LAPTOP: Lantibiotic production, Technology, optimization and improved process

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Introduction. The objective of the FP7-funded LAPTOP project was to develop an economically viable production process for the lantibiotic NAI-107, a promising antibiotic with the potential to treat life-threatening infections caused by multidrug-resistant Gram-positive pathogens. NAI-107 is produced by the actinomycete *Microbispora* sp. Because of its complex chemical structure, NAI-107 cannot be obtained by chemical means, and its production requires robust fermentation processes with optimized strains, media and recovery procedures.

The development of a robust and economically feasible production process for NAI-107 has required the integration of basic knowledge of the physiology and genetics of the producing strain obtained by a combination of classical and post-genomic approaches, with a detailed knowledge of the production process and its scalability to industrial level. This has been achieved by the coordinated work of the seven partners in the project. Due to limited knowledge of the *Microbispora* strain, the objectives of first half of the project were the development of a gene transfer system for *Microbispora* (based on conjugation from *Escherichia coli*) allowing the generation of different mutants; a draft genome sequence of *Microbispora*, which led to the identification of about 8,000 coding sequences, among them the key components of the N- and P-regulons; and a 2D proteome map of the strain.

The physiology of *Microbispora* has been studied under different conditions, leading to the identification of chemically defined media suitable for NAI-107 production, which was also instrumental for carbon and nitrogen flux analysis. The composition of *Microbispora* cell wall has also been established under producing and non-producing conditions.
The mechanism of action of this lantibiotic has been elucidated in detail in sensitive bacterial strains, establishing that it binds to lipid II and related cell wall intermediates.

Fermentation studies have led to the design of improved media and processes for NAI-107 production, which have resulted in production titers up to 10 fold higher than in the starting production process. The recovery and downstream processing of NAI-107 have also been optimized. Finally, the minor components present in the NAI-107 complex have been chemically characterized and evaluated for their antimicrobial activity.

Highlights from the main achievements in the project will be presented.

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LAPTOP website: http://www.jic.ac.uk/laptop/