4.1a An executive summary

The LAPTOP objective was the development of a robust and economically feasible production process for NAI-107, with the final objective of producing Good Laboratory Practice-grade compound. The lantibiotic NAI-107, produced by the actinomycete Microbispora sp, is a new antibiotic with the potential to treat life-threatening infections caused by multidrug-resistant Gram-positive pathogens. It is active against all Gram-positive pathogens including multidrug-resistant isolates, with good potency and efficacy in sophisticated experimental models of infection, such as rat endocarditis caused by methicillin-resistant Staphylococcus aureus. NAI-107 is currently undergoing formal toxicology studies. Because of its complex chemical structure, NAI-107 cannot be produced by chemical means. Thus, the supply of sufficient amounts of product for future clinical studies requires robust fermentation and recovery processes to deliver a high-quality compound at reasonable cost. This is particularly relevant for NAI-107 because of the lack of precedents: no lantibiotic is produced at an industrial scale as a drug for human use and no Microbispora strain is used for industrial production. Due to limited knowledge of the Microbispora strain, the initial objectives were the development of tools instrumental for analyzing the physiology and genetics of the producing strain and for getting insights into NAI-107's mechanism of action. Next, knowledge generated during the previous reporting period was translated into the generation of improved strains, the design of optimized media and recovery procedures. A gene transfer system based on conjugation between E. coli and Microbispora was developed and applied to generate knockout and over-expression mutants. From a draft genome sequence of Microbispora, consisting of about 8,000 coding sequences, key components of the N-regulon and P-regulon were identified and a 2D-protein map was constructed during primary and secondary metabolism, establishing differentially abundant proteins. After the identification of chemically defined media suitable for NAI-107 production, carbon flux analysis established that C-assimilation occurs mostly through the pentose phosphate pathway. Since production of an antibiotic is often limited by the self-resistance mechanism in the producer strain, it was important to investigate in detail the mechanism of action of

NAI-107 in model strains, as well as establishing key features in self-resistance, transport and cell wall composition in Microbispora. In whole cell and cell-free experiments, NAI-107 was demonstrated to inhibit cell wall biosynthesis by binding to Lipid II with high affinity and inhibiting all enzymes utilizing this key biosynthetic intermediate. Pore formation, as observed with some Lipid-II binding lantibiotics, did not occur with NAI-107 in vitro. Analysis of the Microbispora cell wall indicates that it contains stem peptides with either glycine or alanine at the first position and it contains a direct linkage between peptide chains. Differential proteome analyses established that the proteins associated with NAI-107 production are, among others, those required for amino sugar metabolism, cell wall biosynthesis, lantibiotic resistance, and nitrogen metabolism. Previous work on a different Microbispora strain had identified key roles for the regulatory genes, which are also present in the NAI-107 gene cluster: *mib*X encoding a sigma factor, *mibW* encoding the cognate anti-sigma factor, and *mibR* encoding a likely DNA-binding protein. Critical parameters for *Microbispora* growth and NAI-107 production have been delineated and new seed and production media have been designed. These have resulted in production titers up to 10 fold higher than in the starting production process. A simple recovery and purification process has been designed, which allows purification of NAI-107 with few, industrially scalable steps. The minor components present in the NAI-107 complex have been purified, chemically characterized and evaluated for their antimicrobial activity. The fermentation process was successfully transferred to a different laboratory, with satisfactory production levels at both the 15 and the 250-L scale.