

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

Martin Stocks PhD
 martin@pbltechnology.com
 Tel: +44 (0)1603 456500
 Fax: +44 (0)1603 456552
 Tech ID: 07.434

A lead biologic for the treatment of *Clostridium difficile* infection

**Highly active, species-specific lysin.
 Effective for all strains of *C. difficile* tested to date.**

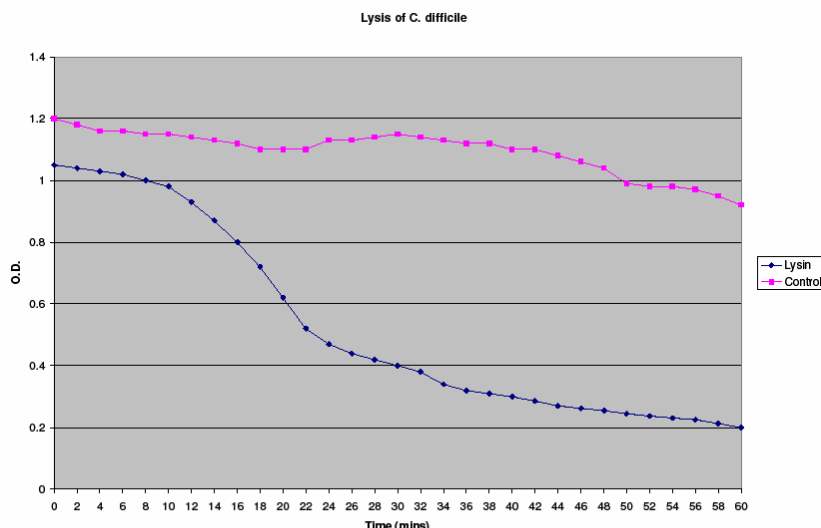
Patent Literature

Not yet published. Further details available under CDA.

Hospital-based nosocomial infections, often associated with antibiotic use, are a growing problem globally. MRSA and MSSA have received most attention to date, but serious infections caused by *Clostridium difficile* (*C. difficile*-associated disease, or CDAD) are a growing problem, with infection rates, morbidity and mortality increasing in the UK and worldwide. In England and Wales alone, CDAD notifications had reached 35,500 by 2003 and in 2004 there were 2,247 deaths associated with *C. difficile* infection.

C. difficile is an anaerobic Gram positive bacterium that is normally present in the gut of approximately 3% of the population. Only certain strains carry the toxin genes necessary for disease production. CDAD is believed to occur when the normal gut flora is disturbed, leading to an overgrowth of *C. difficile* and the production of toxins. *C. difficile* has the capacity to form spores that resist heating, drying and disinfectants. There is some evidence that exposure to non-chlorine based cleaning agents actually increases sporulation. These characteristics contribute to the organism's capacity to persist in the hospital environment, thereby maintaining a reservoir of pathogens with the potential to infect patients. In addition to the threat to human health, *C. difficile* is also a significant cause of morbidity and mortality in animals, particularly in farm animals such as calves and sheep.

There is a pressing need for a therapeutic regimen that will suppress or remove *C. difficile* infection without damaging or disrupting the normal bacterial flora of the gut. Several studies have attempted to isolate and characterize *C. difficile*-specific bacteriophage, in the hope that one could be developed as a therapeutic agent, but to date most phage known are lysogenic and therefore of limited use as therapeutic entities.



Although such lysogenic phage are of little value as agents in themselves, they carry in their genomes an **endolysin** protein that, if functional, could be developed as a therapeutic agent in the absence of phage replication.

Figure:

Timecourse showing the lysis of a suspension of *Clostridium difficile*, following the addition of purified recombinant Φ CD27 endolysin.



Scientists at the Institute of Food Research have recently identified a novel bacteriophage of *C. difficile* (Φ CD27) and have cloned, sequenced and over-expressed a functional **endolysin** from this phage. In the normal bacteriophage life cycle, **endolysins** are produced very late, and act to release mature bacteriophage by breaking down the cell wall of the host bacteria. Although **endolysins** normally act from the inside of the bacterial cell, it is a well known property of Gram+ve bacteria, such as *Clostridia*, that they are susceptible to lysis by exposure to exogenously applied **endolysin**. Furthermore, the unique biochemical makeup of individual bacterial species' cell wall structures results in **endolysins** often exhibiting highly host-specific activity. The **endolysin** isolated from the Φ CD27 bacteriophage selectively and efficiently lyses all *C. difficile* bacteria so far tested (30 isolates, including the highly pathogenic 027 (B1/NAP1) strain). In contrast, testing with a range of other gut commensal bacteria, including several examples of the *Clostridium*-like Firmicutes, showed no sensitivity to lysis. Although other lysogenic bacteriophage have been identified by homology searching in the genome of *C. difficile*, and putative **endolysin** sequences found, to date none of these have been shown to be active when expressed as recombinant protein (unpublished data).

The Φ CD27 **endolysin** is therefore unique in its capacity to be expressed in a highly active form by standard recombinant production technologies. It has significant potential for development as a therapeutic agent for delivery to the gut. An appropriately formulated **endolysin** could be used in a hospital setting either as a prophylactic or therapeutic agent.

PBL is seeking a commercial partner to further develop the **endolysin**, and the intellectual property is available for licence on an exclusive basis to an appropriate party. For further information, or to discuss licensing terms, please contact Dr Martin Stocks at PBL.

References:

Molecular Characterization of a *Clostridium difficile* Bacteriophage and Its Cloned Biologically Active Endolysin.
Melinda J Mayer, Arjan Narbad, and Michael J Gasson.
Journal of Bacteriology, October 2008, p 6734-6740, Vol 190, No 20.