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Omics and multi-omics approaches to study the biosynthesis of secondary metabolites in microorganisms

Emilia Palazzotto and Tilmann Weber

Natural products produced by microorganisms represent the main source of bioactive molecules. The development of high-throughput (omics) techniques have importantly contributed to the renaissance of new antibiotic discovery increasing our understanding of complex mechanisms controlling the expression of biosynthetic gene clusters (BGCs) encoding secondary metabolites. In this context this review highlights recent progress in the use and integration of 'omics' approaches with focuses on genomics, transcriptomics, proteomics metabolomics meta-omics and combined omics as powerful strategy to discover new antibiotics.

Address
Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet bygning 220, 2800 Kgs., Lyngby, Denmark

Corresponding author: Weber, Tilmann (tiwe@biosustain.dtu.dk)

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Introduction

Bacteria, and in particular actinomycetes (Phylum: Actinobacteria), represent one of the most prolific source of bioactive natural products with a wide range of biological activities. Many well-known bioactive molecules, widely used in medicine for treatment of infection diseases, such as tetracyclines, β-lactams, aminoglycosides, macrolides, and glycopeptides have been isolated from the most dominant actinomycetes genus, *Streptomyces*, as products of the secondary metabolism [1].

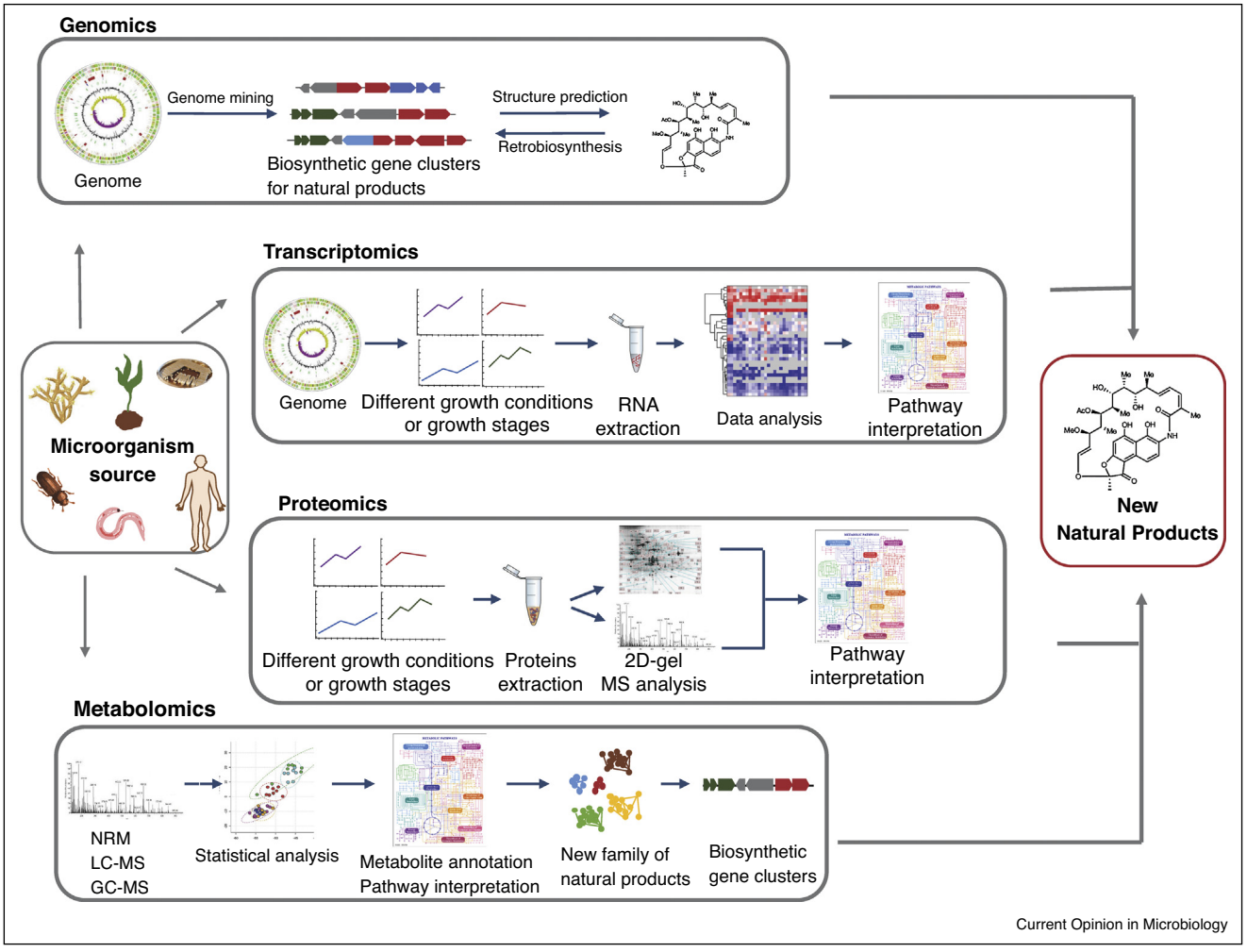
With the advent of next-generation sequencing (NGS) technologies and the possibility to routinely obtain good-quality whole genome sequences, actinomycetes revealed their potential metabolic landscape and thus the far greater potential to produce specialized metabolites than has been discovered by classic screening-based methods in the previous 50 years. In fact, the genome sequences revealed that a typical actinomycete contains on average about 30 secondary metabolite gene clusters and thus theoretically has the genetic potential to produce approximately a 10-times higher number of secondary metabolites than have been detected during screening and chemical analytics [2,3]. These observations strongly contributed to renew the interest for natural product discovery and these prolific microorganisms in the recent years. In addition, the development of high-throughput (omics) techniques, genomics, transcriptomics, proteomics, metabolomics and computational resources have importantly increased our understanding of complex cellular physiology and key pathways affecting secondary metabolites production.

In this context, this review highlights recent progress in the use and integration of 'omics' approaches with focuses on genomics, transcriptomics, proteomics, metabolomics, meta-omics (metagenomics, metatranscriptomics and metaproteomics) and combined omics as powerful strategy to discover new antibiotics in actinomycetes and other microorganisms (Figure 1).

Genomics

The massive development of sequencing technologies over the past decade coupled with efficient bioinformatics tools showed the untapped metabolic potential of actinomycetes triggering a revolution in the drug discovery research. In fact, genomic analyses revealed that silent gene clusters are present even within the genomes of extensively studied species like the model streptomycetes *S. coelicolor* A3(2) or *S. avermitilis* [2,4]. Genome mining has been established as powerful tool to estimate the genetic potential of a strain by scanning the genome of interest and identifying secondary metabolite BGCs [5]. In particular, the antibiotics and Secondary Metabolites Analysis SHell (anti-SMASH) [6••,7] and the connected antiSMASH database [8], Prediction Informatics for Secondary Metabolomes (PRISM) [9,10], Global Alignment for natuRaL-products chemInformatiCs (GARLIC), Generalized Retrobiosynthetic Assembly Prediction Engine (GRAPE) platform [11••] and IMG/ABC [12•] the most well established tools, have been improved with more features to predict and assign functions to enzymes involved in the biosynthesis of secondary metabolites and facilitate the connection of biosynthetic gene clusters to their corresponding natural products [13].

Figure 1



Schematic representation of omics workflow for natural products discovery. The workflow depicted includes the experimental procedures used within genomics, transcriptomics, proteomics and metabolomics approaches.

The availability of these user-friendly genome mining tools has led to the discovery of many novel natural products. Some selected examples published within the last two years are outlined below.

One noteworthy example of new natural product accessed by genomic approach is hexaricins, a new family of polyketides which gene clusters were predicted in the rare marine actinomycete *Streptosporangium* sp. CGMCC 4.7309 [14]. Genome mining analysis was used to explore secondary metabolite diversity of this rare actinomycete revealing 20 cryptic secondary metabolite biosynthetic clusters and among those the unusual type II PKS BGC.

Another example is the identification of the BGC encoding lobosamides A–C in the marine actinobacterium *Micromonospora* sp. These macrolactams, which cluster organization is conserved, displayed growth inhibitory activity against *Trypanosoma brucei* the causative agent of human African trypanosomiasis [15].

In 2017, a study on actinobacteria genome mining lead to discovery of 49 potential producers of Leinamycin-type natural products, a potent antitumor drug [16]. After the first isolation from *Streptomyces atroolivaceus* S-140 in 1989, yet no analogues have been isolated. By mining bacterial genomes novel anticancer drugs producers were identified.

Genome mining and comparative genomic analysis have opened up the prospect of prioritizing gifted strains for metabolic engineering. In *Streptomyces lydicus* 103, producer strain of streptolydigin, the genomics-based bottom up approach have unveiled a biosynthetic potential undetected in standard fermentation condition [17]. The complete genome sequencing and comparative analysis

Table 1

Examples of omics-approaches used within the last 5 years (since 2013) as strategy to discover new antibiotics in actinomycete strains.

Actinomycete strain	Omics strategies	Natural products	References
Streptosporangium sp. CGMCC 4.7309	Genomics	Hexaricins	[14]
Micromonospora sp.	Genomics	Lobosamides A–C	[15]
Streptomyces atroolivaceus S-140	Genomics	Leinamycin-type	[16]
Streptomyces lydicus 103	Genomics	Streptolydigin	[17]
Streptomyces collinus Tü 365	Genomics	Streptocollin	[67,68]
Streptomyces coelicolor A3(2)	Transcriptomics	Analysis of transcriptome and translatome of primary and secondary metabolism	[27••]
Streptomyces coelicolor A3(2)	Transcriptomics	Identification of the AbrC3 regulon	[69]
Streptomyces bingchenggensis	Genomics Transcriptomics	Milbemycin	[70]
Microbispora ATCC-PTA-5024	Proteomics	Molecular mechanisms regulating lantibiotic NAI-107	[30]
Planobispora rosea	Genomics Proteomics metabolomics	Thiopeptitde GE2270	[31]
Streptomyces sp. MBT28	Metabolomics	Prenylated isatin antibiotic	[40]
Streptomyces fradiae MM456M-mF7	Metabolomics	Fradiamines A and B	[41]
Streptomyces coelicolor A3(2)	Metabolomics	Desferrioxamine	[71]
Streptomyces sp. MP131-18	Genomics Metabolomics	Spiroindimicins E and F α-Pyrone lagunapyrone	[62]
Saccharopolyspora spinosa	Proteomics Transcriptomics	Key regulators and enzymes involved in spinosad production	[72]
Streptomyces lilacinus NRRL B-1968	Genomics Proteomics metabolomics	Griseobactin (known) Rakicidin D (orphan Nrps/PKS) Putative Siderophore	[73]

allowed the reconstruction of both primary and secondary metabolic pathways suggesting key metabolic genes for metabolic engineering.

The genomic analysis of a whole 40 *Micromonospora* strains underlined the genetic potential to synthesize novel specialized metabolites of this genus. By antiSMASH, close to one thousand biosynthetic gene clusters were detected, including NRPS, PKS, terpenes and siderophores clusters [18]. The analysis of whole genome sequence data of the genus *Micromonospora* provided insights into the metabolic and biotechnological potential to produce new natural products.

Transcriptomics

Although DNA sequencing has revealed the high abundance of BGCs in actinomycetal genomes, a majority of their actual products have not been characterized. In many cases, these clusters remain silent under laboratory conditions due to the complex regulation at transcriptional, translational and post-translational levels. To unveil the mechanisms controlling the metabolic switch, development differentiation and natural products biosynthesis, transcriptional changes in gene expression levels have been extensively studied [19–21].

A widely used strategy to trigger the expression of cryptic BGCs and thus production of new natural products, is the use of biological (co-cultivation), chemical and molecular elicitors [22].

Different studies highlight the co-cultivation-based elicitation between actinomycetes, actinomycetes with other bacteria or fungi as powerful method to activate the expression of BGCs (Table 1).

An example is represented by the co-cultivation of the soil bacterium *S. coelicolor* with the competitor myxobacterial strain *Corallococcus coralloides* B035. Transcriptional analysis revealed that co-cultivation induced an increased expression of *redX* and *redH* genes and thus an increment of undecylprodigiosin production in *S. coelicolor* [23].

The transcriptomic analysis of the fungus *Aspergillus nidulans* co-cultured with 58 actinomycetes has showed that interspecies 'talk' can activate silent gene clusters in *A. nidulans* such as the polyketide orsellinic acid, lecanoric acid, and the cathepsin K Inhibitors F-9775A and F-9775B [24].

Furthermore, rare earth elements or the alteration of growth conditions or media composition were successfully used as chemical elicitors to induce the expression of actinorhodin BGC in *S. coelicolor* [25] or in order to activate the production of antimicrobial agents against multidrug-resistant clinical isolates in a collection of actinomycetes [26].

Recently, a comprehensive study of transcriptome and translatome, in both primary and secondary metabolism, of the model organism *Streptomyces coelicolor* allowed to elucidate the relationship between transcription and

translation at a genome wide scale during four growth phases [27••]. These findings highlight that some cluster-situated regulator (CSR) genes are translationally induced during the transition phase might facilitate the design of new approaches to manipulate secondary metabolic gene clusters and antibiotic discovery. Thus, understand the BGCs regulatory code by bioinformatics prediction and identification of *cis* elements and their corresponding transcription factors(s) represents a promising strategy to activate the expression of cryptic BGCs [28].

Comparative transcriptomics was used to exploit BGC expression among four strains of marine bacteria belonging to *Salinispora* genus [29•]. The results indicated that a majority of the BGCs analysed in the selected *Salinispora* strains are transcribed at levels that should facilitate product detection. In addition, compounds not previously reported from *S. pacifica* were identified and some BGCs classified as orphan were linked to their products.

Proteomics

In addition to genomics and transcriptomics, also proteomics has been used as powerful method to shed light on the relationship among metabolic pathways and natural products production. Indeed, comparing protein expression levels, proteomics provides information on differential pathways regulation highlighting key players in natural products biosynthesis that can be used as target for rational engineering.

An extensive analysis of the proteomic changes associated with lantibiotic NAI-107 production was performed on *Microbispora* ATCC- PTA-5024 strain. Using differential proteomic analysis it was possible to unveil different regulatory mechanisms controlling morphological differentiation and antibiotic production during different stages of growth [30].

Proteomics represents a complementary approach to transcriptomics and genomics for strain characterization. A genomic, transcriptomic and proteomic analysis was carried out on the unusual actinomycete *Planobispora rosea* the producer of thiopeptide GE2270. The analysis and integration of different 'omics' data unveiled the physiology of the *P. rosea* during GE2270 biosynthesis [31].

In addition, a widely applicable strategy that allows to link all type of natural products to a specific gene cluster is Natural Products Proteomining. This method correlates the change of biosynthetic proteins levels to secondary metabolite production [32].

Metabolomics

Genomics-based methods have revealed that most of the biosynthetic potential of cultured and un-cultured microorganisms remains inaccessible to date. In fact, a major obstacle is that many BGCs are silent or poorly expressed under standard laboratory conditions. Different methodologies have been developed to trigger the expression of such 'silent' gene cluster, enable pathway engineering and heterologous expression new chassis [33,34].

Due to the complex regulatory mechanisms controlling the activation of BGCs expression and the thigh relation between primary and secondary metabolism [35], metabolomics, reflecting the phenotype of genomic, transcriptomic, and proteomic networks, represent a powerful tool to investigate the response to chemical and biological stimuli of secondary metabolite producing strain [36].

Metabolomics, measuring the global levels of low molecular weight metabolites allows a metabolic comparison of different biological samples avoiding chemical redundancy. Thus, thanks to the possibility to obtain data pertaining to differences between culturing condition the identification of secondary metabolites from orphan BGCs has significantly increased.

Numerous new natural products have been identified during the last years by the use of two analytical platforms: Nuclear magnetic resonance (NMR) based metabolomic analyses and mass spectrometry (MS). Multivariate statistical analyses including principal component analysis (PCA), partial least-squares regression (PLS-DA) and the MS/MS database Global Natural Product Social (GNPS) molecular networking platform have greatly improved analysis of mass spectrometry data allowing a rapid dereplication of known molecules and strain prioritization [37,38].

In addition, VarQuest, a novel algorithm for identification of Peptidic natural products variants via database search of mass spectra was developed to extend GNPS by Pevzner and co-workers [39].

An example of metabolomics approach to discover new natural products is the identification of a previously undescribed prenylated isatin antibiotic produced by *Streptomyces* sp. MBT28. This molecule with antibiotic activity against *Bacillus subtilis* was identified by NMR-based metabolomics strategy [40].

New bioactive compounds, a siderophore and its derivative, named fradiamines A and B, were discovered and characterized from the extract of the deep-sea actinomycetes *Streptomyces fradiae* MM456M-mF7 by LC-HRESI-MS based non-targeted metabolomics [41].

Liquid chromatography (LC) LCMS–PCA provide a high-throughput platform to prioritize bacterial strain and isolate two new halimane-type diterpenoids, micromonohalimanes A and B from a *Micromonospora* sp. symbionte of marine ascidian *Symplegma brakenhielmi* [42]. By this method a strain with unique metabolic potential to

produce novel natural products with antibacterial activity was identified.

Meta-omics approaches

Due to the fact that only a small percentage of all existing prokaryotes is amenable to cultivation using standard techniques, a plethora of natural products remain undiscovered. As discussed by Schofield and Sherman [43••], the use of meta-omics technique (metagenomics, metatranscriptomics and metaproteomics) provides a culture-independent approaches to explore this hidden potential.

Metagenomics is one of the most commonly used approaches to study the chemistry of uncultivated bacteria. The analysis of DNA isolated from environmental sample allowed to exploit the 'dark matter', biosynthetic pathways of uncultured marine bacteria such as obligate symbiont of sponges and other marine invertebrates. Thanks to a combination of metagenome sequencing and single cell genomics the prokaryotic BGC encoding onnamide was identified from a marine sponge *Theonella swinhoei*. These results confirmed the role of symbiotic bacteria as 'talented' producers of new polyketides and peptides with antitumor activity [44,45].

Another successful example is represented by the culture-independent natural products discovery platform developed by Brady and co-workers [46]. In fact, using degenerate PCR primers to amplify conserved regions of biosynthetic genes of BGCS in environmental samples, bioinformatics platform environmental Surveyor of Natural Product Diversity (eSNaPD) to analyze sequencing results and heterologous production a new class of calcium dependent antibiotic, malacidins, was discovered.

A big contribution to discovery of new potent antibiotics has been given by the development of new microbiome screening methods to explore previously uncultured bacteria. In 2015 using the innovative *in situ* cultivation method iChip, a new cell wall inhibitor, teixobactin, was identified from the previously uncultured microorganism *Eleftheria terrae* [47]. After structure elucidation, the teixobactin biosynthetic gene cluster was identified by genome sequencing of *E. terrae* and homology searches. Due to the different mode of action of this compound no teixobactin-resistant strains were isolated.

Studies on microbial molecular interactions highlighted the complex signaling network between different organisms and the possible activation of silent biosynthetic gene clusters as response to environmental stimuli. This dynamics of gene expression in a complex microbial community can be investigate using a metatranscriptomics approach. In fact, while metagenomics analysis opened the possibility to investigate the chemical potential of different samples including uncultured or unknown species by mining environmental DNA, metatranscriptomics analysis gives information on the transient responses to environmental condition analyzing the collective set of messenger RNAs that are present in an environmental sample [48–50]. The integration of metagenomics and metatranscriptomic analysis was successfully used for determining the microbial communities and their metabolic relation in marine environments revealing this environment as a significant new source of drug discovery and development [51].

As discussed above, the development of meta-omics technique allowed the exploration of biosynthetic capability of uncultured microorganism or microorganism isolated from un-explored or under-explored habitats. The integration and analysis of metagenomics, metatranscriptomics, and metaproteomics data provides a stable indication of the physiological activity and translational regulation of a complex environmental microbial community in an ecosystem [52–54]. Different metaproteomic tools are available to analyse biosynthetic gene cluster and pathways. Among those, Orthogonal Active Site Identification System (OASIS) and Proteomic Interrogation of Secondary Metabolism (PrISM) [55,56]. Using PrISM method from unsequenced environmental *Bacillus* sp. the Cyclic Imine koranimine [57]. With high throughput (meta)-proteome data, it also possible to investigate the post-translational modifications in *in situ* environments and thus shed a light on the mechanisms used by bacteria regulate physiological processes and natural products production [58]. In 2017, Borriss and co-workers performed a large-scale lysine malonylation analysis in the model Gram-positive plant growth-promoting rhizobacteria (PGPR), *Bacillus amyloliquefaciens* FZB42. The bioinformatic analysis revealed that the enzymes involved in rhizobacterium–plant interaction and responsible for antibiotic production including polyketide synthases (PKSs) and nonribosomal peptide synthases (NRPSs) were highly malonylated [59].

Combined omics approach

The advent of more powerful metabolomics approaches coupled with genomics analysis have enabled the discovery of new natural products and the investigation of their importance in nature, thereby accelerating natural product discovery. Matabologenomics consists in combining genome sequencing and automated gene cluster prediction with mass spectrometry-based metabolomics [60,61••]. Genomic data are used to interrogate the chemical data and *vice versa* to uncover families of molecules with phenotypes of interest in large strain collections. Using this approach, the combined genomics-metabolics profiling of the marine strain *Streptomyces* sp. MP131-18 led to the identification of new biologically active compounds such as the bisindole pyrroles spiroindimicins E and F and two new α-pyrone lagunapyrone [62].

To identify novel phosphonic acids, a large-scale genome mining analysis and screening for unique phosphoenolpyruvate mutase gene (*pepM*) were performed on 10 000 actinomycetes by Metcalf and co-workers [63]. In 278 strains, 64 distinct groups of phosphonate biosynthetic gene clusters were identified. By characterization of strains within these groups a new archetypical pathway for phosphonate biosynthesis and 11 previously undescribed phosphonic acid natural products were discovered.

An extensive analysis of lanthipeptide-related biosynthetic gene clusters in Actinobacteria revealed that lanthipeptide synthetases can produce natural products other than lanthipeptides. In fact, genomics and MS data suggest cross talk between lanthipeptide biosynthetic enzymes and PKS and NRPS systems and thus natural products with new scaffolds [64].

A multi-omics approach was also successfully applied to understand how BGCs are exploited in animal–microbe symbiosis in both marine and terrestrial environments.

One example is the extensive study investigating the natural product diversity produced by the nematode symbionts *Photorhabdus* and *Xenorhabdus*. Despite comparative genomics analysis indicates a high similarity at DNA level, high-resolution mass spectrometry analyses reveal a huge chemical diversity. The use of genomic and metabolomic methods in a complementary manner allowed the rapid identification of potentially interesting bioactive products previously unidentified including the xefoampeptides and tilivalline [65].

A combination of chemistry, genetics, metagenomics, and metatranscriptomics approaches was successfully used by Fischbach and co-workers to study how microorganisms play a key role in human health evaluating 752 metagenomic samples from the NIH Human Microbiome Project [66]. The results revealed that the human-associated bacteria house 3118 BGCs encoding small molecules, many of which are presumably associated with beneficial properties. Among these BGCs for thiopeptide antibiotics, some of which have a similar structure with molecules already in clinical trials.

Conclusions

Enabled by the technological advances in genomics, metabolomics, transcriptomics and other 'omics' the biosynthetic potential of large and complex systems has been explored unveiling new BGCs and discovering new natural products. Tremendous insight into both diversity, distribution and evolution of BGCs can be provided by the integration of multi-omic analysis. Furthermore, the combined use of multi-meta-omics approaches gives a more complete pictures of microbial communities and their metabolism could she a light on molecular signals triggering the expression of silent gene cluster the and the role of natural products in natural habitat.

Conflicts of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Genilloud O: **Actinomycetes: still a source of novel antibiotics**. *Nat Prod Rep* 2017, **34**:1203-1232.

2. Bentley SD, Chater KF, Cerdeño-Tárraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D *et al.*: **Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2)**. *Nature* 2002, **417**:141-147.

3. Challis GL: **Exploitation of the *Streptomyces coelicolor* A3(2) genome sequence for discovery of new natural products and biosynthetic pathways**. *J Ind Microbiol Biotechnol* 2014, **41**:219-232.

4. Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Omura S: **Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis***. *Nat Biotechnol* 2003, **21**:526-531.

5. Ziemert N, Alanjary M, Weber T: **The evolution of genome mining in microbes — a review**. *Nat Prod Rep* 2016, **33**:988-1005.

6. •• Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de Los Santos ELC, Kim HU, Nave M *et al.*: **antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification**. *Nucleic Acids Res* 2017, **45**:W36-W41.
AntiSMASH currently is the most widely software to computationally mine for secondary metabolite biosynthetic gene clusters.

7. Medema MH, Breitling R, Bovenberg R, Takano E: **Exploiting plug-and-play synthetic biology for drug discovery and production in microorganisms**. *Nat Rev Microbiol* 2011, **9**:131-137.

8. Blin K, Medema MH, Kottmann R, Lee SY, Weber T: **The antiSMASH database, a comprehensive database of microbial secondary metabolite biosynthetic gene clusters**. *Nucleic Acids Res* 2017, **45**:D555-D559.

9. Skinnider MA, Dejong CA, Rees PN, Johnston CW, Li H, Webster AL, Wyatt MA, Magarvey NA: **Genomes to natural products PRediction Informatics for Secondary Metabolomes (PRISM)**. *Nucleic Acids Res* 2015, **43**:9645-9662.

10. Skinnider MA, Merwin NJ, Johnston CW, Magarvey NA: **PRISM 3: expanded prediction of natural product chemical structures from microbial genomes**. *Nucleic Acids Res* 2017, **45**:W49-W54.

11. •• Dejong CA, Chen GM, Li H, Johnston CW, Edwards MR, Rees PN, Skinnider MA, Webster AL, Magarvey NA: **Polyketide and nonribosomal peptide retro-biosynthesis and global gene cluster matching**. *Nat Chem Biol* 2016, **12**:1007-1014.
Description and application of the GRAPE/GARLIC algorithms that allows automatic matching of polyketide and NRPS structural data to predicted biosynthetic gene clusters.

12. • Hadjithomas M, Chen IA, Chu K, Huang J, Ratner A, Palaniappan K, Andersen E, Markowitz V, Kyrpides NC, Ivanova NN: **IMG-ABC: new features for bacterial secondary metabolism analysis and targeted biosynthetic gene cluster**

discovery in thousands of microbial genomes. *Nucleic Acids Res* 2017, **45**:D560-D565.
The IMG-ABC is the largest database of automatically mined biosynthetic gene clusters identified in microbial genomes.

13. Kim HU, Blin K, Lee SY, Weber T: **Recent development of computational resources for new antibiotics discovery**. *Curr Opin Microbiol* 2017, **39**:113-120.

14. Tian J, Chen H, Guo Z, Liu N, Li J, Huang Y, Xiang W, Chen Y: **Discovery of pentangular polyphenols hexaricins A–C from marine *Streptosporangium* sp. CGMCC 4.7309 by genome mining**. *Appl Microbiol Biotechnol* 2016, **100**:4189-4199.

15. Schulze CJ, Donia MS, Siqueira-Neto JL, Ray D, Raskatov JA, Green RE, McKerrow JH, Fischbach MA, Linington RG: **Genome-directed lead discovery: biosynthesis, structure elucidation, and biological evaluation of two families of polyene macrolactams against *Trypanosoma brucei***. *ACS Chem Biol* 2015, **10**:2373-2381.

16. Pan G, Xu Z, Guo Z, Hindra, Ma M, Yang D, Zhou H, Gansemans Y, Zhu X, Huang Y *et al.*: **Discovery of the leinamycin family of natural products by mining actinobacterial genomes**. *Proc Natl Acad Sci U S A* 2017, **114**:E11131-E11140.

17. Jia N, Ding MZ, Luo H, Gao F, Yuan YJ: **Complete genome sequencing and antibiotics biosynthesis pathways analysis of *Streptomyces lydicus* 103**. *Sci Rep* 2017, **7**:44786.

18. Carro L, Nouioui I, Sangal V, Meier-Kolthoff JP, Trujillo ME, Montero-Calasanz MDC, Sahin N, Smith DL, Kim KE, Peluso P *et al.*: **Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential**. *Sci Rep* 2018, **8**:525.

19. Liu G, Chater KF, Chandra G, Niu G, Tan H: **Molecular regulation of antibiotic biosynthesis in *Streptomyces***. *Microbiol Mol Biol Rev* 2013, **77**:112-143.

20. Palazzotto E, Renzone G, Fontana P, Botta L, Scaloni A, Puglia AM, Gallo G: **Tryptophan promotes morphological and physiological differentiation in *Streptomyces coelicolor***. *Appl Microbiol Biotechnol* 2015, **99**:10177-10189.

21. Vockenhuber MP, Sharma CM, Statt MG, Schmidt D, Xu Z, Dietrich S, Liesegang H, Mathews DH, Suess B: **Deep sequencing-based identification of small non-coding RNAs in *Streptomyces coelicolor***. *RNA Biol* 2011, **8**:468-477.

22. Abdelmohsen UR, Grkovic T, Balasubramanian S, Kamel MS, Quinn RJ, Hentschel U: **Elicitation of secondary metabolism in actinomycetes**. *Biotechnol Adv* 2015, **33**:798-811.

23. Schäberle TF, Orland A, König GM: **Enhanced production of undecylprodigiosin in *Streptomyces coelicolor* by co-cultivation with the corallopyronin A-producing myxobacterium, *Corallococcus coralloides***. *Biotechnol Lett* 2014, **36**:641-648.

24. Schroeckh V, Scherlach K, Nützmann HW, Shelest E, Schmidt-Heck W, Schuemann J, Martin K, Hertweck C, Brakhage AA: **Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans***. *Proc Natl Acad Sci U S A* 2009, **106**:14558-14563.

25. Kawai K, Wang G, Okamoto S, Ochi K: **The rare earth, scandium, causes antibiotic overproduction in *Streptomyces* spp.**. *FEMS Microbiol Lett* 2007, **274**:311-315.

26. Zhu H, Swierstra J, Wu C, Girard G, Choi YH, van Wamel W, Sandiford SK, van Wezel GP: **Eliciting antibiotics active against the ESKAPE pathogens in a collection of actinomycetes isolated from mountain soils**. *Microbiology* 2014, **160**:1714-1725.

27. •• Jeong Y, Kim JN, Kim MW, Bucca G, Cho S, Yoon YJ, Kim BG, Roe JH, Kim SC, Smith CP *et al.*: **The dynamic transcriptional and translational landscape of the model antibiotic producer *Streptomyces coelicolor* A3(2)**. *Nat Commun* 2016, **7**:11605.
A comprehensive analysis of the transcriptome and translatome of the model streptomycete *S. coelicolor* A3(2).

28. Rigali S, Anderssen S, Naômé A, van Wezel GP: **Cracking the regulatory code of biosynthetic gene clusters as a strategy for natural product discovery**. *Biochem Pharmacol* 2018 http://dx.doi.org/10.1016/j.bcp.2018.01.007. S0006-2952(18)30007-8.

29. • Amos GCA, Awakawa T, Tuttle RN, Letzel AC, Kim MC, Kudo Y, Fenical W, Moore SB, Jensen PR: **Comparative transcriptomics as a guide to natural product discovery and biosynthetic gene cluster functionality**. *Proc Natl Acad Sci U S A* 2017, **114**:E11121-E11130.
The comparative analysis of transcriptomics data obtained from diverse *Salinispora* strains facilitated the detection and characterization of compounds synthesised by previously orphan BGCs.

30. Gallo G, Renzone G, Palazzotto E, Monciardini P, Arena S, Faddetta T, Giardina A, Alduina R, Weber T, Sangiorgi F *et al.*: **Elucidating the molecular physiology of lantibiotic NAI-107 production in *Microbispora* ATCC-PTA-5024**. *BMC Genomics* 2016, **17**:42.

31. Tocchetti A, Bordoni R, Gallo G, Petiti L, Corti G, Alt S, Cruz JC, Salzano AM, Scaloni A, Puglia AM *et al.*: **A genomic, transcriptomic and proteomic look at the GE2270 producer *Planobispora rosea*, an uncommon actinomycete**. *PLoS One* 2015, **10**:e0133705.

32. Gubbens J, Zhu H, Girard G, Song L, Florea BI, Aston P, Ichinose K, Filippov DV, Choi YH, Overkleeft HS *et al.*: **Natural product proteomining, a quantitative proteomics platform, allows rapid discovery of biosynthetic gene clusters for different classes of natural products**. *Chem Biol* 2014, **21**:707-718.

33. Zhu H, Sandiford SK, van Wezel GP: **Triggers and cues that activate antibiotic production by actinomycetes**. *J Ind Microbiol Biotechnol* 2014, **41**:371-386.

34. Liu J, Zhu X, Seipke RF, Zhang W: **Biosynthesis of antimycins with a reconstituted 3-formamidosalicylate pharmacophore in *Escherichia coli***. *ACS Synth Biol* 2015, **4**:559-565.

35. Nieselt K, Battke F, Herbig A, Bruheim P, Wentzel A, Jakobsen Ø, Sletta H, Alam MT, Merlo ME, Moore J *et al.*: **The dynamic architecture of the metabolic switch in *Streptomyces coelicolor***. *BMC Genomics* 2010, **11**:10.

36. Covington BC, McLean JA, Bachmann BO: **Comparative mass spectrometry-based metabolomics strategies for the investigation of microbial secondary metabolites**. *Nat Prod Rep* 2017, **34**:6-24.

37. Crüsemann M, O'Neill EC, Larson CB, Melnik AV, Floros DJ, da Silva RR, Jensen PR, Dorrestein PC, Moore BS: **Prioritizing natural product diversity in a collection of 146 bacterial strains based on growth and extraction protocols**. *J Nat Prod* 2017, **80**:588-597.

38. Hou Y, Braun DR, Michel CR, Klassen JL, Adnani N, Wyche TP, Bugni TS: **Microbial strain prioritization using metabolomics tools for the discovery of natural products**. *Anal Chem* 2012, **84**:4277-4283.

39. Gurevich A, Mikheenko A, Shlemov A, Korobeynikov A, Mohimani H, Pevzner PA: **Increased diversity of peptidic natural products revealed by modification-tolerant database search of mass spectra**. *Nat Microbiol* 2018, **3**:319-327.

40. Wu C, Du C, Gubbens J, Choi YH, van Wezel GP: **Metabolomics-driven discovery of a prenylated isatin antibiotic produced by Streptomyces species MBT28**. *J Nat Prod* 2015, **78**:2355-2363.

41. Takehana Y, Umekita M, Hatano M, Kato C, Sawa R, Igarashi M: **Fradiamine A, a new siderophore from the deep-sea actinomycete *Streptomyces fradiae* MM456M-mF7**. *J Antibiot (Tokyo)* 2017, **70**:611-615.

42. Zhang Y, Adnani N, Braun DR, Ellis GA, Barns KJ, Parker-Nance S, Guzei IA, Bugni TS: **Micromonohalimanes A and B: antibacterial halimane-type diterpenoids from a marine *Micromonospora* species**. *J Nat Prod* 2016, **79**:2968-2972.

43. •• Schofield MM, Sherman DH: **Meta-omic characterization of prokaryotic gene clusters for natural product biosynthesis**. *Curr Opin Biotechnol* 2013, **24**:1151-1158.
A comprehensive review describing the use of metagenomics, metatranscriptomics and metaproteomics to discover prokaryotic biosynthetic gene clusters from environmental samples.

44. Wilson MC, Mori T, Rückert C, Uria AR, Helf MJ, Takada K, Gernert C, Steffens UA, Heycke N, Schmitt S *et al.*: **An environmental bacterial taxon with a large and distinct metabolic repertoire**. *Nature* 2014, **506**:58-62.

45. Piel J, Hui D, Wen G, Butzke D, Platzer M, Fusetani N, Matsunaga S: **Antitumor polyketide biosynthesis by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei***. *Proc Natl Acad Sci U S A* 2004, **101**:16222-16227.

46. Hover BM, Kim SH, Katz M, Charlop-Powers Z, Owen JG, Ternei MA, Maniko J, Estrela AB, Molina H, Park S *et al.*: **Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-positive pathogens**. *Nat Microbiol* 2018.

47. Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schäberle TF, Hughes DE, Epstein S *et al.*: **A new antibiotic kills pathogens without detectable resistance**. *Nature* 2015, **517**:455-459.

48. Gilbert JA, Field D, Huang Y, Edwards R, Li W, Gilna P, Joint I: **Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities**. *PLoS One* 2008, **3**:e3042.

49. Carvalhais LC, Dennis PG, Tyson GW, Schenk PM: **Application of metatranscriptomics to soil environments**. *J Microbiol Methods* 2012, **91**:246-251.

50. Jiang Y, Xiong X, Danska J, Parkinson J: **Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiome-specific functionality**. *Microbiome* 2016, **4**:2.

51. Wu J, Gao W, Johnson RH, Zhang W, Meldrum DR: **Integrated metagenomic and metatranscriptomic analyses of microbial communities in the meso- and bathypelagic realm of north pacific ocean**. *Mar Drugs* 2013, **11**:3777-3801.

52. Wilmes P, Bond PL: **The application of two-dimensional polyacrylamide gel electrophoresis and downstream analyses to a mixed community of prokaryotic microorganisms**. *Environ Microbiol* 2004, **6**:911-920.

53. Frias-Lopez J, Shi Y, Tyson GW, Coleman ML, Schuster SC, Chisholm SW, Delong EF: **Microbial community gene expression in ocean surface waters**. *Proc Natl Acad Sci U S A* 2008, **105**:3805-3810.

54. Jansson JK, Baker ES: **A multi-omic future for microbiome studies**. *Nat Microbiol* 2016, **1**:16049.

55. Meier JL, Niessen S, Hoover HS, Foley TL, Cravatt BF, Burkart MD: **An orthogonal active site identification system (OASIS) for proteomic profiling of natural product biosynthesis**. *ACS Chem Biol* 2009, **4**:948-957.

56. Meier JL, Burkart MD: **Proteomic analysis of polyketide and nonribosomal peptide biosynthesis**. *Curr Opin Chem Biol* 2011, **15**:48-56.

57. Evans BS, Ntai I, Chen Y, Robinson SJ, Kelleher NL: **Proteomics-based discovery of koranimine, a cyclic imine natural product**. *J Am Chem Soc* 2011, **133**:7316-7319.

58. Li Z, Wang Y, Yao Q, Justice NB, Ahn TH, Xu D, Hettich RL, Banfield JF, Pan C: **Diverse and divergent protein post-translational modifications in two growth stages of a natural microbial community**. *Nat Commun* 2014, **5**:4405.

59. Fan B, Li YL, Li L, Peng XJ, Bu C, Wu XQ, Borriss R: **Malonylome analysis of rhizobacterium *Bacillus amyloliquefaciens* FZB42 reveals involvement of lysine malonylation in polyketide synthesis and plant–bacteria interactions**. *J Proteomics* 2017, **154**:1-12.

60. Goering AW, McClure RA, Doroghazi JR, Albright JC, Haverland NA, Zhang Y, Ju KS, Thomson RJ, Metcalf WW, Kelleher NL: **Metabologenomics: correlation of microbial gene clusters with metabolites drives discovery of a nonribosomal peptide with an unusual amino acid monomer**. *ACS Cent Sci* 2016, **2**:99-108.

61. •• Maansson M, Vynne NG, Klitgaard A, Nybo JL, Melchiorsen J, Nguyen DD, Sanchez LM, Ziemert N, Dorrestein PC, Andersen MR *et al.*: **An integrated metabolomic and genomic mining workflow to uncover the biosynthetic potential of bacteria**. *mSystems* 2016:1.
Efficient workflow to combine chemical/metabolomics data and informations obtained from genome mining approaches.

62. Paulus C, Rebets Y, Tokovenko B, Nadmid S, Terekhova LP, Myronovskyi M, Zotchev SB, Rückert C, Braig S, Zahler S *et al.*: **New natural products identified by combined genomics-metabolomics profiling of marine *Streptomyces* sp. MP131-18**. *Sci Rep* 2017, **7**:42382.

63. Ju KS, Gao J, Doroghazi JR, Wang KK, Thibodeaux CJ, Li S, Metzger E, Fudala J, Su J, Zhang JK *et al.*: **Discovery of phosphonic acid natural products by mining the genomes of 10,000 actinomycetes**. *Proc Natl Acad Sci U S A* 2015, **112**:12175-12180.

64. Zhang Q, Doroghazi JR, Zhao X, Walker MC, van der Donk WA: **Expanded natural product diversity revealed by analysis of lanthipeptide-like gene clusters in actinobacteria**. *Appl Environ Microbiol* 2015, **81**:4339-4350.

65. Tobias NJ, Wolff H, Djahanschiri B, Grundmann F, Kronenwerth M, Shi YM, Simonyi S, Grün P, Shapiro-Ilan D, Pidot SJ *et al.*: **Natural product diversity associated with the nematode symbionts Photorhabdus and Xenorhabdus**. *Nat Microbiol* 2017, **2**:1676-1685.

66. Donia MS, Cimermancic P, Schulze CJ, Wieland Brown LC, Martin J, Mitreva M, Clardy J, Linington RG, Fischbach MA: **A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics**. *Cell* 2014, **158**:1402-1414.

67. Iftime D, Jasyk M, Kulik A, Imhoff JF, Stegmann E, Wohlleben W, Süssmuth RD, Weber T: **Streptocollin, a Type IV lanthipeptide produced by *Streptomyces collinus* Tü 365**. *Chembiochem* 2015, **16**:2615-2623.

68. Iftime D, Kulik A, Härtner T, Rohrer S, Niedermeyer TH, Stegmann E, Weber T, Wohlleben W: **Identification and activation of novel biosynthetic gene clusters by genome mining in the kirromycin producer *Streptomyces collinus* Tü 365**. *J Ind Microbiol Biotechnol* 2016, **43**:277-291.

69. Rico S, Santamaría RI, Yepes A, Rodríguez H, Laing E, Bucca G, Smith CP, Díaz M: **Deciphering the regulon of *Streptomyces coelicolor* AbrC3, a positive response regulator of antibiotic production**. *Appl Environ Microbiol* 2014, **80**:2417-2428.

70. Wang XJ, Zhang B, Yan YJ, An J, Zhang J, Liu CX, Xiang WS: **Characterization and analysis of an industrial strain of *Streptomyces bingchenggensis* by genome sequencing and gene microarray**. *Genome* 2013, **56**:677-689.

71. Traxler MF, Watrous JD, Alexandrov T, Dorrestein PC, Kolter R: **Interspecies interactions stimulate diversification of the *Streptomyces coelicolor* secreted metabolome**. *MBio* 2013:4.

72. Yang Q, Ding X, Liu X, Liu S, Sun Y, Yu Z, Hu S, Rang J, He H, He L *et al.*: **Differential proteomic profiling reveals regulatory proteins and novel links between primary metabolism and spinosad production in *Saccharopolyspora spinosa***. *Microb Cell Fact* 2014, **13**:27.

73. Albright JC, Goering AW, Doroghazi JR, Metcalf WW, Kelleher NL: **Strain-specific proteogenomics accelerates the discovery of natural products via their biosynthetic pathways**. *J Ind Microbiol Biotechnol* 2014, **41**:451-459.