting animal health and growth.

Organic acids

Antimicrobial effects

- Bactericidal and bacteriostatic**
- Bacterial cell membrane perturbation**
- Osmotic stress**
- Disruption of biomolecule synthesis**

Host response

- Anti-inflammatory response:** Chemotactic effect, modulation of inflammatory cytokines.
- Epithelial cell response:** Energy source, increased proliferation and host defense peptide production.
- Some compounds such as butyric acid and valeric acid have a pungent odor, but their derivatives monobutyrim and monovalerin lack odor and are therefore potentially more practical for use in the livestock industry.

Objective

To test the antimicrobial activity of organic acids and their derivatives against gram-positive and gram-negative bacterial strains.

Materials and methods

- The **minimum inhibitory concentration (MIC)** of individual organic acids on different bacterial strains was tested in triplicates using micro-broth dilution method modified from Stoddard et al. (2008) and Li et al. (2015).
- The same strains of bacteria were tested for susceptibility to common antimicrobial drugs. Common drugs for gram-negative (G-) and gram-positive (G+) strains were tested respectively on **National Antimicrobial Susceptibility Monitoring System (NARMS)** plates.

Tested concentrations of organic acids and derivatives

- Monopropionin: 0.5-25.0 mg/mL
- Monovalerin: 0.01-25.0 mg/mL
- Monobutyrim: 0.01-50.0 mg/mL
- Monolaurin: 0.01-25.0 mg/mL
- Valeric acid: 0.01-3.5 mg/mL
- Butyric acid: 0.01-3.5 mg/mL
- Sodium formate: 0.5-20.0 mg/mL
- *Prophorce: 0.01-10.0 mg/mL

Bacterial strains

- Six G- strains**
 - Escherichia coli* ATCC 25822, *Salmonella enterica* Typhimurium ATCC 14028, *Campylobacter jejuni* ATCC 33560 (CIP 702)
 - Wild-types: *E. coli* F18, *S. enterica* Typhimurium (isolated from cull dairy cattle in California), *C. jejuni*, Campy 8DLIS D-12-1 (isolated from environmental water in California).
- Four G+ strains**
 - Enterococcus faecalis* ATCC 29212, *Clostridium perfringens* ATCC 12915, *Streptococcus pneumoniae* ATCC49619, *Streptococcus suis* ATCC 43765.

*Prophorce: Sodium formate and free formic acid (40:60 w/v).

Results

Table 1. MIC (mg/mL) values of organic acids on tested G- and G+ bacterial strains

Compound	G(-)		G(+)		G(-)		G(+)		G(-)		G(+)	
	ATCC 25922	F18	ATCC 14028	ID# 4286	ATCC 33560	Campy 8DLIS D12-1	ATCC 29212	ATCC 12915	ATCC 49619	ATCC 43765		
Monopropionin	10.0	10.0	10.0	10.0	10.0	10.0	10.0	11.3	> 25.0	> 25.0		
Monovalerin	6.7	5.0	10.0	15.0	2.5	3.7	10.0	3.1	2.4	2.0		
Monobutyrim	15.0	10.0	11.7	10.0	>50 ^a	10.0	10.0	2.6	7.7	7.8		
Monolaurin	10.0	10.0	10.0	10.0	0.6	5.0	0.5	0.3	0.01	0.4		
Valeric acid	2.7	2.8	2.7	2.6	0.5	0.7	2.0	1.3	1.0	1.0		
Butyric acid	2.3	2.5	2.5	2.3	0.8	0.5	2.0	1.2	1.0	0.7		
Sodium formate	> 20.0	> 20.0	> 20.0	> 20.0	2.0	2.0	> 20.0	18.8	15.8	11.0		
Prophorce	2.0	2.2	2.2	2.2	0.7	1.0	1.0	1.0	1.0	1.9		

Table 2. MIC (mg/L) values of antimicrobial drugs on tested G- bacterial strains

Antimicrobial drug	<i>E. coli</i> ATCC 25922	<i>E. coli</i> F18	<i>S. enterica</i> Typhimurium ATCC 14028	<i>S. enterica</i> Typhimurium ID# 4286	<i>C. jejuni</i> ATCC 33560	<i>C. jejuni</i> Campy 8DLIS D12-1
Amikacin	NI	8	NI	NI	NI	NI
Piperacillin/tazobactam constant 4	NI	4	NI	NI	4	NI
Tigecycline	NI	1	NI	NI	NI	NI
Ticarcillin/clavulanic acid constant 2	2	2	NI	2	NI	NI
Levofloxacin	NI	NI	NI	NI	NI	NI
Nitrofurantoin	NI	32	NI	NI	NI	NI
Tetracycline	NI	NI	NI	NI	NI	NI
Doripenem	NI	1	NI	NI	NI	NI
Minocycline	NI	8	NI	NI	NI	NI
Ertapenem	8	1	NI	NI	NI	NI
Trimethoprim/sulfamethoxazole	NI	NI	NI	NI	NI	NI
Imipenem	4	2	NI	NI	NI	NI
Piperacillin	NI	64	NI	NI	NI	NI
Meropenem	4	0.5	NI	NI	NI	2
Gentamicin	NI	2	NI	NI	NI	NI
Cefazolin	8	NI	NI	NI	NI	NI
Tobramycin	NI	4	NI	NI	NI	NI
Ceftazidime	16	NI	NI	NI	NI	NI
Ampicillin/sulbactam 2:1 ratio	NI	8	NI	8	NI	NI
Aztreonam	NI	NI	NI	NI	NI	NI
Ampicillin	NI	NI	NI	16	NI	NI
Cefepime	NI	4	NI	NI	NI	NI
Ciprofloxacin	NI	0.5	NI	NI	NI	NI
Ceftriaxone	4	NI	NI	16	NI	NI

Gram-negative summary

- Antimicrobial activity:** Butyric acid, valeric acid, and Prophorce > monovalerin, monolaurin, and monobutyrim > monopropionin and sodium formate.
- S. enterica* Typhimurium and *C. jejuni* strains **resistant** to most drugs.

Conclusions

- Butyric acid, valeric acid, Prophorce** inhibited growth of all strains.
- Monovalerin, monolaurin, monobutyrim** inhibited growth of all strains, except monobutyrim did not inhibit *C. jejuni* (ATCC 33560) at 50 mg/mL.
- Sodium formate** and **monopropionin** had weakest antimicrobial activity.

Table 3. MIC (mg/L) values of antimicrobial drugs on tested G+ bacterial strains

Antimicrobial drug	<i>E. faecalis</i> ATCC 29212	<i>C. perfringens</i> ATCC 12915	<i>S. pneumoniae</i> ATCC 49619	<i>S. suis</i> ATCC 43765
Tigecycline	NI	NI	0.015	0.015
Erythromycin	4	4	0.25	0.25
Tetracycline	NI	NI	NI	4
Ciprofloxacin	NI	NI	0.12	0.12
Chloramphenicol	NI	NI	2	2
Penicillin	16	2	0.25	0.25
Dapomycin	16	8	0.25	0.25
Vancomycin	32	NI	1	0.25
Streptomycin	NI	1024	2048	1024
Nitrofurantoin	NI	NI	NI	2
Tylosin tartrate	NI	NI	2	0.25
Gentamicin	1024	512	128	128
Quinupristin/dalfopristin	NI	16	0.5	0.5
Lincomycin	2	1	1	1
Linezolid	NI	NI	0.5	0.5
Kanamycin	1024	1024	128	128

Gram-positive summary

- Antimicrobial activity:** Monolaurin and Prophorce > monovalerin, monobutyrim, butyric acid, and valeric acid > monopropionin and sodium formate.
- G+ strains were **susceptible** to majority of antimicrobial drugs (except *E. faecalis* was susceptible to 7 out of 16 drugs).

References

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