Characterisation of the activity of a novel DNA ligase enzyme encoded by a crAss-like bacteriophage from the human gut

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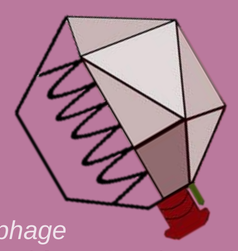
Introduction

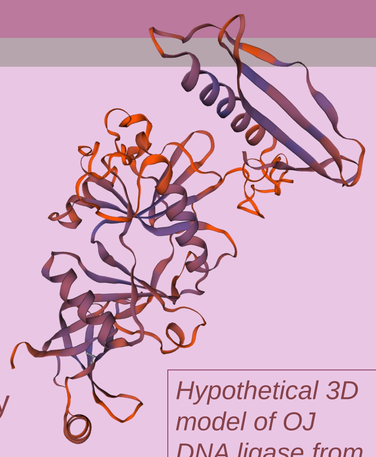
DNA ligases are essential enzymes in all forms of life and a valuable tool in the biotech industry. They help joining pieces of DNA during DNA replication and repair. As highly divergent organisms are a rich source of novel enzyme activities, the recently discovered family of viruses – crAss-like bacteriophages (crAssphages), are of an increasing interest for scientists. This family are also the most abundant viruses in human gut. Some members of the family appear to encode their own ATP-dependent DNA ligases, but these have not been studied before.



crAssphage (adapted from Guerin et al., 2018).

Aim

Purify and biochemically characterise the **first example of a crAssphage DNA ligase enzyme**, as a prelude to exploring its utility in biotech applications.



Hypothetical 3D model of OJ DNA ligase from a crAss-like phage

Methods

- **Molecular cloning:** genetically engineered bacteria (*E. coli*) to express the crAss-like phage DNA ligase enzymes.
- **Mutagenesis**: created an inactive mutant form of one of OJ DNA ligase enzyme, named OJ_m.
- **Purified** OJ and OJ_m DNA ligases from the mixture of native bacterial proteins.
- Tested the **ligation activity** of OJ, compared to the commercially available standard T4 phage DNA ligase and to OJ_m.

The adenylation domain (green) of OJ DNA ligase, where the mutation was introduced.

Lab Results

Expression and Purification:

Four DNA ligases were successfully expressed (fig.1). The expression of OJ DNA ligase was scaled-up and its catalytically inactive mutant, called OJm, was successfully expressed and purified in parallel (fig.2).

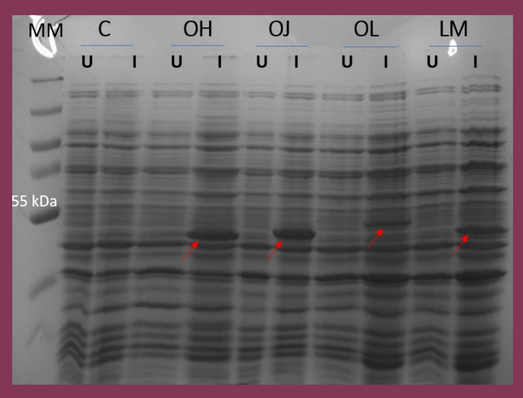


Fig.1. All crAss-like phage enzymes: OH, OJ, OL and LM, indicated by an arrow. Samples before (U) and after induction (I) of the expression. (C - control)

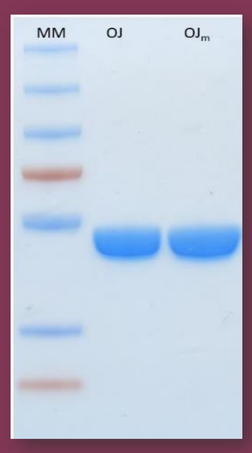
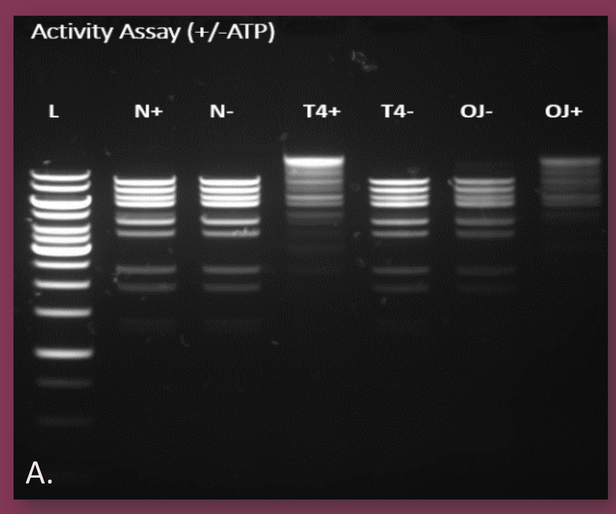


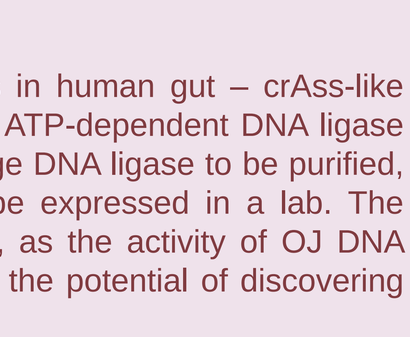
Fig.2. The purified OJ and mutant OJ_m DNA ligase enzymes

Activity Assays:

In the three assays the ligation activity of OJ DNA ligase was comparable with the activity of the commercially available standard T4 phage DNA ligase. The results show that the OJ ligase is a highly active ATP-dependent DNA ligase that is capable of ligating both cohesive and blunt DNA ends.

Fig.3. Catalytic activity assays testing the ligation activity of OJ DNA ligase. A. Testing the dependence of ATP (+/- indicate f ATP was present); B. and C. Testing the ligation activity on different substrates (B. λ DNA-BstEII Digest; C. λ DNA HindIII and HaeIII Digest).





Bioinformatics

We generated a phylogenetic tree of the distribution of DNA ligase enzymes across crAss-like phages, using 98 hypothetical DNA ligase sequences (fig.4). As crAssphages from the same genera were more closely related, the tree was similar to the ones found in literature, using the sequences of other enzymes. The next step will be to apply the findings in a wider context and see the position of crAss-like phages in the DNA ligase tree of viruses. This might help us understand the reasons for the diversity of DNA ligases across crAssphages.

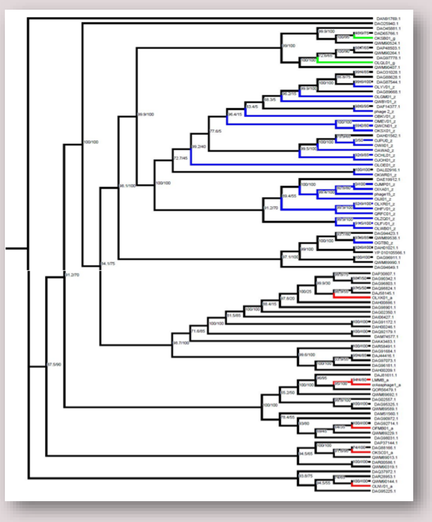


Fig.4. DNA ligase distribution across crAss-like phages. Genera shown with colour (green – gamma, red – alpha; blue – zeta). Phylogeny was inferred using IQ-Tree (Minh et al., 2020)

Conclusion

This study shows that the most abundant viruses in human gut – crAss-like bacteriophages can encode soluble and functional ATP-dependent DNA ligase enzymes. OJ DNA ligase is the first crAss-like phage DNA ligase to be purified, and one of the first crAss-like phage protein to be expressed in a lab. The project offers a solid base for future development, as the activity of OJ DNA ligase can be tested under various conditions with the potential of discovering novel properties of the enzyme.

Acknowledgments

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