# Endonuclease Compliance

Applications in red biotechnology are getting easier to submit as the market broadens for endonucleases. Now, new derivatives are being welcomed into the sector with fewer limitations

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A novel fermentative process, free of animal-based derivatives, uses endotoxin-free *Bacillus amyloliquefaciens* to economically produce the endonuclease from *Serratia marcescens* in high quantities. The new derivative is the first current Good Manufacturing Process (cGMP) product of its type, allowing its use in red biotechnology without regulatory restrictions.

Nucleases comprise a broad family of hydrolases that cleave nucleic acids. Several biotechnological applications have been reported, for example, in removing nucleic acids in biosynthetic processes to reduce viscosity associated to cell disruption or in the cost-effective elimination of nucleic acid impurities of a final (biosynthetic) product to comply with tight safety regulations. The production of the endonuclease from *S marcescens* has recently been successfully demonstrated in *Bacillus subtilis* – a gram-positive, endotoxin-free bacterium. Moreover, the fermentation is conducted in the absence of animal-based derivatives, thus, the novel derivative is an animal-free ingredient without the risk of bovine spongiform encephalopathy and transmissible spongiform encephalopathy (BSE/TSE). On that basis, the new endonuclease fulfils all cGMP requirements and may significantly broaden the applications of nucleases in biopharmaceuticals, promoting new markets and stimulating established ones.

## Applying Nucleases

Nucleases are hydrolases that cleave the phosphodiester bonds between monomers of nucleic acids (DNA and RNA), digesting them into smaller oligonucleotides. In nature, they are responsible for the proper maintenance of nucleic acids in cells, keeping the genetic quality control and being actively involved in DNA replication and reparation. Some unspecific nucleases display the cleavage of all forms of nucleic acids, namely DNA and RNA, single- or double-stranded, linear or circular, and without any defined sequence-specificity. In the realm of red biotechnology and molecular biology sciences, such nucleases have become useful biocatalysts for a broad number of applications. A remarkable example is the purification of biopharmaceuticals – for instance, biosynthesised proteins, vaccines, etc – by removing the nucleic acid impurities generated along the bioprocess, thus saving costs associated to the downstream processing and purification units. Another useful application is the reduction of viscosity in biological samples caused by the presence of nucleic acids when cells are disrupted during the work-up steps.

## An Efficient Alternative

An outstanding example is the endonuclease secreted by *S marcescens*, a gram-negative bacterium. That nuclease (a dimeric form) results highly active and promiscuous, being able to efficiently cleave all forms of nucleic acids, RNA and DNA, single- and double-stranded, as well as linear or circular sequences, leading to oligonucleotides of two to five base pairs. Besides such remarkably efficient catalytic performances, the enzyme displays a long-term stability at room temperature, remaining fully active in the presence of ionic and nonionic detergents, as well as when reducing and chaotropic agents are aggregated. Based on that robustness, its use in red biotechnology has been successfully demonstrated, as the enzyme can be adapted to many real processing conditions in which the presence of deleterious agents may be frequent.

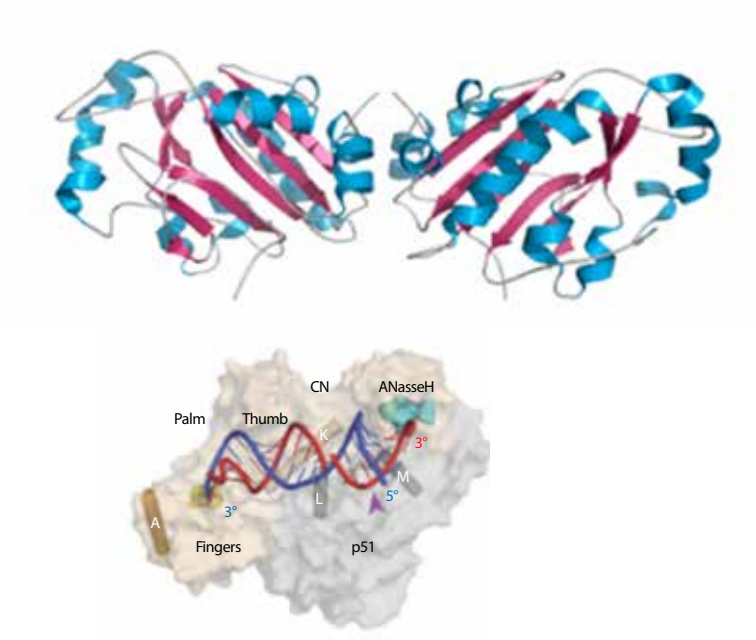


Figure 1: Dimeric structure and catalytic performance of the unselective endonuclease of *S marcescens*

In this respect, a genetically engineered form of this endonuclease, produced using gram-negative *Escherichia coli* as host, was commercialised years ago.

### Exploring a New Derivative

Due to its excellent features and performances, a new genetically engineered form of *S marcescens* endonuclease has been developed. The new product displays the expected excellent broad operational window of pH and temperature of the endonuclease. Quite remarkably, the new derivative offers unique selling points. The patented production host is not *E coli*, but a gram-positive *Bacillus sp*, an endotoxin-free strain. Moreover, the fermentation broth is free of animal-derived feedstocks and antibiotics, thus the endonuclease can be regarded as a BSE/TSE-free product with a high viral safety (as no animal derivatives are involved in the production). Conclusively, the endonuclease from *S marcescens* can now be delivered in full compliance with the EU cGMP requirements. Last, but not least, it can be produced in high quantities with an excellent quality-price ratio. These outstanding features may open new market lines related to endonucleases – apart from stimulating the established applications – resulting as attractive for many sectors needing such cGMP requirements, like medical and biopharma manufacturing, as well as in cell therapy, oncology, CAR-T-cell development, etc.

A successful case-study is the DNA/RNA removal in production processes of biopharmaceuticals, vaccines, or biologicals through biosynthetic approaches. For the manufacture of such compounds – and to reach their ultimate commercialisation – FDA regulations restrict the nucleic acid levels to less than 100pg per dose (applied to the end-product sample). These endonucleases can rapidly and unselectively degrade nucleic acids up to two to five base pairs, assuring the full compliance with regulations after the cleavage step.

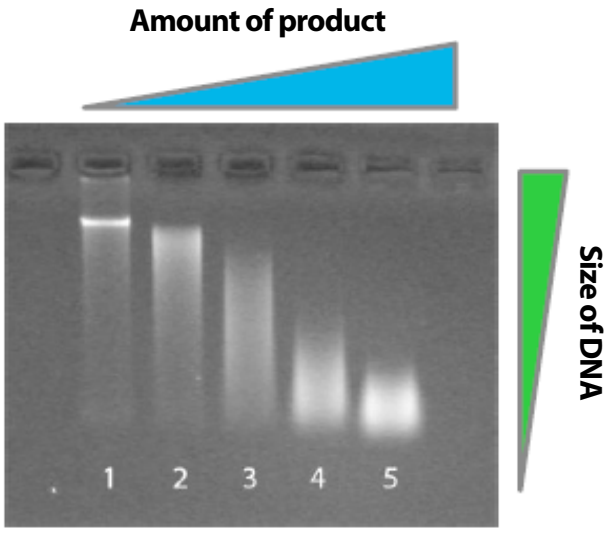


Figure 2: Use of endonucleases to remove excess of nucleic acids with applications in biopharmaceuticals

Furthermore, in the production and purification of biological compounds such as antibodies, adenovirus particles, antigenic products, vaccines, binding proteins, etc, the cell disruption may lead to the release of macromolecular nucleic acids to the fermentation broth, generating a considerable increase in the viscosity, thus hampering the subsequent manipulation of the derivative. To circumvent this, endonucleases rapidly degrade nucleic acids, and the handling of process fluids, eg through ultrafiltration, can be then simplified, reducing downstream costs significantly. To further emphasise the potential of endonucleases in this area, it must be noted that the established traditional methods to remove nucleic acids are often based on extraction, sonication, or precipitation


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procedures. These more-or-less successful strategies are typically conducted under more severe processing parameters (temperature, solvents, addition of chemical agents, etc) than those applied for endonucleases. Those conditions may partly degrade the desired biological targets, such as leading to a loss of the desired protein or vaccine, as they are not selective for the nucleic acid removal. Overall, such methods may increase costs associated to downstream significantly. These aspects strongly reinforce the unique propositions that endonucleases may offer to the biopharma industry.

In summary, the unspecific endonuclease from *S marscescens* has shown a successfully broad use in biopharmaceuticals, as this enzyme displays an ample operational framework (eg pH, temperature, etc). It is largely robust to cope with many challenging processing conditions and deleterious agents, and it is extremely efficient in nucleic acid hydrolysis. Gratifyingly, a novel derivative of that enzyme is now produced using an endotoxin-free gram-positive *Bacillus sp* as a host. Moreover, the fermentation procedure does not use animal-derived feedstocks, conferring the enzyme the consideration of BSE/TSE-free product with a high viral safety. This uniqueness, in full compliance with the EU cGMP requirements, will enable the broadening of the market options for endonucleases, generating added value to biotech and biopharma applications where the need of cGMP is required, like in nucleic acid removal, biosynthesis, cell therapy, oncology, CAR-T-cell development, and more.

## About the authors

Dr Pablo Domínguez de María holds BScs in pharmacy and chemistry and a PhD in biocatalysis. He worked in the industry for 6.5 years at Evonik from and AkzoNobel BV, being involved in projects related to sustainable chemistry, organocatalysis, biorefineries, and biotech. In 2009, Pablo joined the RWTH Aachen University, Germany, as group leader, defending his habilitation in 2015. Since 2014, he is the CEO of Sustainable Momentum, a consultancy firm providing technical support in areas related to biorefineries, biotech, and sustainable chemistry. He has delivered several technical books, is co-inventor of some patents, and has published more than 100 scientific publications.

Dr Stefan Schoenert is Head of Strain and Process Development at c-LEcta. He was the developer of DENARASE nuclease and is responsible for development and improvement of biotech-relevant expression systems based on microorganisms including *Bacillus sp*, *E coli*, and *Pichia pastoris* and their use in production of enzymes and organic chemicals. Stefan has a PhD in biology from the University of Konstanz, Germany.
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