The Journal of Antibiotics (2019) 72:54–56
https://doi.org/10.1038/s41429-018-0109-0

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

CORRESPONDENCE

MS network-based screening for new antibiotics discovery

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Received: 6 September 2018 / Revised: 2 October 2018 / Accepted: 4 October 2018 / Published online: 23 October 2018

Microbial products have a rich history of serving as natural sources of bioactive molecular scaffolds. Recent advances in genomic approaches have prompted the development of new microbial products, but characterizing new bioactive compounds from complex microbial mixtures remains challenging, as evidenced by the frequent rediscovery of known compounds. We hypothesized that an MS network derived from 1000 microbial metabolomes constructed in a previous study [1] could serve as a platform that would enable more efficient selection of microbial culture broths containing new bioactive compounds. In this study, we verified the effectiveness and usefulness of the MS network-based indexing approach in screening for new antibiotics.

Our MS network-based screening workflow is summarized in Fig. 1a. First, bioactive samples are screened using various conventional assays, and they are then analyzed using LC-MS/MS to generate an MS network using the global natural products social molecular networking (GNPS) data analysis tool [2–5]. Next, the MS network data are integrated with previous MS network data derived from 1000 microbial metabolomes in order to analyze the production frequency. This enables concomitant dereplication of natural products using a reference spectra library database containing over 22,000 natural products. Through this step, it becomes clear what metabolites contained in bioactive samples are rare and possibly new, allowing for the selection of highly unique strains. Subsequently, small batches of screening broths of the strains selected are fractionated by HPLC, and the fractionated samples are then evaluated using a bioassay to confirm the relatedness of target metabolites to the bioactivity. Thus, the MS network-based indexing approach enables us to prioritize strains for further scale-up isolation.

One of our screening efforts focused on the discovery of new compounds exhibiting anti-mycobacterial activity against *Mycobacterium avium* and *M. intracellulare*. These organisms cause an infection known as *M. avium* complex (MAC), which is associated with high unmet medical needs in Japan and other countries [6, 7]. To validate the MS network-based indexing approach, this screening program of anti-MAC agents was performed. Over 6000 microbial culture broths were evaluated using a liquid microdilution assay according to our established method [8]. In addition to this assay, the selectivity of the cytotoxic activity of the active samples was evaluated against mammalian cells, resulting in the selection of 41 microbial culture broths. A subsequent MS network-based comparison of the metabolites in these samples with 1000 microbial metabolomes resulted in the selection of one particular actinomycete strain that produced the largest number of rare metabolites. As shown by the red-colored nodes in Fig. 1b, this strain produced 44 metabolites that did not match the more than 7000 microbial metabolites in the MS network. In addition, these metabolites were not dereplicated by the reference spectra. Among the selected metabolites, we found that one molecular network with a total of 22 nodes was related to anti-MAC activity (Fig. 1b, circled area). Indeed, a mass-guided purification study led to the isolation of target metabolites from the culture broth of the strain. Various spectroscopic analyses, including NMR, indicated that some of the metabolites were new compounds. Furthermore, anti-mycobacterial activity of the compounds against *M. tuberculosis* as well as the causative agents of MAC was confirmed. Further studies directed toward drug development are now in progress with the assistance of a Japanese medical research and development organization. Detailed data regarding the new antibiotics will be reported elsewhere.

In conclusion, we demonstrated the effectiveness of our MS network-based indexing approach for rapidly identifying potential microbial sources of new compounds in screening for antibiotics. The MS spectral data for 1000 microbial metabolomes, which can be accessed via the public GNPS database, should also be useful for natural product chemists. To maximize the utility of the MS network-based indexing approach, it

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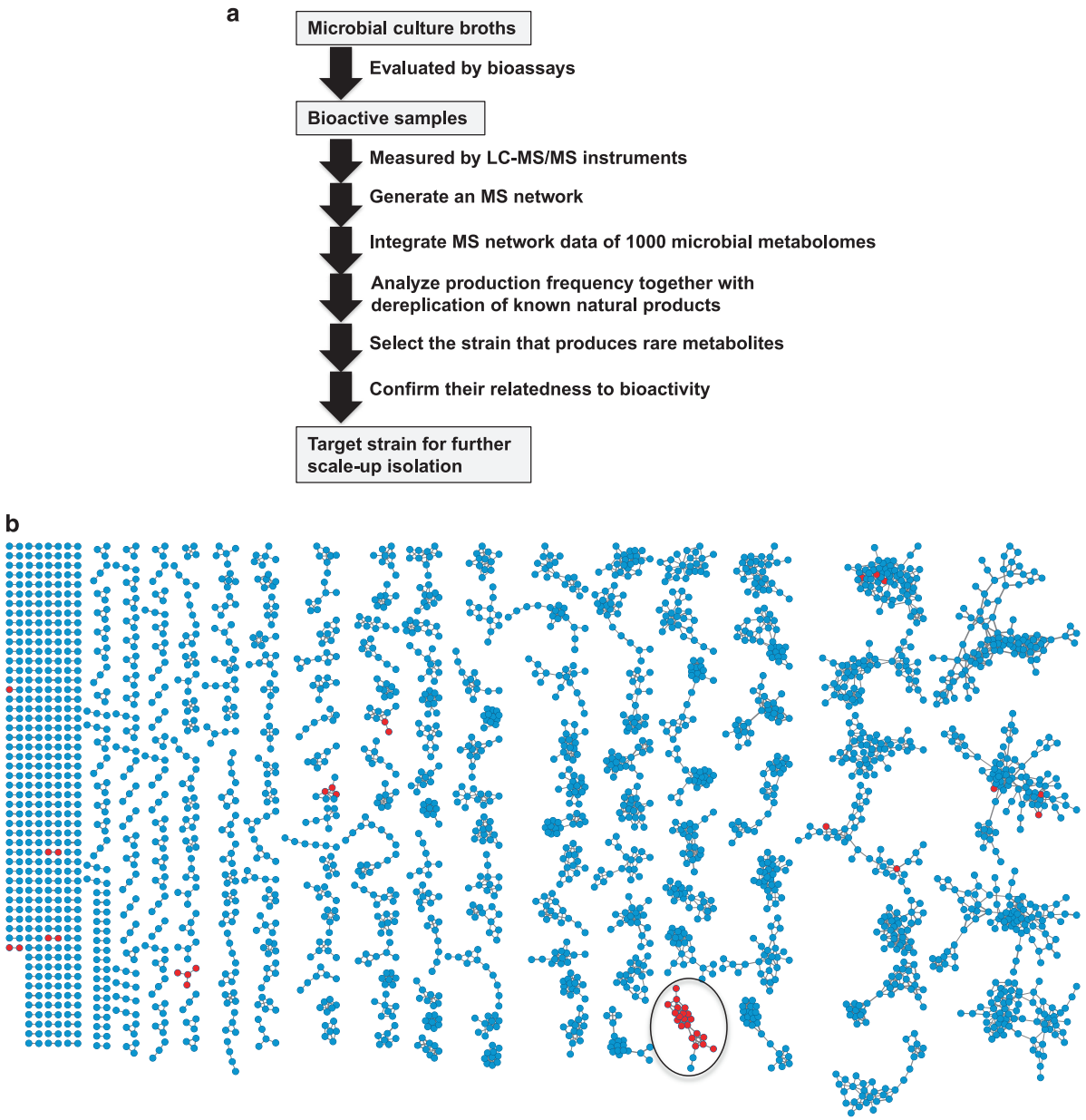

Fig. 1 **a** Workflow of MS network-based screening of bioactive compounds. **b** Molecular network generated by integration of data for 1000 microbial metabolomes, from which 41 bioactive samples were selected in the screening for anti-MAC agents. These data were generated using a cosine similarity score of 0.7 and parent mass tolerance of ±2.0 Da. Each node represents one consensus MS/MS spectrum-labeled parent mass. Edge thickness indicates similarity in cosine score. Red color indicates nodes related to metabolites produced by the actinomycete strain selected in our MS network-based screening. Blue color indicates nodes shared with other strains. The molecular network related to the new anti-MAC agents is shown in the circled area

is helpful to accumulate MS spectral data for a diverse array of microbial samples in the database. In particular, expanding the number of reference spectra of bioactive natural products within the database would enhance its usefulness and effectiveness in screening for new bioactive compounds.

Acknowledgements We are grateful to Mr. A. Kanamoto, OP Bio Factory Co., Ltd., for providing the microbial culture collection. We thank Dr. H. Onaka, Graduate School of Agricultural and Life Sciences, The University of Tokyo, for use of LC-MS/MS instruments. We also thank Mr. K. Hosoda, School of Pharmacy, Kitasato University, for measurement of anti-mycobacterial activity. This study was supported by JSPS KAKENHI grant number 15K07867 (NK) from the Ministry of Education, Culture, Sports, Science, and a Kitasato University Research Grant for Young Researchers (NK).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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