

## Coupling Proteomics and Fermentation Technology for the Improvement of Bioactive Molecule Production Yield in Actinomycetes

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Most bioactive molecules (like anticancers, antitumors, antibiotics, immunosuppressants, insecticides, antivirals, herbicides, antifungals) with valuable industrial and market value are naturally produced by actinomycetes [1-4], Gram-positive filamentous bacteria widespread in both terrestrial and aquatic environments [5,6]. Out of thousands of bioactive molecules, also known as secondary metabolites since they are not essential for actinomycete growth in standard laboratory condition at least, more than 50% are synthesised by strains of *Streptomyces* genus [4]. Despite the cellular and ecological role of secondary metabolites is still debated [3,7], microbial fermentation is widely exploited to produce these compounds at industrial level. Although they have many different activities and range within a vast chemical complexity and diversity, there are two main common issues which could be addressed for the establishment of a cost-effective microbial fermentation process:

1) bioactive production is strictly coordinated with a complex physiological differentiation program, as it has been extensively demonstrated in *Streptomyces coelicolor*, the model strain of this group of bacteria [8,9].

2) under typical laboratory conditions bioactive molecule yield is usually low-level or null at all in some cases.

Thus, the development of a robust and economically feasible production process for secondary metabolites implies the study of strain physiology combined with a detailed knowledge of the production process and its scalability to industrial level. Anyhow, each actinomycete species requires its own optimised fermentation conditions to obtain high production titre of any specific secondary metabolite. Therefore, there are few general strategies and basic guidelines which could lead to yield improvement. At the industrial level most efforts for the development of an efficient fermentation process have been principally done in medium composition optimization by using time-consuming empirical trials. Effective computational and statistical approaches were recently used as predictive methods which could replace traditional one-factor-at-a-time technique used for optimizing actinomycete growth conditions [10,11]. Anyhow, these approaches could take further advantages from a more comprehensive understanding of strain physiology. On the other hand, generations of high yielding strains through, i) combination of random chemical mutagenesis and recombination [12-14], ii) expression of secondary metabolite genes in heterologous hosts [15,16] and iii) spontaneous mutations and antibiotic resistance selection [17-19] are well documented too. However, with the advent of "omics" technologies a change in strategy happens, since the chance of managing huge amount of molecular insights on strain physiology, gene expression and biochemical capabilities introduced new perspectives as never before [20].

In particular, although proteomics is characterized by expensive and multi-step labour-intensive techniques it is actually the most powerful "omics" tool (Table 1), since it can give answers to more than one biological question [21,22], such as: gene expression (which and how much gene product is present and presumably works); protein regulation and turn-over (covalent modification occurrence and degradation); interacting partners (multi protein complexes);

topological distribution (cytoplasm, membrane localization). Besides that, the functional clusterization of proteins (according to metabolic pathway databases) can give a clear picture of which biochemical pathways and cellular processes happen or are necessary as cellular response during a particular condition, growth stage or perturbation. In other words, proteomics can give an account of which molecular strategy can be performed by organisms, cells or tissues during a specific physiological need. Thus the study at the proteome level of changes occurring in actinomycetes between unproductive growth stages (or growth conditions) and those in which secondary metabolism production is switched on can reveal [23-25]: molecular event(s) or metabolic signal(s) triggering or stopping bioactive molecule production; relationships between primary metabolite supply and bioactive molecule biosynthesis; hierarchical cascade of regulatory effectors controlling secondary metabolism biosynthetic genes (including resistance mechanisms in the case of antibiotic production). Knowledge gained from proteomics can be then used as background to drive rational approaches for growth condition modifications (i. e. changes in medium composition, oxygen supply and pH) and/or strain improvement (engineering of targeted regulatory, metabolic, stress response and biosynthetic genes)

Due to the vast range of scientific aspects that could arise especially when working on rare actinomycetes, a multidisciplinary approach is often required to have good chances of success in getting significant results in terms of improved production at the industrial level. The establishment of "omics" (genome sequencing is necessary and metabolomics is complimentary to proteomics) and also classical molecular genetic tools (i.e. transformation protocols), cultivation protocols (mycelium storage, growth parameters, medium recipes), end-product recovery and process scaling-up are all examples of the variety and complexity of matters that have to be managed and integrated. Thus, scientific consortia, made by companies, like Small or Medium Enterprise (SME) and academic research laboratories, can successfully address many of these scientific problems. As an example, the LAPTOP scientific project (<http://www.jic.ac.uk/laptop/>), financed by EU from July 2010 to June 2013, combined the experiences and expertises from SMEs and academy research laboratories with the aim to develop a production process for the lantibiotic NAI-107, a new promising antibiotic with the potential to treat life-threatening infections caused

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“omics” tool	Advantages	Disadvantages
Genomics	Qualitative. Relatively cheap; <i>in silico</i> predictive models may be generated concerning strain biochemistry and metabolic pathways.	No information about gene expression and regulation thereof.
Transcriptomics	Quantitative and qualitative. Relatively cheap. Number of genes to be analysed may include all the predicted ORFs or just a restricted number of them.	Genome sequence must be available. Abundance levels may be misinterpreted due to post-transcriptional regulatory events.
Proteomics	Quantitative and qualitative information. Analysis of post-translational regulatory event(s). Topological distribution insights. Interaction study.	Genome sequence must be available. Labour-intensive. Expensive. Not the whole protein complement can be analysed. Set-up and optimization of procedures are often required.
Metabolomics	Quantitative and qualitative information. Detection of end-products of cellular processes.	Labour-intensive. Expensive. Not the whole metabolite complement can be analysed.

Table 1: Comparison between different “omics”.

by multi-drug resistant Gram-positive pathogens [26,27]. NAI-107 is produced by fermentation of the actinomycete *Microbispora* sp. Since no lantibiotics are industrially produced as drugs for human use and there are no examples of industrial use of *Microbispora* genus, delivering a high quantity of high quality compound is extremely challenging. By means of proteomics many basic physiological aspects of *Microbispora* sp. growth and NAI-107 production were elucidated and, in just three years, this collaborative project produced enough knowledge and genetic tools that can lead to an efficient production process by generating and utilizing high producing strains, improved production media coupled with an efficient recovery process.

Thus, such similar combined and cooperative strategies may become standard models for industrial development based on modern molecular genetic tools and classical actinomycete fermentation techniques.

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