Introduction

NAI-107 is a modified peptide antibiotic (lantibiotic) made by a *Microbispora* species. It is active against a broad range of Gram-positive pathogens, and shows much promise for development into a clinically useful anti-infective. The aim of this project was to develop a robust and economically feasible production process for NAI-107, an essential requirement for further clinical development. This required the application of microbiology, molecular biology, genetics, biochemistry, physiology, metabolic flux analysis, fermentation technology and process scale-up to achieve. The results of this multi-disciplinary project are summarised here.

Characterization of the NAI-107 complex of lantibiotics

The minor congeners (F0, F1, F2, A0, B1 and B2) were purified, structurally characterised and assayed for their antibiotic activity in comparison to the main congeners A1 and A2.

Development of genetic tools for *Microbispora*

A gene transfer system based on conjugation from *Escherichia coli* was developed to construct mutants in *Microbispora* sp. and to over-express regulatory genes.

An annotated draft genome sequence of *Microbispora* sp. was generated.

A 2D-protein map of *Microbispora* during primary and secondary metabolism was constructed and differential proteins identified by mass spectrometry. An interactive proteome web site will soon be available.

A chemically defined medium allowing NAI-107 production was designed and used for carbon flux analysis.
Regulation of NAI-107 biosynthesis

*mlbR*, *mlbX* and *mlbW* were identified as key regulatory genes for NAI-107 biosynthesis. Over-expression of *mlbR* or *mlbX* resulted in higher levels of NAI-107 production under laboratory conditions.

ppGpp synthesis was identified as a key intracellular signalling molecule in triggering *mlbR* expression and NAI-107 production in a feed forward regulatory mechanism.

NAI-107 was shown to act as a signalling molecule, triggering its own production, presumably coordinating biosynthesis in the population as a whole.

Immunity to NAI-107

Both an ABC transporter (MlbEF) and a lipoprotein (MlbQ) were implicated in immunity to NAI-107 in the producing organism.

Changes in cell wall composition in *Microbispora* sp. do not appear to play a role in immunity.

Interaction of NAI-107 with its target

Whole-cell experiments confirmed that NAI-107 inhibits cell wall biosynthesis. *In vitro* assays identified Lipid II, the immediate precursor for cell wall biosynthesis, as the molecular target of NAI-107.

The proteome response profile of *Bacillus subtilis* to NAI-107 showed most similarity to the lantibiotic gallidermin, which also binds to Lipid II.

Regulators of nitrogen and phosphate metabolism

GlnR and PhoP were identified as key regulators of nitrogen and phosphate metabolism, respectively.

Nitrogen excess correlated positively with both NAI-107 production (specific productivity) and biomass accumulation, and increased expression of *mlbA*, encoding the NAI-107 precursor peptide, was observed in defined medium supplemented with ammonia or nitrate.

Overexpression of *glnR*, *nnaR* and *glnK* resulted in higher levels of NAI107 production in chemically defined medium.

Phosphate excess also correlated with biomass accumulation and NAI-107 production, but not with specific productivity. Unlike many actinomycete antibiotics, NAI-107 production was not stimulated by phosphate limitation.

Key enzymes in carbon metabolism identified

Carbon flux analysis revealed greatest flux through the pentose phosphate pathway. Fluxes through the tricarboxylic acid cycle and Embden-Meyerhof-Parnas pathway were lower.

Isolation of higher level producers of NAI-107

A variety of antibiotic resistant mutants were isolated that produced increased levels of NAI-107. Streptomycin-resistant mutant S13 was selected for bioreactor analysis using production medium.
Conclusions

By bringing together a range of multidisciplinary skills, we now have a good understanding of how NAI-107 is produced by *Microbispora* sp. and how its biosynthesis is regulated. We have developed substantial understanding of the physiology of the producing organism, particularly of carbon and nitrogen metabolism. We have confirmed the mechanism of action of the lantibiotic and identified its molecular target. This knowledge has been brought together to achieve the aim of the project, namely the design of an industrial scale production process for NAI-107.

For further details see [http://www.jic.ac.uk/laptop](http://www.jic.ac.uk/laptop) or contact the project coordinator, Dr Margherita Sosio at msosio@naicons.com

This project involved the research groups of the following participants (students and postdoctoral fellows funded by the project are shown in yellow):

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