Cloning and expression of the listerial bilE operon (encoding a membrane encoded bile exclusion system) in Lactobacillus salivarius resulted in a significant increase in in vitro bile tolerance; the transformed strain exhibiting a 3 log increased survival relative to the control strain. Furthermore, in vivo studies using Balb/c mice showed that the transformed strain persisted for longer and at significantly higher numbers than the control which lacks bilE – this improved persistence translated to an increased anti- C. difficile effect, presumably outcompeting the pathogen in the gut.
Current Knowledge and Background

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 etiological agents of nosocomial infections.

One of the most notorious of this emerging group is *Clostridium difficile*; the most common identifiable cause of bacterial 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). Indeed it has been estimated that *C. difficile* infections affect as many as 1.2% of hospitalized patients in the US, representing an estimated cost of $1.1 billion (Kyne et al., 2002). In Ireland outbreaks of *C. difficile* have affected major hospitals and community acquired infections have been reported (Kyne et al., 1998). While the current cost of *C. difficile* in the global health care system is not clear, the financial and social costs are likely to be significant.

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 hyper-virulent ribotype 027, this epidemic is characterised by increased frequency and severity of enteric disease and is significantly more recalcitrant to standard antibiotic therapy. Faced with this epidemic clinicians are now struggling to find viable therapeutic alternatives (McFarland, 2005). One such alternative involves the use of probiotics; 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, 2008). 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 healthcare costs, morbidity and mortality if used routinely in patients aged over 50.

‘Designer probiotics’ therefore 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, 2008). 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* 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*. 
The study hypothesis
Certain probiotic bacteria have been shown to stimulate the mucosal immune system and inhibit gut associated bacterial infections. Rational genetic manipulation of such cultures, improving delivery and clinical efficacy, will ultimately lead to the development of effective therapeutics; targeting chronic gut associated pathogens such as *C. difficile* for which few adequate therapeutic agents exist, resistance is increasing and effective vaccines are currently not available.

The overall aim of the study was to develop an effective alternative to antibiotic therapy for the control of *C. difficile*-associated disease (CDAD).

The main findings
Cloning and expression of the listerial *bilE* operon (encoding a membrane encoded bile exclusion system) in *Lactobacillus salivarius* resulted in a significant increase in *in vitro* bile tolerance; the transformed strain exhibiting a 3 log increased survival relative to the control strain. Furthermore, *in vivo* studies using Balb/c mice showed that the transformed strain persisted for longer and at significantly higher numbers than the control which lacks *bilE* – this improved persistence translated to an increased anti- *C. difficile* effect, presumably outcompeting the pathogen in the gut.

Unfortunately, despite several attempts, we failed to clone and express either the thuricin or lactisin bacteriocins in our *L. salivarius* strain.

Conclusions
Despite failing to heterologously express anti-*C. difficile* bacteriocins against our *L. salivarius* background, we have none the less proved that rational genetic manipulation to improve stress tolerance; facilitating improved gut persistence is effective in ameliorating *C. difficile* infection and may be used as a viable alternative to conventional antibiotic therapy.