Designer probiotics: a potential therapeutic for *Clostridium difficile*?

The continuing demographic shift towards a more elderly society, coupled with an ever increasing dependence on conventional therapeutics and an alarming escalation in antibiotic resistance, has facilitated the emergence of a new faction of bacterial adversaries, the 'superbugs', the aetiological agents of nosocomial infections. One of the most notorious of this emerging group is *Clostridium difficile*, the most common identifiable cause of bacteria-associated diarrhoea in the United States and the major cause of gastroenteritis in nursing homes and health-care facilities for the elderly (Crogan & Evans, 2007).

In addition to hospitalization, the most significant predisposing factors for *C. difficile* infection include advanced age (>65 years) and antibiotic therapy. The most common antibiotic inducing agents implicated to date include the broad-spectrum cephalosporins, while the only remaining effective therapeutic agents are metronidazole and vancomycin (McFarland, 2005). Despite being the most effective antibiotic, vancomycin use carries with it the added risk of secondary colonization by vancomycin-resistant enterococci and/or the spread of vancomycin resistance to other potentially dangerous hospital 'superbugs' such as methicillin-resistant *Staphylococcus aureus*.

Against this backdrop, the last decade has seen the emergence of a new epidemic of *C. difficile*-associated disease (CDAD) (Kuijper *et al.*, 2007). Linked to the hypervirulent ribotype 027, this epidemic is characterized by increased frequency and severity of enteric disease and is significantly more recalcitrant to standard antibiotic therapy. Faced with this epidemic, clinicians and researchers alike are now struggling to find viable therapeutic alternatives (McFarland, 2005). One such alternative involves the use of probiotics; these are defined as 'live microorganisms, which when consumed in

adequate amounts, confer a health benefit on the host'. Probiotic therapy has become the focus of considerable research efforts in recent times (Sleator & Hill, 2007a). Indeed, numerous clinical studies have attributed a myriad of impressive health-promoting effects to probiotics, including effective treatment of certain digestive and metabolic disorders as well as antagonistic activities against a variety of microbial pathogens. Hickson *et al.* (2007) recently reported that consumption of a commercially available probiotic drink can reduce the incidence of CDAD in a hospital setting and has the potential to decrease health-care costs, morbidity and mortality if used routinely in patients aged over 50.

While the exact mechanisms by which probiotic bacteria inhibit pathogens such as *C. difficile* are as yet poorly understood, some advances have nevertheless been made in our understanding of probiotic function. Recent work in our laboratory, for example, revealed that the therapeutic potential of the probiotic strain *Lactobacillus salivarius* is due, at least in part, to its ability to produce a potent two-peptide bacteriocin, Abp118 (Corr *et al.*, 2007). Furthermore, Rea *et al.* (2007) recently showed significant anti-*C. difficile* potential for yet another bacteriocin, the two-component lantibiotic lacticin 3147, produced by *Lactococcus lactis*. Significantly, and in contrast to conventional broad-spectrum antibiotics, lacticin 3147 completely eliminates 10^6 c.f.u. *C. difficile* ml⁻¹ within 30 min (at concentrations as low as 18 µg ml⁻¹) without dramatically impacting on the normal resident microflora. While this work involved *in vitro* studies in model faecal environments, *in vivo* sensitivity of the bacteriocin to gastric acidity creates a technological/delivery hurdle which will have to be overcome if this bacteriocin is to achieve its potential as an effective oral therapeutic (Gardiner *et al.* 2007).

A novel solution to the loss of activity associated with direct ingestion of the

bacteriocin is to clone and express the genes for bacteriocin production (and immunity) into an appropriate bacterial carrier, such as *Lactobacillus salivarius*. This approach circumvents *in vivo* degradation of the bacteriocin during gastric transit and facilitates continued bacteriocin production at the site of infection (the sigmoid colon), while at the same time dramatically improving the clinical efficacy of the probiotic (Fig. 1a). Such 'designer probiotics' may be further manipulated, using a patho-biotechnology-based approach (Sleator & Hill, 2006, 2007b), to improve growth and survival potential both *ex vivo* (Sheehan *et al.*, 2006) and *in vivo* (Sheehan *et al.*, 2007).

In addition to modulating their antimicrobial spectrum and improving their physiological stress tolerance, recent studies have led to the development of probiotic strains engineered to express receptor mimics on their surface. These surface proteins specifically target enteric infections by blocking crucial ligand-receptor interactions between the pathogen and host cell (Paton *et al.*, 2006; Fig. 1b). Blocking this adherence reduces infection, while toxin neutralization ameliorates symptoms until the pathogen is eventually overcome by the immune system, as a result of bacteriocin production, or combined antibiotic therapy. Given that a significant feature of the *C. difficile* epidemic strain 027 is increased production of toxin A and B (Warny *et al.*, 2005), the ability to neutralize or 'mop up' these toxins would result in a significantly improved prognosis (Fig. 1c). In support of this proposal, Paton *et al.* (2000) described the construction of a probiotic *Escherichia coli* strain expressing a chimeric lipopolysaccharide terminating in a shiga toxin (Stx) receptor, 1 mg dry weight of which can neutralize >100 µg Stx1 and Stx2. Designer probiotics with receptor blocking potential against enterotoxigenic *E. coli* toxin LT and cholera toxin (Ctx) have also been described (Paton *et al.*, 2006).

Fig. 1. Overview of the anti-*C. difficile* potential of designer probiotics. Bacteriocin produced by the probiotic (dark shading) can lyse invading *C. difficile* cells (light shading) (a), while heterologously expressed receptor mimics on the surface of probiotic cells can antagonize pathogen adherence to the host (b) and neutralize toxin production (c).

In conclusion then, while conventional medical research continues in its attempts to develop effective therapeutic and prophylactic compounds against *C. difficile*, their application is often complicated by *in vivo* sensitivity and rising production costs. 'Designer probiotics' on the other hand provide an effective means of circumventing the short half-life and fragility of conventional therapeutics, providing a cost-effective alternative which will ultimately contribute to health and social gain (Sleator & Hill, 2007c). Indeed, McFarland (2005) in her seminal review on alternative approaches to the control of *C. difficile* proposed that effective treatment of CDAD needs to 'reduce the burden of *C. difficile* and its toxins in the intestine, restore the normal colonic microflora and assist the host's immune system'. Designer probiotics satisfy all three of these requirements, thus making them an ideal alternative treatment for *C. difficile*. However, while consumer acceptance of genetically engineered micro-organisms still remains a significant challenge, this obstacle should eventually be overcome by the application of rigorous scientific controls, such as adequate

biological containment, and proper risk-benefit analysis of the potential advantages of such a strategy.

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