



LETTER TO THE EDITOR

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Compatible solutes: the key to *Listeria's* success as a versatile gastrointestinal pathogen?

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Abstract

Recently we reported a role for compatible solute uptake in mediating bile tolerance and increased gastrointestinal persistence in the foodborne pathogen *Listeria monocytogenes* [1]. Herein, we review the evolution in our understanding of how these low molecular weight molecules contribute to growth and survival of the pathogen both inside and outside the body, and how this stress survival mechanism may ultimately be used to target and kill the pathogen.

The Gram-positive foodborne pathogen *Listeria monocytogenes* is a causative agent of gastroenteritis [2,3] and in severe cases, listeriosis, which ranges from a mild flu-like illness to meningitis, or as infection of the foetus in pregnant women. Described as a 'Jekyll and Hyde' character [4], *L. monocytogenes* exhibits saprophytic and parasitic lifestyles; residing both in decaying plant matter in the soil [5], and as a transient inhabitant of the gastrointestinal (GI) tract of several animal species including man [6].

This physiological robustness (the ability to adapt to a variety of different environments) results from an ability to sense and respond rapidly to changes in the external environment [7]; a response mediated by a complex arsenal of genes encoding proteins linked to survival both within and outside of the host [8]. One such response, which has been the focus of significant research efforts in our laboratories, is the accumulation (either by transport [9] or synthesis [10,11]) of compatible solutes - low-molecular-weight molecules which when amassed to high intracellular concentrations help ameliorate the effects of several stressful conditions [12].

The preferred compatible solutes for the majority of bacteria and those most effective in *L. monocytogenes* are the trimethylammonium compounds; betaine, which is found in relatively high concentrations in foods of plant origin [13,14] and carnitine, which is most abundant in animal tissues [15]. Functional genomic studies,

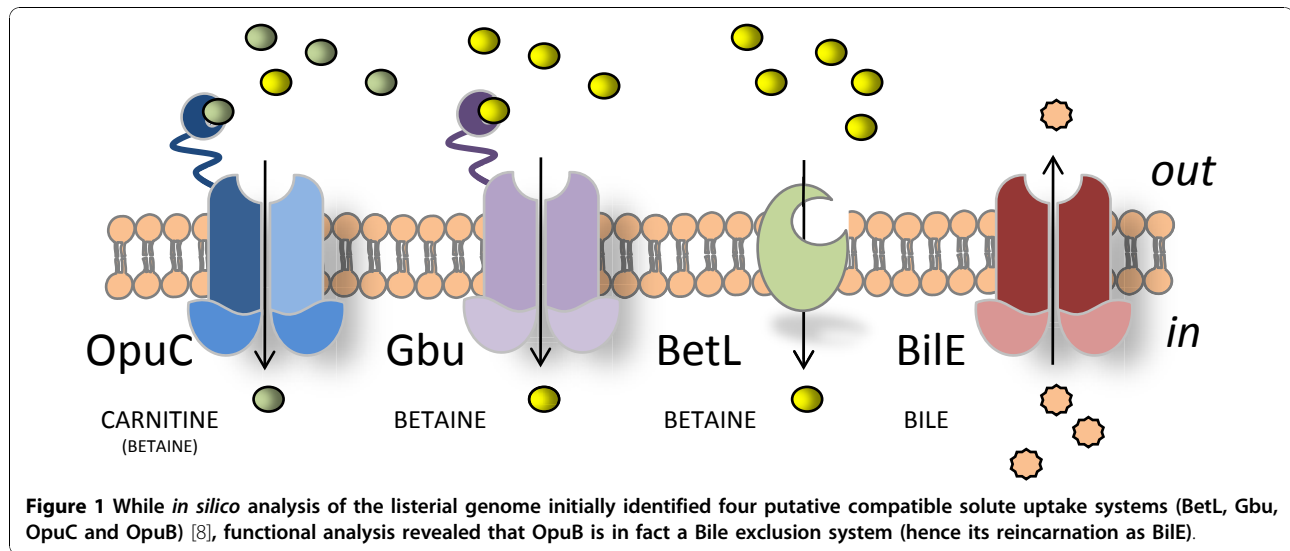
coupled with *in silico* analysis of genome sequences [8], revealed four putative compatible solute uptake systems in *L. monocytogenes*: BetL and Gbu (dedicated to betaine uptake), OpuC (which transports carnitine and to a lesser extent betaine) and OpuB which was designated as a putative compatible solute uptake system solely on the basis of sequence homology to the betaine uptake system BusA (OpuA) of *Lactococcus lactis* (Figure 1) [16].

Although initially identified as osmoprotective compounds (facilitating growth of the pathogen in low a_w environments), subsequent studies revealed a multitude of beneficial effects arising from compatible solute accumulation; including protection against desiccation [17], low temperature [18] and high pressure [19] stresses encountered in foods and/or food processing environments. However, in addition to facilitating growth and survival in external environments, we have revealed a significant role for OpuC (and more specifically carnitine uptake) in enabling growth and survival of the pathogen within the host GI tract [15]. Inactivating the *opuC* gene, and thus reducing carnitine uptake, resulted in a significant reduction in the ability of *L. monocytogenes* to colonize the upper small intestine and cause subsequent systemic infection following oral inoculation. Given that the osmolarity of the gut (equivalent to 0.3 M NaCl) represents an osmotic challenge to the pathogen and that carnitine is the most abundant compatible solute in that environment (0.05 to 0.2% on a fresh weight basis), this finding was perhaps predictable. But is this the full story? Is osmotolerance alone responsible for the increased gut colonization and persistence ascribed to *opuC* in

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L. monocytogenes, or indeed *betL* when heterologously expressed in *Bifidobacterium breve*? [20]

Double, Double, BilE and Trouble

Despite exhibiting significant sequence similarity to members of the betaine carnitine choline transporter (BCCT) family (hence the original Opu nomenclature for osmoprotectant uptake) OpuB has to date failed to display any appreciable compatible solute uptake, suggesting an alternative role for the protein. Indeed, a more detailed bioinformatic analysis of the sequence revealed two ATP-dependent bile acid permease signature sequences in the first gene of the operon [21]. Common to bile efflux pumps these motifs suggested a possible role for OpuB in listerial bile tolerance. Produced in the liver, stored interdigestively in the gall bladder and secreted into the duodenum, bile represents a far more immediate challenge to the pathogen than osmolarity and, as such, is the foremost innate immune defense mechanism of the upper small intestine [22]. Phenotypic analysis of the *in silico* findings using radiolabelled bile efflux studies revealed that OpuB did in fact function as a bile exclusion system - actively extruding bile from the bacterial cell - a phenotype which significantly modulates the virulence potential of the pathogen. That OpuB functions as a bile tolerance locus, as opposed to an osmolyte uptake system as was originally believed, led to its reincarnation as BilE (for BilE Exclusion) [21].

Notwithstanding its newly ascribed function as a bile resistance mechanism, the similarity of BilE to compatible solute uptake systems, together with the fact that it is transcriptionally regulated by the alternative sigma factor σ^B [22], along with BetL, Gbu and OpuC [8],

suggested a common function for all four proteins; if not in osmotolerance then perhaps in bile tolerance...

In support of this hypothesis a systematic analysis of strains with mutations in the primary compatible solute uptake systems also revealed roles for OpuC, and to a lesser extent BetL, in resisting the acute toxicity of bile [1]. Furthermore, real-time gene expression profiling in the presence of bile, using a *lux* gene reporter system, revealed that both *betL* and *opuC* are induced by bile. Interestingly, while *opuC* is more highly expressed *in vitro*, *betL* exhibits higher expression levels *in vivo*. Significantly, in addition to BetL, Gbu, OpuC and BilE; σ^B has also been shown to regulate the expression of BSH (a bile detoxification system) and, as such, may act as the master regulator of bile tolerance in the GI tract.

Listeria's Achilles heel?

The fact that compatible solutes protect *L. monocytogenes* at all stages of its lifecycle, from saprophyte to parasite, makes them a potentially important target for controlling the pathogen. Regulating the levels and/or availability of specific compatible solutes in high risk foods, e.g. baby formula (where carnitine is often added as a vitamin-like supplement), is an obvious first step [23]. While pathogen control during infection may be mediated by 'smugglin technology' - the application of toxic analogues - bactericidal compounds which, because they resemble compatible solutes, are accumulated by and ultimately kill the pathogen. Another approach which has received considerable attention in recent times is based on the patho-biotechnology concept [24-29] - the application of pathogen derived virulence or stress survival factors for the construction of improved probiotic strains as biological control agents [30]. These alternative approaches to pathogen control,

borne out of a clear understanding of how the pathogen adapts to its specific environment (both inside and outside the host) may ultimately provide us with a viable alternative to antibiotics for controlling old adversaries such as *L. monocytogenes*, as well as new and emerging pathogens - the so called "super bugs" [26,28].

Acknowledgements

The authors wish to acknowledge the continued financial assistance of the Health Research Board (HRB) and the Alimentary Pharmabiotic Centre (APC) through funding by Science Foundation Ireland (SFI). This letter is based on a manuscript entitled Compatible solutes: A listerial passe-partout? In *Gut Microbes* 1(2), 77-79.

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Authors' contributions

RDS and CH conceived of the study and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 6 December 2010 Accepted: 10 December 2010

Published: 10 December 2010

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doi:10.1186/1757-4749-2-20

Cite this article as: Sleator and Hill: Compatible solutes: the key to *Listeria's* success as a versatile gastrointestinal pathogen?. *Gut Pathogens* 2010 **2**:20.

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