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Ligowska, Malgorzata Anna; Cohn, Marianne Thorup; Brøndsted, Lone

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Role of Clp proteolytic complex in stress response of *Campylobacter jejuni*

Małgorzata Ligowska, Marianne Thorup Cohn and Lone Brøndsted

Campylobacter jejuni is a Gram-negative pathogen, recognized as the leading cause of bacterial foodborne infection in humans worldwide. It is a spiral-shaped, motile, microaerophilic bacterium that colonizes chicken intestine.

Similarly to other pathogens, it owes its ability to survive stress in the environment to various molecular mechanisms as well as physiological adaptation.

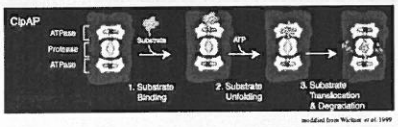
The Clp proteolytic complex is one of the mechanisms that allow *Campylobacter jejuni* to survive at various unfavourable conditions present inside, as well as outside of the host. It is responsible for degradation of misfolded proteins, which in turn prevents the formation of protein aggregates in the cell.

It was shown by Cohn *et al.* (2007) that in *C. jejuni*, ClpX and ClpP proteins are responsible for degradation of heat-damaged proteins and that ClpX and ClpP are required for growth at 42 and 44°C.

We will now further investigate the role of the Clp proteolytic complex in survival of *C.jejuni* under different stress conditions encountered by this bacterium in the environment and in the host.

C. jejuni strains with mutations in genes *clpP*, *clpX*, *clpA* and *clpS* will be tested for growth and survival in the conditions mentioned above, both *in vitro* and using different food models.

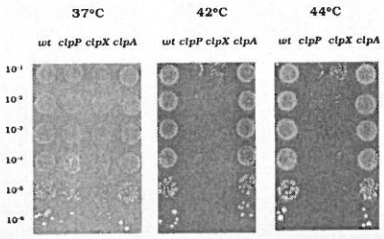
Clp PROTEASES ARE IMPORTANT FOR BACTERIA



- Involved in general and regulatory proteolysis
- Important for virulence and stress tolerance i many pathogens
- Their specificity may be modified by an adaptor protein

GROWTH OF *clp* MUTANTS AT DIFFERENT TEMPERATURES

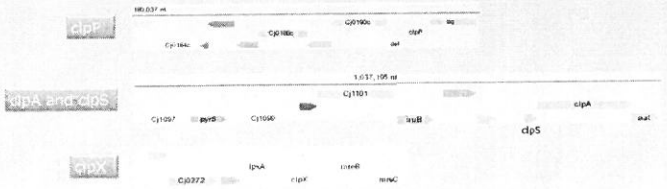
ClpX and ClpP are required for growth at 42 and 44°C



Cohn et al. 2007

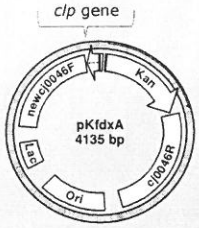
Cohn *et al.* (2007) observed that mutants lacking *clpX* or *clpP* gene were growing much poorer than the wild type at high temperatures (42°C and 44°C), suggesting that ClpX and ClpP are required for growth of *C. jejuni* at these temperatures. A ClpA mutant, however, could grow just as well as the wild type.

clp GENES IN *C. jejuni* ARE EXPRESSED IN OPERONS



Northern blot analysis of *ΔclpP::cat* mutant showed a slight reduction in expression of the downstream *def* gene, indicating a polar effect of the insert on *def*.
Therefore, in order to verify that the observed phenotype of mutants is due to lack of one of the Clp proteins, we will complement the gene of interest.

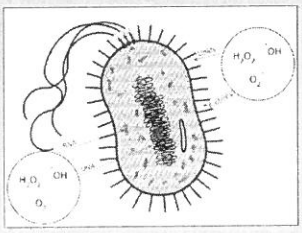
METHOD FOR CREATING NEW MUTANTS – COMPLEMENTATION OF *clp* GENES



clp gene is inserted into the *C. jejuni* chromosome

The phenotype of complemented mutant can be compared to *Δclp* and wild type

ROLE OF CLP PROTEINS IN OXIDATIVE STRESS



Oxygen stress is the main type of stress encountered by *Campylobacter* during the poultry processing chain. Strains are exposed to atmospheric oxygen immediately after evisceration of a chicken in a slaughter house.

Exposure to oxidative stress leads to many, often irreversible, alterations in bacterial proteins. Clp proteases can therefore play a major role in response to this type of stress.

We will test:
- Survival of mutants after exposure to different oxidative agents
  - Hydrogen peroxide
  - Cumene hydroperoxide
  - Methyl viologen
- Survival of mutants after exposure to atmospheric oxygen

We will also investigate types of protein modifications that can occur in *Campylobacter* during oxidative stress and the specific mechanisms, by which Clp proteases could act on these modified proteins.

ROLE OF CLP PROTEINS IN ADAPTATION TO STATIONARY PHASE

The following is known about *clpP* mutant behaviour in stationary phase (M. Cohn, unpublished results):

- *clpP* mutant cells have aberrant morphologies in stationary phase
- In late stationary phase, *clpP* mutant has lower viability than the wild type

This indicates that there is a possible role for ClpP in the stationary phase.

Campylobacter jejuni does not have a stationary phase response regulator typical in other bacteria, RpoS. However, a characteristic behaviour is observed under prolonged incubation. A rapid decrease in CFU/ml is followed by a rapid increase in cell numbers, probably caused by appearance of dynamic populations (Kelly *et al.*).

We would like to examine the viability of *C. jejuni* strains when *clpP*, *clpA*, *clpX* or *clpS* are absent and also to investigate whether there is a relation between exposure to oxygen and stationary phase survival.

ROLE OF Clp PROTEINS IN COLONIZATION OF THE HOST

- In *H.pylori*, a double *clpAP* mutant was unable to colonize the murine host (Loughlin *et al.*)
- Single *clpA* and *clpP* mutants colonized just as well as the parental strain

The ability to get rid of damaged proteins seems to be crucial in order for an infection to establish.

We would like to test the ability of *clp* mutants to colonize mice, compared to the wild type.

References
Cohn *et al.* 2007 *Applied and Environmental Microbiology*, **73**:7803-7813
Kelly *et al.* 2001 Applied and Environmental Microbiology, **67**: 2248-2254
Loughlin *et al.* 2009 *Microbial Pathogenesis*, **46**: 53-57
Wickner *et al.* 1999 *Science*, **286**

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