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Sequence and comparative analysis of *Lactococcus lactis* c2 dairy bacteriophages with different thermal inactivation

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Introduction

The prolate headed *Lactococcus lactis* c2 phages are among the 3 most common species isolated in the dairy industry. It was previously believed that the c2 phage species showed a very narrow heat inactivation spectrum, many inactivated by traditional pasteurization treatment of cheese milk. We recently characterized the thermal inactivation of eight c2 phages [Marvig *et al.* (2011) Int. Dairy J. 21, 556-560] and showed that c2 phages had an inactivation span (8-log reduction) of at least 10°C from 70°C to 80°C.

Comparative genomics of c2 phages

We have recently sequenced five c2 phages using 454 Next Generation Sequencing Technology. The draft sequence was aligned in Figure 1 using the Artemis Comparison Tool. The published sequences of c2 and bIL67 were included in the comparison. The comparison showed that c2 phages like the 936 phages have conserved genomes.

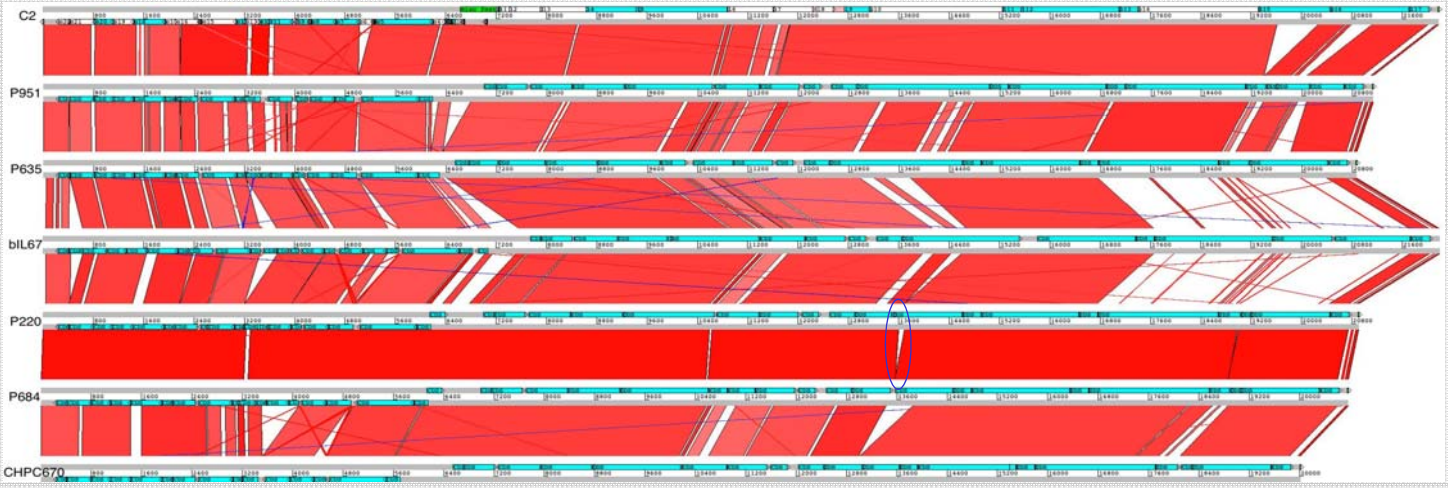

Figure 1: Comparison of the putative ORF's from the c2 phages c2, P951, P635, bIL67, P220, P684 and CHPC670 using the Artemis Comparison Tool. Increasing colour intensity indicates increasing homologies. Blue ring indicate region in I10 homologue with deletion in P684 compared to P220.

Comparative genomics of c2 phages with different thermal inactivation

The phages P220 and P684 had identical host range (data not shown) and highly similar DNA sequence as seen from Figure 1 and Figure 2B. However, the two phages have different thermal inactivation as seen in Figure 2A. At 70°C for 5 min P220 was completely inactivated (8 log reduction) while P684 only had a 4.5 log reduction.

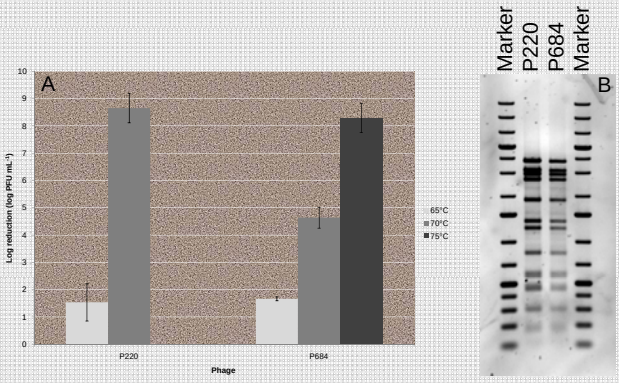

Figure 2. A. Thermal inactivation of P220 and P684 for 5 min at given temperature. B. Restriction endunuclease digestion pattern of P220 and P684 with HinfI. Marker is 1 kb ladder

Conclusion

- Phages of the c2 species have a conserved genome structure (Figure 1).
- Phage P220 and P684 have almost identical genome sequence but differences in thermal inactivation (Figure 2).
- The more stable P684 have a 93 bp deletion (31 a.a.) in the I10 gene homologue (Figure 3)
- L10 has in phage c2 been localized to the tail tip [Lubbers *et al.* (1995)]
- L10 may be involved in tail initiation complex and thereby control of phage stability

Acknowledgement

We thank Chr. Hansen A/S and Danisco for providing the phages CHPC670 and P951, respectively

Is the late expressed L10 gene product involved in thermal stability?

When the genomes of P220 and P684 are compared the main difference in the structural part of the genome is a 93 bp deletion in the I10 gene homologue (Figure 3A). This gives a 31 a.a. deletion (Figure 3B)

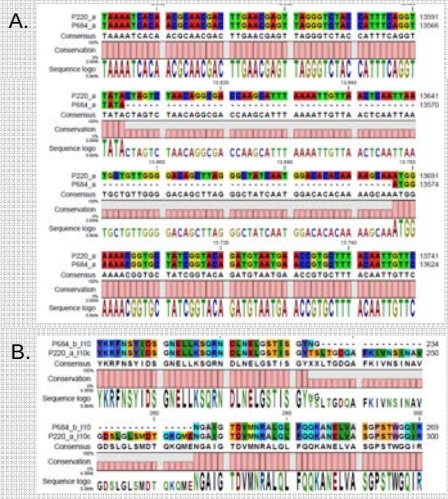

Figure 3. Comparaison of part of I10 gene sequence (A) and corresponding a.a. sequence (B)

The L10 gene product has in phage c2 has been described as a protein located by immuno-gold labeling to the tip of the tail [Lubbers *et al.* (1995) Appl. Environ. Microbiol., 61, 4348-4356], and suggested to be the anti-receptor . However, later L15 was shown to be the anti-receptor [Stuer-Lauridsen *et al.* (2003) Virology, 309, 10-17]. We speculate that L10 could be part of the tail initiator complex and as such have confound influence of stability of the phage particle. Further work is needed to support this hypothesis.