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Loving the poison: the methylcitrate cycle and bacterial pathogenesis

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Abstract

Propionate is an abundant catabolite in nature and represents a rich potential source of carbon for the organisms that can utilize it. However, propionate and propionate-derived catabolites are also toxic to cells, so propionate catabolism can alternatively be viewed as a detoxification mechanism. In this review, we summarize recent progress made in understanding how prokaryotes catabolize propionic acid, how these pathways are regulated and how they might be exploited to develop novel antibacterial interventions.

INTRODUCTION

Propionate is one of the most abundant short-chain fatty acids (SCFAs) and is a common by-product of bacterial fermentation. Consequently, propionate is produced in large quantities (50–100 mM) in the gastrointestinal tract of mammals [1, 2]. Plants, insects and micro-organisms utilize several alternative pathways for metabolizing propionate as a carbon source. For example, propionyl-CoA is the by-product of β-oxidation of odd-chain fatty acids, of branched-chain fatty acid catabolism, of branched-chain amino acid degradation and cholesterol catabolism. Remarkably, though, propionate (or one of its derived catabolites) is lethally toxic to cells if it is allowed to accumulate. Therefore, there is a trade-off between the beneficial effects of propionate catabolism (its skeleton is a useful carbon source) and propionate toxicity. In this review, we address recent advances in our understanding of how cells catabolize propionic acid, what regulates this and why propionate and its catabolites are toxic. We also discuss how propionate catabolism plays an important role in virulence, and how this can be targeted for the development of new antimicrobial interventions.

To begin with, we will discuss how cells metabolize propionate. Three different mechanisms have been proposed: (i) the 2-methylcitrate cycle, (ii) the methylmalonyl pathway and (iii) the incorporation of propionyl moieties into cell wall lipids. In mammalian cells, the methylmalonyl-CoA pathway is the principal means by which propionyl-CoA toxicity is alleviated. In contrast, and in most bacteria, mycobacteria, yeast and filamentous fungi, the 2-MC cycle is the main pathway used for the detoxification and catabolism of propionate [3], and we will focus mainly on this pathway for the remainder of this review.

THE METHYLCITRATE CYCLE

The methylcitrate cycle was originally thought to be present only in fungal species such as *Candida lipolytica* and *Aspergillus nidulans* [4]. However, in the late 1990s, the methylcitrate cycle was reported in *Salmonella enterica* and *Escherichia coli* [5, 6]. As shown in Fig. 1, propionate and oxaloacetate undergo an aldol condensation reaction to form 2-methylcitrate (2-MC), followed by an isomerization to yield 2-methyl*iso*citrate, and finally, a cleavage reaction yielding succinate and pyruvate. This sequence of reactions is catalyzed by three core enzymes; methylcitrate synthase (MCS, encoded by *prpC*), methylcitrate dehydratase (MCD, encoded by *prpD*) and 2-methyl*iso*citrate lyase (MCL, encoded by *prpB*) [7]. These reactions in the MCC are directly analogous to those catalyzed by citrate synthase, aconitase and *iso*citrate lyase in the glyoxylate cycle (Fig. 2). However, several variations upon this theme exist, and some organisms encode additional MCC-related enzymes such as PrpE and PrpF (Fig. 3). *PrpE* encodes propionyl-CoA synthase. In *S. enterica*, acetyl-CoA synthase can substitute for PrpE, so *prpE* only becomes essential for growth on propionate in an *acs* mutant background [8]. In contrast, PrpF plays an altogether more interesting role. Whereas PrpD functions to

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Abbreviations: CoA, coenzyme A; ICL, isocitrate lyase; 2-MC, 2-methylcitrate; 2MCA, 2-methyl-*cis*-aconitate; 4MCA, 4-methyl-*cis*-aconitate; MCC, methylcitrate cycle; MCD, methylcitrate dehydratase; MCL, methylcitrate lyase; MCS, methylcitrate synthase; MS, malate synthase; SCFAs, short chain fatty acids; TCA, tricarboxylic acid.

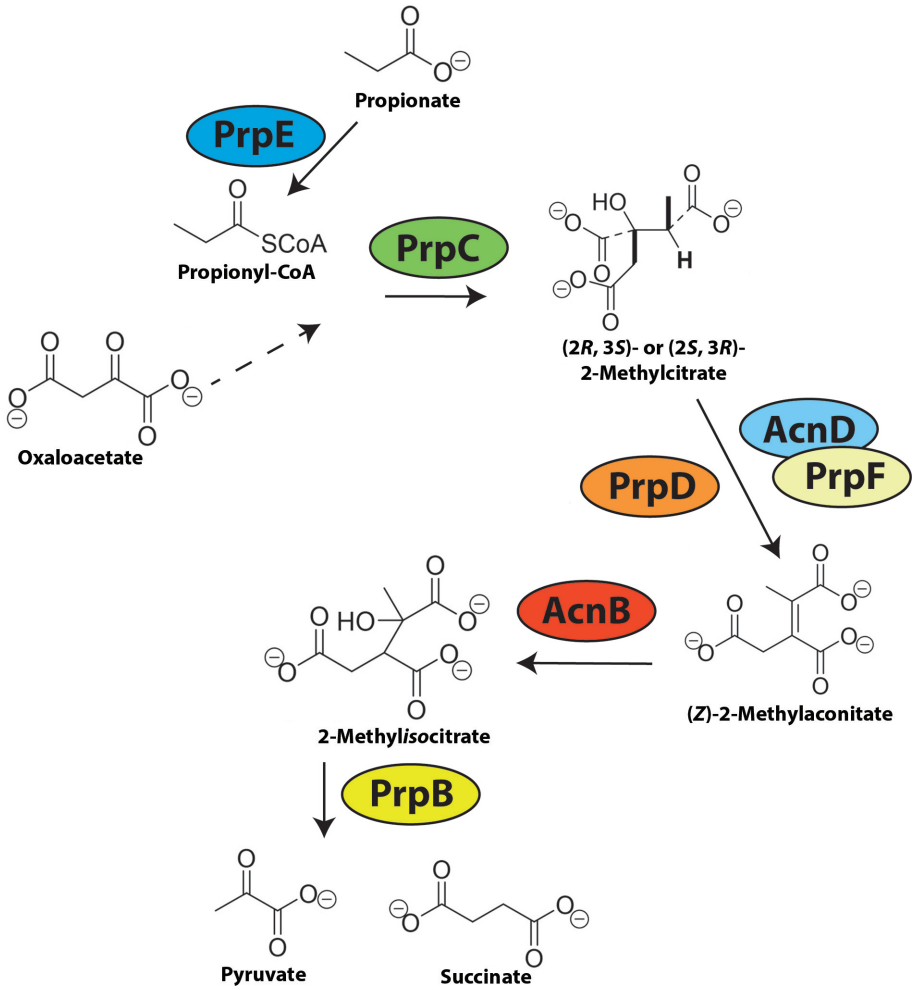

Fig. 1. In the majority of MCC arrangements, propionate is first activated to propionyl-CoA by propionyl-CoA synthase (PrpE). The propionyl-CoA is then condensed with oxaloacetate by methylcitrate synthase (PrpC), forming 2-methylcitrate. 2-MC is subsequently dehydrated to yield methylaconitate. This step is accomplished by either methylcitrate dehydratase/hydratase (PrpD) or by the combined activity of methylcitrate dehydratase (AcnD) and methylaconitate *cis-trans* isomerase (PrpF). Following dehydration, methylaconitate is rehydrated by aconitase (AcnB) to yield methyl*iso*citrate. This is then cleaved by methyl*iso*citrate lyase (PrpB) or by *iso*citrate lyase (AceA in *M. tuberculosis*) to yield pyruvate and succinate.

dehydrate 2-methylcitrate (2MC) directly to 2-methyl-*cis*-aconitate (2MCA) (Fig. 1), an alternative route is to convert 2-methylcitrate into 4MCA (4-methyl-*cis*-aconitate, in a reaction catalyzed by AcnD) first and then isomerize the 4MCA into 2MCA (in a reaction catalyzed by PrpF) [9]. Quite why these different strategies for converting 2MC into 2MCA are employed is not clear, although one possibility is that since AcnD is an oxygen-sensitive Fe-S protein, PrpD functions when oxygen levels are high [10]. Some organisms, such as *Neisseria meningitides*, have also been shown to encode additional genes outside of this 'extended set', presumably providing even greater flexibility in propionate metabolism [11]. It is noteworthy that none of these gene clusters encode any apparent propionate uptake mechanism, so a passive diffusion mechanism has been suggested. However, in at least one case (*Corynebacterium glutamicum*) a proton-driven monocarboxylic acid transporter (MctC) has been shown to have high affinity for both acetate and propionate [12].

REGULATION OF METHYLCITRATE CYCLE GENE EXPRESSION – PRPR

PrpR is a transcriptional activator of the *prp* genes in some *Gammaproteobacteria* (*Enterobacteriales* and *Xanthomonadales*) and *Betaproteobacteria* (*Burkholderiales*). In most *Enterobacteriales* the propionate metabolism genes are organized in the divergon *prpR prpBCDE*, although in the majority of *Xanthomonadales* and *Betaproteobacteria* the propionate divergon has a slightly different arrangement – *prpR prpBC-acnD-prpF*. In some *Betaproteobacteria*, additional transcription factors regulate propionate metabolism [13].

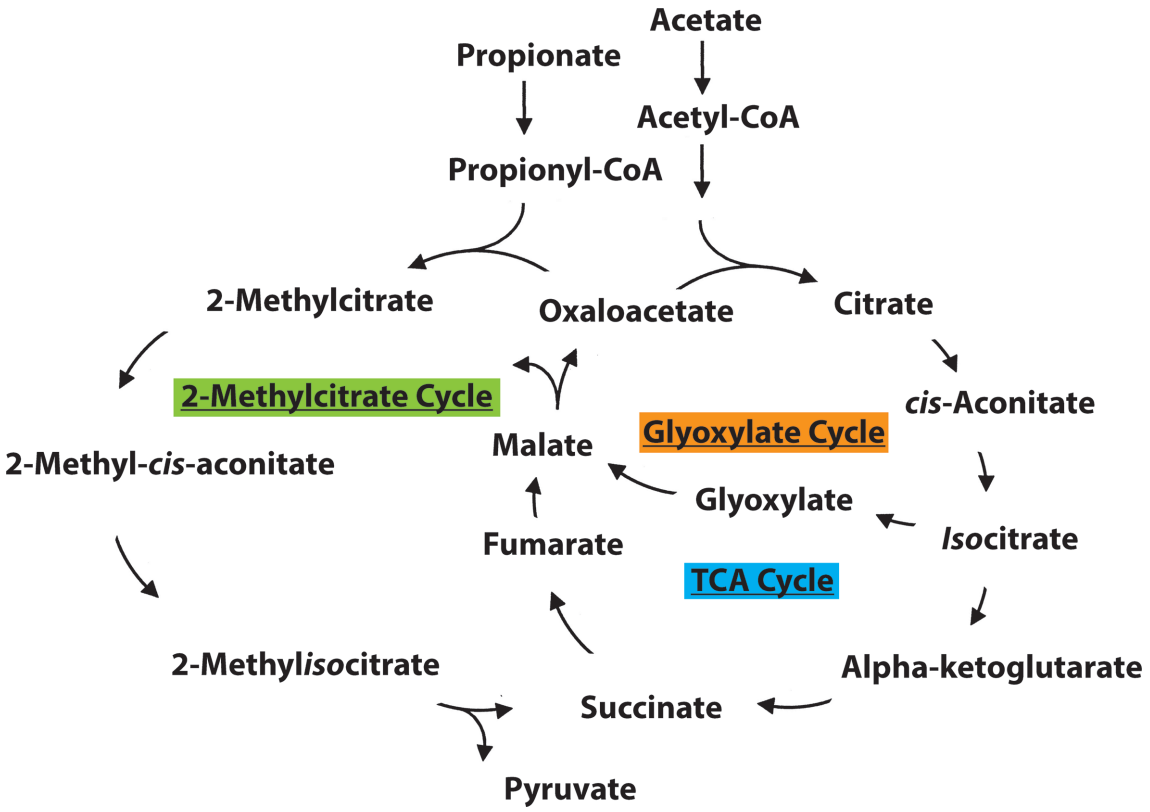

Fig. 2. The overlapping reactions encompassing the citric acid (TCA), glyoxylate and methylcitrate cycles in bacteria. Odd-chain fatty acids are degraded via the β-oxidation cycle to yield acetyl-CoA and propionyl-CoA units. These are then further oxidized by the citric acid cycle or the methylcitrate cycle, respectively. Anaplerosis during growth on even-chain fatty acid substrates is achieved by metabolism of acetyl-CoA via the glyoxylate cycle, which consists of the enzymes *iso*citrate lyase (ICL) and malate synthase (MS).

PrpR is widely conserved across many species, although it was first described in *Salmonella enterica* serovar Typhimurium [14]. *S. enterica* PrpR is a sigma-54 family transcriptional regulator that requires 2-methylcitrate as a co-activator [15]. This accumulates during growth on propionate, and following binding of 2-methylcitrate to PrpR, transcription of the MCC gene cluster is rapidly induced, thereby decreasing the toxicity of propionate-derived metabolites [16]. However, a detailed analysis of the *C. glutamicum* transcriptional regulatory network revealed no obvious homologue of PrpR from *E. coli* or *S. enterica* [17]. Instead, the *C. glutamicum prpR* gene was found to encode a regulatory protein belonging to the HTH_XRE family, and a Δ*prpR* mutant of *C. glutamicum* was unable to induce expression of the *prpDBC2* genes in the presence of propionate [18]. Intriguingly, *C. glutamicum* PrpR was noted to have moderate similarities to the regulator of acetate metabolism, RamB [19]. However, whereas RamB can act as both a repressor and an activator [20], PrpR acts exclusively as an activator. Nevertheless, the similarity in the amino acid sequence between RamB and PrpR hinted at a common ancestry, with each regulator apparently evolving separately to eventually control degradation of the structurally closely related but different carboxylic acids, acetate or propionate [18].

Additional insight into the genetic regulatory control of propionate catabolism came from studies on *Mycobacterium tuberculosis* (Mtb). In this organism, PrpR was shown to act as a transcriptional activator of the *prpDC* and *icl1* genes (*icl1* encodes *iso*citrate lyase – one of the enzymes involved in the glyoxylate shunt). Strikingly, *ramB* was shown to be negatively regulated by PrpR. This suggested that PrpR plays a key role in coordinating expression of the enzymes involved in the glyoxylate shunt and methylcitrate pathways [21]. PrpR was also shown to regulate Mtb *dnaA* expression, indicating that it may also be involved in controlling the initiation of chromosomal DNA replication. Consequently, PrpR appears to be an important element of the complex regulatory system(s) required for the persistence of Mtb within macrophages, controlling both the catabolism of host-derived fatty acids and the initiation of chromosomal replication [22].

OTHER REGULATORS OF *PRP* GENE EXPRESSION

Although PrpR is the principal regulator of *prp* operon expression in enterobacteria, several other proteins are also involved. These include σ^{54}, integration host factor (IHF)

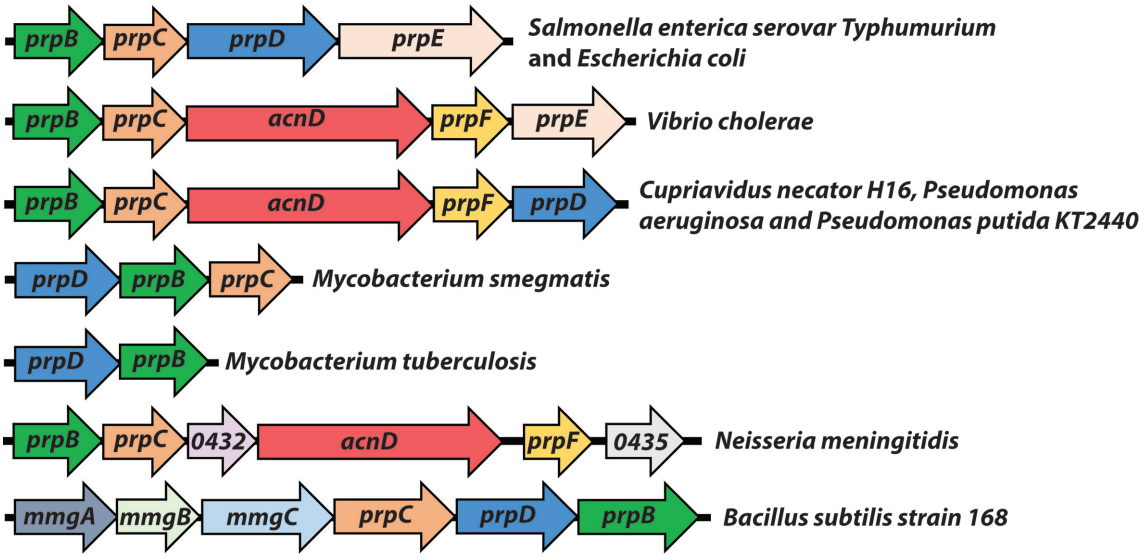

Fig. 3. At least seven structural variations of the *prp* operons have been described thus far for bacteria. The combined activity of AcnD and PrpF replaces the activity of PrpD in some organisms. Interestingly, though, and in spite of the apparent redundancy, some *prp* operons encode both AcnD/PrpF and PrpD. Further variations include the *N. meningitidis prp* operon, which contains two additional uncharacterized genes, NMB0432 (a putative membrane protein) and NMB0435 (a putative acetate kinase). Mutants deficient in NMB0432 or NMB0435 are unable to utilize propionic acid. The *P. aeruginosa prp* operon appears to lack an obvious *prpE* homologue; presumably this function is encoded by an unlinked gene external to the operon. Similarly, *B. subtilis* strain 168 also does not appear to encode a PrpE homologue. Instead, this function is encoded within the mother cell metabolic gene (*mmg*) operon, which is involved in fatty acid degradation. Crucially, the *M. tuberculosis* genome does not encode the main terminal enzyme of the MCC, PrpB (methyl*iso*-citrate lyase). This is because in Mtb the glyoxylate shunt enzyme, *iso*citrate lyase, is capable of both *iso*citrate lyase and methyl*iso*citrate lyase activity, so no additional enzyme is required.

and the cyclic AMP receptor protein (CRP) [23]. *E. coli* cells with defects in CRP or adenylate cyclase activity (*crp* or *cya* mutants, respectively) exhibit reduced transcriptional activation of *prpR* and *prpBCDE*, and the effect on *prpBCDE* is independent of *prpR*. This suggests that propionate metabolism is subject to catabolite repression, and that this is likely mediated through CRP-dependent control of both *prpR* and the *prpBCDE* operon [24].

Expression of the *prp* genes is also known to be regulated by small RNA molecules. *Neisseria* metabolic switch regulators [NmsRs] act as switches controlling the transition between cataplerotic and anaplerotic metabolism in *N. meningitidis*. The expression of several TCA cycle-associated genes (including *prpF*, *prpB*, *acnB* and *prpC*) were down-regulated by the direct action of NmsRs. Expression of the NmsRs themselves was under the control of the stringent response through the action of the (p)ppGpp synthase, RelA [25], suggesting that the use of propionate may become crucial in this organism under conditions of nutrient limitation [26].

The activity of many small RNAs in bacterial pathogens depends on the RNA chaperone Hfq. Among the direct targets of $NmsR_A$, three proteins (GltA, PrpB and PrpC) were also found to be overexpressed in an Hfq deletion strain of *N. meningitidis* [11]. This suggested that NmsRs may act in concert with Hfq. Hfq-dependent repression of *prpB* expression was subsequently shown to be mediated by two *trans*-acting sRNAs, RcoF1 and RcoF2 [27]. Northern blot analysis revealed that both the single *prpB* and the bicistronic *prpB-prpC* mRNAs were up-regulated upon the deletion of *hfq* or a double deletion of *rcoF1 and rcoF2* [28], thereby confirming the genetic link between RcoF1/2, Hfq and *prp* gene expression. By regulating *prpB*, RcoF1 and RcoF2 appear to be the first *trans*-acting sRNAs with a direct host-associated function in *N. meningitidis* [27].

In *S. enterica*, the *prpBCDE* genes undergo antagonistic regulation by σ^S at the RNA and protein levels. Expression of the *prpBCDE* operon was stimulated by σ^S at the RNA level, yet at the same time, also down-regulated at the protein level. This negative effect of σ^S on the abundance of the Prp proteins is consistent with the fact that Δ*rpoS* mutants grow better (compared with the wild-type) on propionate as a sole carbon source. In *E. coli* K12, the *prp* genes are also positively controlled by σ^S at the RNA level [29]. However, and since *rpoS* disruption did not improve *E. coli* K12 growth on propionate, *prp* regulation appears to be different in *E. coli* and *S. enterica*. This may be related to the finding that in contrast to the situation in *S. enterica*, the *prp* operon of *E. coli* is interrupted by repetitive elements [30]. Dual antagonistic control of *prp* expression at both the RNA and protein levels may be a mechanism that allows *S. enterica* σ^S to

maintain finely balanced Prp expression, with greater responsiveness to changing environmental conditions [30].

In *E. coli*, RNase R expression is fourfold higher under anoxic compared with oxic conditions. Intriguingly, RNase R has been shown to hydrolyze *prpBCDE* transcripts, potentially to ensure that propionate degradation only occurs under oxic and not anoxic conditions. Thus, the *prp*-operon of *E. coli* K12 is also regulated at least partly at a post-transcriptional level to prevent the anaerobic metabolism of propionate [31]. This may be a method to prevent futile expenditure of metabolic energy, 2-MC toxicity and/or metabolic roadblocks during anoxic conditions.

Recent studies have demonstrated that propionate catabolism is also regulated post-translationally. For example, propionate metabolism in *Mycobacterium smegmatis* is regulated by lysine acetylation [32] and a cAMP-dependent protein lysine acetyltransferase in *Mycobacterium bovis* BCG (KATmt) has been shown to be necessary for growth on propionate as a sole carbon source [33].

ALLEVIATING PROPIONATE TOXICITY

Although many aerobes (and also some anaerobes) can use propionate as a sole carbon source, the addition of propionate into growth media stunts the growth of most micro-organisms, even in the presence of other carbon sources [34, 35]. Due to this property, SCFAs such as propionate are widely used as preservatives in the food industry. However, and in spite of its widespread use, we still do not have a clear mechanistic insight as to how propionate prevents microbial growth.

The importance of the 2-MC cycle in propionate detoxification has been demonstrated in *E. coli*, *M. smegmatis*, *M. tuberculosis*, *C. glutamicum, S. enterica* and several other bacteria. However, the mechanisms of propionate toxicity appear to be extremely diverse. Early studies argued that growth inhibition might be caused by generic, non-specific mechanisms such as the dissipation of the proton motive force, decreased intracellular pH or accumulation of the propionate anion in the cytoplasm [1]. More recent work has challenged these notions. For example, in *Rhodobacter sphaeroides* propionyl-CoA inhibits pyruvate dehydrogenase, and in *S. enterica* 2-MC inhibits the gluconeogenic enzyme, fructose 1,6 bisphosphatase (FBPase) [7]. 2-MC has also been shown to be toxic in *Aspergillus nidulans* [36]. Another potentially toxic effect was described for *E. coli*, where it was found that homocysteine accumulates after propionate addition [37]. Consistent with this hypothesis, enhanced expression of the potential homocysteine-consuming enzyme, MetE, was noted in *C. glutamicum* [38]. In addition, propionyl-CoA has been described as toxic in *E. coli* [39], *R. sphaeroides* [40] and *Aspergillus nidulans* [41].

B. SUBTILIS

Recent work has examined growth on propionate or the scavenging of branched fatty acids and branched-chain amino acids as separate drivers for the evolution of a methylcitrate cycle. In *B. subtilis*, lack of an obvious propionyl-CoA synthase and a general inability to grow on propionate suggest that the function of the methylcitric acid cycle in this organism is to cope specifically with propionyl-CoA, and not propionate, making this pathway potentially distinct from the propionate-metabolizing pathways of other species [42]. What, then, is the source of the propionyl-CoA in these circumstances? Instead of a PrpE-like propionyl-CoA synthase homologue, the *B. subtilis* mother cell metabolic gene (*mmg*) operon comprises six ORFs, *mmgABCDEF*, the first three of which were shown to encode enzymes involved in fatty acid β-oxidation. The final round of the β-oxidation cycle of methyl-branched fatty acids or the carbon skeleton derived from isoleucine catabolism would yield one acetyl-CoA and one propionyl-CoA. The acetyl-CoA would enter the citric acid cycle via the citrate synthase activity of MmgD. However, MmgD also has 2-methylcitrate synthase activity, and can likewise condense propionyl-CoA with oxaloacetate to begin the 2-MC cycle. The remaining steps are encoded by *mmgEF* and *citB* (aconitase) [42]. Further atypical methylcitrate cycles may be present in organisms that do not grow on propionate as a sole carbon source. A key question is whether metabolic defence against toxic levels of propionate acts as an additional driver to maintain this operon.

ENTEROBACTERIA

Although the reasons for retaining the methylcitrate cycle are clearly diverse, genomics has also revealed the repeated loss of this cycle in some species. *Shigella* sp. and the enteroinvasive *E. coli* arose from distinct *Escherichia* ancestors, acquiring the capacity to invade epithelial cells. The genome sequences of these species have undergone substantial reduction, with multiple metabolic pathways becoming lost. One such victim of genome reduction is the methylcitrate pathway, which appears to have been specifically deleted from many of these enteroinvasive pathogens, although it is found in all other *E. coli* genomes [43, 44]. Additionally, four genes (*prpDBCE*) found in *Campylobacter coli* but not in *C. jejuni* are involved in the methylcitrate cycle, which could account for the enhanced ability of *C. coli* strains to grow on odd-chain fatty acids compared with *C. jejuni* [45, 46]. However, the precise driver(s) for the loss of this pathway in certain pathogens remains to be elucidated.

Exploring the molecular basis for propionate toxicity has led to unexpected insights into microbial metabolism. In *S. enterica*, deletion of the methylcitrate synthase, PrpC, results in severe growth inhibition when propionate is added to the growth media. This is because, in the absence of PrpC, the production of 2-MC by citrate synthase (GltA) [1], which has a relatively relaxed substrate specificity, inhibits fructose-1,6-bisphosphatase (FBPase), a key enzyme in gluconeogenesis. The lack of growth due to inhibition of FBPase by 2-MC^{GltA} could be overcome by increasing the expression of FBPase or by adding micromolar amounts of glucose (the ultimate product of the pathway in which FBPase is involved) to the medium – thus providing a definitive target of 2-MC inhibition [47]. Similarly, in

M. tuberculosis, the sole annotated FBPase (encoded by *glpX*), is also non-competitively inhibited by 2-MC [48].

The relaxed substrate specificity of certain 2-MC cycle enzymes suggests that the methylcitrate cycle is potentially capable of overcoming bottlenecks in cellular metabolism. For example, due to its promiscuous catalytic activity, PrpC has been shown to complement a deletion in *gltA* (encoding citrate synthase) in both *S. enterica* [1] and *E. coli* [49]. Such observations indicate that the methylcitrate cycle can fulfil atypical functionalities when required [49]. The metabolic redundancy of PrpC may also explain why the citrate synthase of *Pseudomonas aeruginosa* (GltA) is not generally essential for growth [50].

MYCOBACTERIA

Mycobacterial sp. have an unusual way of dealing with excess propionyl-CoA. The mycobacterial cell wall has a high lipid content and contains several important lipid groups, including poly- and di-acyltrehaloses (PAT/DAT), sulfolipids (SL-1) and phthiocerol dimycocerosates (PDIM) [51]. These lipids are produced and actively secreted during infection to subvert the host immune system, eventually leading to *M. tuberculosis* persistence. These bioactive lipids can be esterified with up to five multiple methyl-branched long-chain fatty acids, which provides an effective 'sink' for excess propionyl-CoA. Like PDIM, the storage lipid triacylglycerol (TAG) can also serve as a sink to alleviate the accumulation of potentially toxic propionyl-CoA [51–53].

The mycobacteria also employ an intriguing feedback mechanism to regulate free propionyl-CoA levels in the cell. Propionylation of acyl-CoA synthase in *M. bovis* BCG leads to diminished synthesis of propionyl-CoA from propionate [33]. Indeed, a considerable number of propionylated proteins have been identified in this species, possibly defining another 'sink' for excess propionyl-CoA. Moreover, because the 2-methyl*iso*citrate lyase activity of *iso*citrate lyase (ICL, a key component of glyoxylate shunt) is essential for the methylcitrate cycle in mycobacterial sp., propionylation of ICL may have a dual impact, i.e. to simultaneously modulate ICL activity in response to the propionyl-CoA concentration and sequester excess of propionyl-CoA to relieve the toxicity of this compound [54].

An *M. tuberculosis iso*citrate lyase mutant, Δ*icl1*, has a synthetic lethal phenotype and fails to grow in rich media containing cholesterol. In *M. tuberculosis*, ICL also doubles as a 2-methyl*iso*citrate lyase. It was therefore hypothesized that this growth inhibition might be linked to the accumulation of one or more toxic cholesterol-derived intermediates produced by the methylcitrate cycle, which accumulate in the bacteria when *icl1* is non-functional. Recent work has shown that Δ*icl1* 'bypass' mutants, which either alleviate methylcitrate toxicity or reduce the catabolic flux of propionyl-CoA, are able to rescue the growth defect by alleviating the accumulation of toxic metabolites. This led to the identification of a previously uncharacterized protein, Rv3723/LucA, which functions to integrate cholesterol and fatty acid uptake in *M. tuberculosis* [55]. Similar experiments in other key pathogens will likely uncover more uncharacterized proteins dedicated to propionate metabolism and/or alleviating toxicity.

PROPIONATE METABOLISM AS AN ANTIMICROBIAL TARGET

Given the abundance of propionate in many infection scenarios, the 2-MC cycle has been identified as an attractive target for the development of new anti-bacterial agents. Blocking this pathway in several notable pathogens results in extreme sensitivity to propionate and reduced virulence. For example, several studies have indicated that the primary carbon source for *M. tuberculosis* is fatty acids/cholesterol when residing within host macrophages [56–58]. In support of this, PrpC was found to be required for intracellular growth in macrophages, indicating that propionyl-CoA metabolism is critical for maintaining the intracellular lifestyle [34, 58, 59]. Indeed, deletion of *prpC* impaired the ability of *M. tuberculosis* to grow in media containing cholesterol as a primary carbon source, and recent work has established that an *M. tuberculosis prpCD* double mutant also exhibits a growth defect in murine bone marrow-derived macrophages [60]. These observations are consistent with the notion that *M. tuberculosis* obtains a significant amount of its carbon requirements from cholesterol [61]. Building on these observations, an unbiased chemical screen was recently used to identify compounds that inhibit propionate catabolism and thereby diminish *M. tuberculosis* survival within macrophages. One such compound, V-13–009920, inhibited PrpC and prevented *M. tuberculosis* growth in cholesterol-containing media [60]. These results highlight the potential of targeting this pathway in prominent human pathogens as an antimicrobial strategy.

In *S. enterica* serovar Typhimurium, propionate exposure has been shown to decrease the expression of the *Salmonella* pathogenicity island 1 (SPI-1). This effect is dependent upon the transcriptional regulator HilD, which is post-translationally modified by propionyl-CoA [62]. Furthermore, pre-incubation of *S. enteritidis* with propionate and butyrate resulted in decreased epithelial cell invasion [63]. Moving to a whole-animal infection model (pigs), supplementation of the diet with a mixture of organic acids including propionate resulted in decreased *Salmonella* recovery from the mesenteric lymph nodes [64]. Thus, simple dietary supplementation with SCFAs, especially propionate, may be a promising intervention strategy for decreasing *Salmonella* infection in farm animals [65]. Interestingly, it has also been suggested that the production of propionate by intestinal microflora may be a protective mechanism to control the host response to commensals. This way, the host would avoid local inflammation and tissue damage [66].

The methylcitrate cycle gene cluster also plays an important role in *N. meningitides* infection and transmission. In this organism, the *prp* genes are located on a large genomic island, absent from closely related non-pathogenic *Neisseria* species such as *N. lactamica*. This may be important because

the ability to utilize propionic acids may confer an advantage in colonizing the adult nasopharynx, which is rich in propionic acid-generating bacteria [25, 67]. From an evolutionary perspective, this plays an important role in the transmission and disease epidemiology of *N. meningitides* by providing a selection pressure for the retention of a horizontally acquired propionate utilization pathway [25]. Recent work has shown that another respiratory pathogen, *Pseudomonas aeruginosa*, also utilizes the methylcitrate cycle to catabolize and detoxify propionate in the airway environment. Here, the propionate is derived from the breakdown of tracheobronchial mucin by cohabiting fermentative anaerobes [68]. However, the precise contribution of the *P. aeruginosa* methylcitrate cycle to propionate detoxification and virulence has not been explored further.

In summary, the last few years have yielded major advances in our understanding of how different species of bacteria detoxify propionate – an abundant SCFA in many infection scenarios. Moreover, it is also becoming clear that a better understanding of these pathways, and of the ecology of infection sites, can potentially lead to new antimicrobial interventions. Along the way, we are also learning much about the evolution, acquisition, or loss of metabolic pathways; a voyage of discovery that is being catalyzed by unprecedented access to genome sequence information.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. **Horswill AR, Dudding AR, Escalante-Semerena JC.** Studies of propionate toxicity in *Salmonella enterica* identify 2-methylcitrate as a potent inhibitor of cell growth. *J Biol Chem* 2001;276:19094–19101.
2. **Cummings JH, Pomare EW, Branch WJ, Naylor CP, MacFarlane GT.** Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987;28:1221–1227.
3. **Limenitakis J, Oppenheim RD, Creek DJ, Foth BJ, Barrett MP *et al.*** The 2-methylcitrate cycle is implicated in the detoxification of propionate in *Toxoplasma gondii*. *Mol Microbiol* 2013;87:894–908.
4. **Tabuchi T, Serizawa N, Uchiyama H.** A novel pathway for the partial oxidation of propionyl-CoA to pyruvate via seven-carbon tricarboxylic acids in yeasts. *Agric Biol Chem* 1974;38:2571–2572.
5. **Horswill AR, Escalante-Semerena JC.** *Salmonella typhimurium* LT2 catabolizes propionate via the 2-methylcitric acid cycle. *J Bacteriol* 1999;181:5615–5623.
6. **London RE, Allen DL, Gabel SA, Derose EF.** Carbon-13 nuclear magnetic resonance study of metabolism of propionate by *Escherichia coli*. *J Bacteriol* 1999;181:3562–3570.
7. **Rocco CJ, Escalante-Semerena JC.** In *Salmonella enterica*, 2-methylcitrate blocks gluconeogenesis. *J Bacteriol* 2010;192:771–778.
8. **Horswill AR, Escalante-Semerena JC.** The prpE gene of *Salmonella typhimurium* LT2 encodes propionyl-CoA synthetase. *Microbiology* 1999;145:1381–1388.
9. **Rocco CJ, Wetterhorn KM, Garvey GS, Rayment I, Escalante-Semerena JC.** The PrpF protein of *Shewanella oneidensis* MR-1 catalyzes the isomerization of 2-methyl-cis-aconitate during the catabolism of propionate via the AcnD-dependent 2-methylcitric acid cycle. *PLoS One* 2017;12:e0188130.
10. **Grimek TL, Escalante-Semerena JC.** The acnD genes of *Shewenella oneidensis* and *Vibrio cholerae* encode a new Fe/S-dependent 2-methylcitrate dehydratase enzyme that requires prpF function in vivo. *J Bacteriol* 2004;186:454–462.
11. **Pannekoek Y, Huis In 't Veld R, Hopman CT, Langerak AA, Speijer D *et al.*** Molecular characterization and identification of proteins regulated by Hfq in *Neisseria meningitidis*. *FEMS Microbiol Lett* 2009;294:216–224.
12. **Jolkver E, Emer D, Ballan S, Krämer R, Eikmanns BJ *et al.*** Identification and characterization of a bacterial transport system for the uptake of pyruvate, propionate, and acetate in *Corynebacterium glutamicum*. *J Bacteriol* 2009;191:940–948.
13. **Suvorova IA, Ravcheev DA, Gelfand MS.** Regulation and evolution of malonate and propionate catabolism in proteobacteria. *J Bacteriol* 2012;194:3234–3240.
14. **Horswill AR, Escalante-Semerena JC.** Propionate catabolism in *Salmonella typhimurium* LT2: two divergently transcribed units comprise the prp locus at 8.5 centisomes, prpR encodes a member of the sigma-54 family of activators, and the prpBCDE genes constitute an operon. *J Bacteriol* 1997;179:928–940.
15. **Palacios S, Escalante-Semerena JC.** 2-Methylcitrate-dependent activation of the propionate catabolic operon (prpBCDE) of *Salmonella enterica* by the PrpR protein. *Microbiology* 2004;150:3877–3887.
16. **Palacios S, Escalante-Semerena JC.** 2-Methylcitrate-dependent activation of the propionate catabolic operon (prpBCDE) of *Salmonella enterica* by the PrpR protein. *Microbiology* 2004;150:3877–3887.
17. **Brune I, Brinkrolf K, Kalinowski J, Pühler A, Tauch A.** The individual and common repertoire of DNA-binding transcriptional regulators of *Corynebacterium glutamicum*, *Corynebacterium efficiens*, *Corynebacterium diphtheriae* and *Corynebacterium jeikeium* deduced from the complete genome sequences. *BMC Genomics* 2005;6:86.
18. **Plassmeier J, Persicke M, Pühler A, Sterthoff C, Rückert C *et al.*** Molecular characterization of PrpR, the transcriptional activator of propionate catabolism in *Corynebacterium glutamicum*. *J Biotechnol* 2012;159:1–11.
19. **Gerstmeir R, Cramer A, Dangel P, Schaffer S, Eikmanns BJ.** RamB, a novel transcriptional regulator of genes involved in acetate metabolism of *Corynebacterium glutamicum*. *J Bacteriol* 2004;186:2798–2809.
20. **Auchter M, Cramer A, Hüser A, Rückert C, Emer D *et al.*** RamA and RamB are global transcriptional regulators in *Corynebacterium glutamicum* and control genes for enzymes of the central metabolism. *J Biotechnol* 2011;154:126–139.
21. **Masiewicz P, Brzostek A, Wolański M, Dziadek J, Zakrzewska-Czerwińska J.** A novel role of the PrpR as a transcription factor involved in the regulation of methylcitrate pathway in *Mycobacterium tuberculosis*. *PLoS One* 2012;7:e43651.
22. **Masiewicz P, Wolański M, Brzostek A, Dziadek J, Zakrzewska-Czerwińska J.** Propionate represses the dnaA gene via the methylcitrate pathway-regulating transcription factor, PrpR, in *Mycobacterium tuberculosis*. *Antonie van leeuwenhoek* 2014;105:951–959.
23. **Palacios S, Escalante-Semerena JC.** *prpR*, *ntrA*, and *ihf* functions are required for expression of the prpBCDE operon, encoding enzymes that catabolize propionate in *Salmonella enterica* serovar typhimurium LT2. *J Bacteriol* 2000;182:905–910.
24. **Lee SK, Newman JD, Keasling JD.** Catabolite repression of the propionate catabolic genes in *Escherichia coli* and *Salmonella enterica*: evidence for involvement of the cyclic AMP receptor protein. *J Bacteriol* 2005;187:2793–2800.
25. **Catenazzi MC, Jones H, Wallace I, Clifton J, Chong JP *et al.*** A large genomic island allows *Neisseria meningitidis* to utilize

propionic acid, with implications for colonization of the human nasopharynx. *Mol Microbiol* 2014;93:346–355.

26. Pannekoek Y, Huis In 't Veld RA, Schipper K, Bovenkerk S, Kramer G *et al.* *Neisseria meningitidis* uses sibling small regulatory RNAs to switch from cataplerotic to anaplerotic metabolism. *MBio* 2017;8:e02293-16.
27. Heidrich N, Bauriedl S, Barquist L, Li L, Schoen C *et al.* The primary transcriptome of *Neisseria meningitidis* and its interaction with the RNA chaperone Hfq. *Nucleic Acids Res* 2017;45:6147–6167.
28. Mellin JR, McClure R, Lopez D, Green O, Reinhard B *et al.* Role of Hfq in iron-dependent and -independent gene regulation in *Neisseria meningitidis*. *Microbiology* 2010;156:2316–2326.
29. Wong GT, Bonocora RP, Schep AN, Beeler SM, Lee Fong AJ *et al.* Genome-wide transcriptional response to varying RpoS levels in *Escherichia coli* K-12. *J Bacteriol* 2017;199:e00755-16.
30. Lago M, Monteil V, Douche T, Guglielmini J, Criscuolo A *et al.* Proteome remodelling by the stress sigma factor RpoS/σS in *Salmonella*: identification of small proteins and evidence for post-transcriptional regulation. *Sci Rep* 2017;7:2127.
31. Simonte FM, Dötsch A, Galego L, Arraiano C, Gescher J. Investigation on the anaerobic propionate degradation by *Escherichia coli* K12. *Mol Microbiol* 2017;103:55–66.
32. Hayden JD, Brown LR, Gunawardena HP, Perkowski EF, Chen X *et al.* Reversible acetylation regulates acetate and propionate metabolism in *Mycobacterium smegmatis*. *Microbiology* 2013;159:1986–1999.
33. Nambi S, Gupta K, Bhattacharyya M, Ramakrishnan P, Ravikumar V *et al.* Cyclic AMP-dependent protein lysine acylation in mycobacteria regulates fatty acid and propionate metabolism. *J Biol Chem* 2013;288:14114–14124.
34. Munoz-Elias EJ, Upton AM, Cherian J, McKinney JD. Role of the methylcitrate cycle in *Mycobacterium tuberculosis* metabolism, intracellular growth, and virulence. *Mol Microbiol* 2006;60:1109–1122.
35. Savvi S, Warner DF, Kana BD, McKinney JD, Mizrahi V *et al.* Functional characterization of a vitamin B12-dependent methylmalonyl pathway in *Mycobacterium tuberculosis*: implications for propionate metabolism during growth on fatty acids. *J Bacteriol* 2008;190:3886–3895.
36. Brock M. Generation and phenotypic characterization of Aspergillus nidulans methylisocitrate lyase deletion mutants: methylisocitrate inhibits growth and conidiation. *Appl Environ Microbiol* 2005;71:5465–5475.
37. Roe AJ, O'Byrne C, McLaggan D, Booth IR. Inhibition of *Escherichia coli* growth by acetic acid: a problem with methionine biosynthesis and homocysteine toxicity. *Microbiology* 2002;148:2215–2222.
38. Hüser AT, Becker A, Brune I, Dondrup M, Kalinowski J *et al.* Development of a *Corynebacterium glutamicum* DNA microarray and validation by genome-wide expression profiling during growth with propionate as carbon source. *J Biotechnol* 2003;106:269–286.
39. Man WJ, Li Y, O'Connor CD, Wilton DC. The binding of propionyl-CoA and carboxymethyl-CoA to *Escherichia coli* citrate synthase. *Biochim Biophys Acta* 1995;1250:69–75.
40. Maruyama K, Kitamura H. Mechanisms of growth inhibition by propionate and restoration of the growth by sodium bicarbonate or acetate in *Rhodopseudomonas sphaeroides* S. *J Biochem* 1985;98:819–824.
41. Brock M, Buckel W. On the mechanism of action of the antifungal agent propionate. *Eur J Biochem* 2004;271:3227–3241.
42. Reddick JJ, Sirkisoon S, Dahal RA, Hardesty G, Hage NE *et al.* First biochemical characterization of a methylcitric acid cycle from *Bacillus subtilis* strain 168. *Biochemistry* 2017;56:5698–5711.
43. Touchon M, Hoede C, Tenaillon O, Barbe V, Baeriswyl S *et al.* Organised genome dynamics in the *Escherichia coli* species results in highly diverse adaptive paths. *PLoS Genet* 2009;5:e1000344.
44. Sahl JW, Morris CR, Emberger J, Fraser CM, Ochieng JB *et al.* Defining the phylogenomics of *Shigella* species: a pathway to diagnostics. *J Clin Microbiol* 2015;53:951–960.
45. Méric G, Yahara K, Mageiros L, Pascoe B, Maiden MC *et al.* A reference pan-genome approach to comparative bacterial genomics: identification of novel epidemiological markers in pathogenic *Campylobacter*. *PLoS One* 2014;9:e92798.
46. Wagley S, Newcombe J, Laing E, Yusuf E, Sambles CM *et al.* Differences in carbon source utilisation distinguish *Campylobacter jejuni* from *Campylobacter coli*. *BMC Microbiol* 2014;14:262.
47. Rocco CJ, Escalante-Semerena JC. In *Salmonella enterica*, 2-methylcitrate blocks gluconeogenesis. *J Bacteriol* 2010;192:771–778.
48. Eoh H, Rhee KY. Methylcitrate cycle defines the bactericidal essentiality of isocitrate lyase for survival of *Mycobacterium tuberculosis* on fatty acids. *Proc Natl Acad Sci USA* 2014;111:4976–4981.
49. Digianantonio KM, Korolev M, Hecht MH. A non-natural protein rescues cells deleted for a key enzyme in central metabolism. *ACS Synth Biol* 2017;6:694–700.
50. Lee SA, Gallagher LA, Thongdee M, Staudinger BJ, Lippman S *et al.* General and condition-specific essential functions of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 2015;112:5189–5194.
51. Singh A, Crossman DK, Mai D, Guidry L, Voskuil MI *et al.* *Mycobacterium tuberculosis* WhiB3 maintains redox homeostasis by regulating virulence lipid anabolism to modulate macrophage response. *PLoS Pathog* 2009;5:e1000545.
52. Lee W, Vanderven BC, Fahey RJ, Russell DG. Intracellular *Mycobacterium tuberculosis* exploits host-derived fatty acids to limit metabolic stress. *J Biol Chem* 2013;288:6788–6800.
53. Martinot AJ, Farrow M, Bai L, Layre E, Cheng TY *et al.* Mycobacterial metabolic syndrome: LprG and Rv1410 regulate triacylglyceride levels, growth rate and virulence in *Mycobacterium tuberculosis*. *PLoS Pathog* 2016;12:e1005351.
54. Singhal A, Arora G, Virmani R, Kundu P, Khanna T *et al.* Systematic analysis of mycobacterial acylation reveals first example of acylation-mediated regulation of enzyme activity of a bacterial phosphatase. *J Biol Chem* 2015;290:26218–26234.
55. Nazarova EV, Montague CR, La T, Wilburn KM, Sukumar N *et al.* Rv3723/LucA coordinates fatty acid and cholesterol uptake in *Mycobacterium tuberculosis*. *elife* 2017;6 e26969.
56. Zimmermann M, Kogadeeva M, Gengenbacher M, McEwen G, Mollenkopf HJ *et al.* Integration of Metabolomics and Transcriptomics Reveals a Complex Diet of *Mycobacterium tuberculosis* during Early Macrophage Infection. *mSystems* 2017;2:e00057-17.
57. Pandey AK, Sassetti CM. Mycobacterial persistence requires the utilization of host cholesterol. *Proc Natl Acad Sci USA* 2008;105:4376–4380.
58. Griffin JE, Pandey AK, Gilmore SA, Mizrahi V, McKinney JD *et al.* Cholesterol catabolism by *Mycobacterium tuberculosis* requires transcriptional and metabolic adaptations. *Chem Biol* 2012;19:218–227.
59. Muñoz-Elías EJ, Upton AM, Cherian J, McKinney JD. Role of the methylcitrate cycle in *Mycobacterium tuberculosis* metabolism, intracellular growth, and virulence. *Mol Microbiol* 2006;60:1109–1122.
60. Vanderven BC, Fahey RJ, Lee W, Liu Y, Abramovitch RB *et al.* Novel inhibitors of cholesterol degradation in *Mycobacterium tuberculosis* reveal how the bacterium's metabolism is constrained by the intracellular environment. *PLoS Pathog* 2015;11:e1004679.
61. Yang X, Nesbitt NM, Dubnau E, Smith I, Sampson NS. Cholesterol metabolism increases the metabolic pool of propionate in *Mycobacterium tuberculosis*. *Biochemistry* 2009;48:3819–3821.
62. Hung CC, Garner CD, Slauch JM, Dwyer ZW, Lawhon SD *et al.* The intestinal fatty acid propionate inhibits *Salmonella* invasion through the post-translational control of HilD. *Mol Microbiol* 2013;87:1045–1060.
63. van Immerseel F, de Buck J, Pasmans F, Velge P, Bottreau E *et al.* Invasion of *Salmonella enteritidis* in avian intestinal epithelial

cells *in vitro* is influenced by short-chain fatty acids. *Int J Food Microbiol* 2003;85:237–248.

64. **Argüello H, Carvajal A, Costillas S, Rubio P.** Effect of the addition of organic acids in drinking water or feed during part of the finishing period on the prevalence of *Salmonella* in finishing pigs. *Foodborne Pathog Dis* 2013;10:842–849.
65. **Gart EV, Suchodolski JS, Welsh TH, Alaniz RC, Randel RD *et al.*** *Salmonella* Typhimurium and multidirectional communication in the gut. *Front Microbiol* 2016;7:1827.
66. **Ciarlo E, Heinonen T, Herderschee J, Fenwick C, Mombelli M *et al.*** Impact of the microbial derived short chain fatty acid propionate on host susceptibility to bacterial and fungal infections *in vivo*. *Sci Rep* 2016;6:srep37944.
67. **Brook I.** The role of anaerobic bacteria in upper respiratory tract and other head and neck infections. *Curr Infect Dis Rep* 2007;9:208–217.
68. **Flynn JM, Niccum D, Dunitz JM, Hunter RC.** Evidence and role for bacterial mucin degradation in cystic fibrosis airway disease. *PLoS Pathog* 2016;12:e1005846.