THE ROLE OF NUCLEASE 46L IN DEGRADING NUCLEIC ACIDS FOR PRECISION FERMENTATION

Nucleases are a class of hydrolase enzymes (EC 3.1) that degrade nucleic acids. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two main classes of nucleic acids. DNA and RNA are macromolecules essential for any living form and they contain the genetic information that drive the synthesis of proteins and are responsible for the function of an organism/microorganism.

A nucleotide represents the building block of nucleic acids and it is made of i) a sugar molecule (either ribose in RNA or deoxyribose in DNA); ii) a phosphate group, iii) a nitrogen-containing base (adenine (A), cytosine (C), guanine (G) in both DNA and RNA, thymine (T) in DNA, uracile (U) in RNA. Nucleic acids are linear polymers in which the nucleotides are linked by phosphodiester linkages. DNA is a double stranded molecule, while RNA is a single stranded molecule.

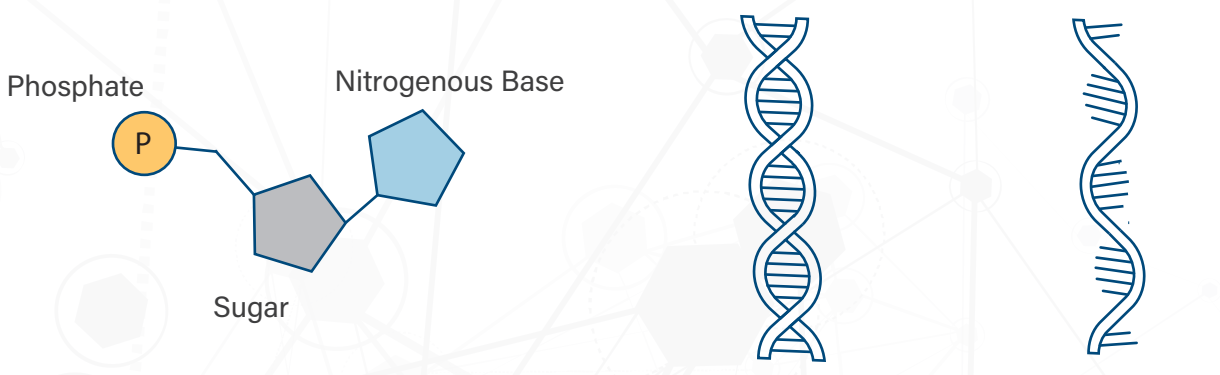

Figure 1: Structural representation of a nucleotide and the two main classes of nucleic acids, DNA and RNA.

Nucleases specifically target the phosphodiester bonds between the nucleotides in the nucleic acids, resulting in the break down of nucleic acid structure.

Industrial biotechnology exploits the power of nucleic acids in numerous applications including microbial strain development for small molecule (chemical) manufacturing and large molecule (proteins and enzymes) manufacturing via microbial fermentation. This underpins innovation in food enzymes, food proteins, pharmaceutical molecules and diagnostic applications.

Microbial fermentation as a technology has been utilised across industries for centuries. More recently precision fermentation has been adopted as an economical technology for the production of highly specific functional proteins and ingredients at vast commercial volumes using microbial hosts as cell factories. In microbial precision fermentation, nucleic acids exist both as the host microorganism nucleic acids, and the recombinant DNA included for the production of the protein of interest.

Cell lysis is the disruption to the microbial cells which break down the cell walls and membranes and this can occur at the final stages of a fermentation process. Cell lysis during fermentation can present many challenges including adversely impacting primary recovery. The disruption of the cells causes the release of the nucleic acids present in the host cell microorganism. The presence of nucleic acids in the fermentation broth increases the viscosity which can negatively affect downstream processing and, as a consequence, the overall yields and protein recovery.

In addition to the reduced performance of the operational process and product loss, the presence of nucleic acids can contribute to impurities in the final product. Removal of any contaminating residual nucleic acids can reduce these impurities and result in a higher quality end product.

Nucleic Acid Hydrolysis Using Nuclease 46L

Nucleases can be divided into endonucleases (cleavage within a nucleic acid chain) and exonucleases (cleavage of the nucleic acid at either end of the chain).

Figure 2: The action of endonucleases and exonucleases

Nuclease 46L is a broad specificity food grade endonuclease that is highly efficient for the degradation and removal of DNA and RNA in a wide range of biotechnology applications. Nuclease 46L is a recombinant *Serratia marcescens* nuclease (EC 3.1.30.2) produced in *Pichia pastoris* by Biocatalysts Ltd.

The hydrolysis of nucleic acids (particularly DNA) using Nuclease 46L is an efficient solution for improving downstream processing, reducing downstream processing time and ensures higher protein recovery.

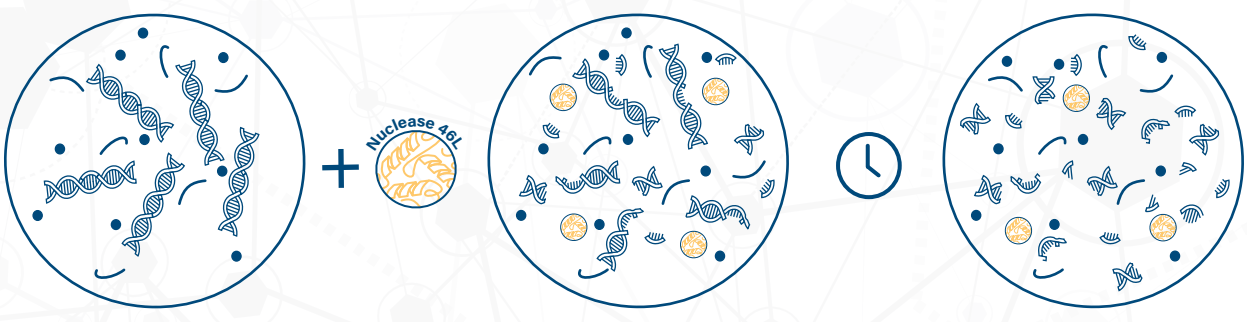

Figure 3: The action of Nuclease 46L in the degradation of nucleic acids

There are certain instances where it might be desirable to reduce or even remove any residual DNA in fermented proteins and enzymes, in addition to improving downstream processing efficiencies.

As a broad specificity food grade endonuclease, Nuclease 46L is highly efficient for the removal or reduction of nuleic acids. Figures 4 and 5. show the effective degradation of nucleic acids when Nuclease 46L is applied to either purified DNA (Figure 4), or complex protein samples such as final enzyme products of a *Pichia pastoris* production process (Figure 5). Nuclease 46L can be dosed at a range of 250-700 units of endonuclease per mL of liquid material, with the addition of magnesium chloride at a concentration range 1-2mM.

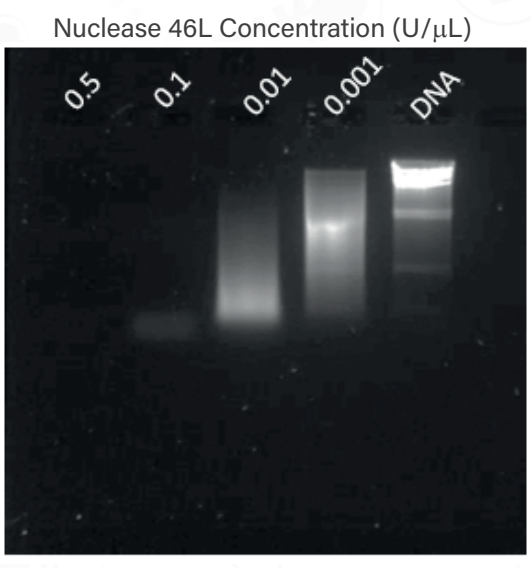

Figure 4. Agarose gel (1%): purified DNA treated with Nuclease 46L. λ DNA Hind III was incubated for 1 hour at 37°C with Nuclease 46L at different concentrations.

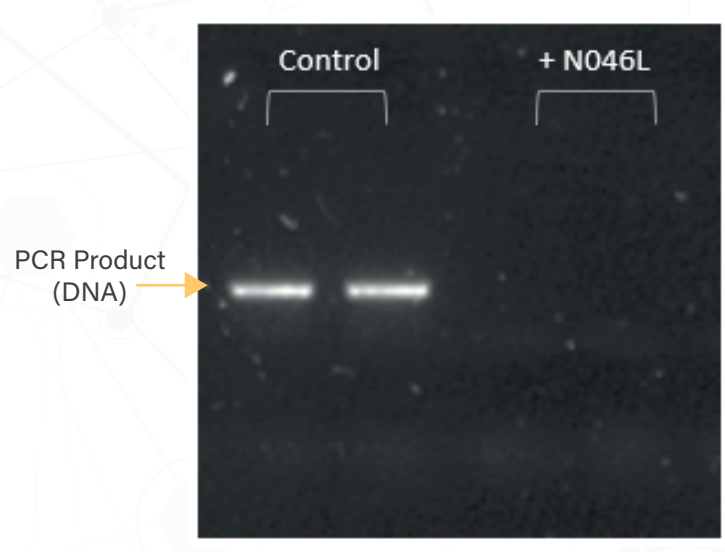

Figure 5. Agarose gel (1%): PCR of DNA extracted from an enzyme expressed in *Pichia pastoris* incubated (in duplicate) for 18 hours at 10°C with (+N046L) and without (control) Nuclease 46L.

Trials are recommended to determine the exact dosage for each application. Lower dosages may be sufficient for increasing the efficiency of downstream processing and other general applications where DNA reduction or removal is desirable.

CONCLUSION

The information in this paper demonstrates the importance of nucleic acids in biotechnology applications and makes reference to the requirement of reducing and removing nucleic acids to improve the efficiency of downstream processing and produce higher quality and purity end products.

If you could benefit from enzymatic degradation of nucleic acids, contact Biocatalysts Ltd for a free sample of Nuclease 46L. If you require a bespoke enzyme for your unique process, Biocatalysts Ltd can develop or scale up novel enzymes as part of our Enzyme Development and Manufacture service.

Disclaimer: It is the responsibility of the end user to ensure country and application specific compliance. Enzyme legislation is in place in various countries, please contact Biocatalysts for assistance on country specific regulations. Nuclease 46L is not authorised for use in a process as claimed in US20200140838A1 and its international counterparts.