KAVAKA 57 : 1-5 (2021) doi: 10.36460/Kavaka/57/2021/1-5

Increasing the chances of finding novel antibiotics to manage multidrug-resistant pathogens

Trichur S. Suryanarayanan[1*] and Florenz Sasse[2]

[1]*Vivekananda Institute of Tropical Mycology (VINSTROM), Ramakrishna Mission Vidyapith, Chennai 600 004, Tamil Nadu, India*

[2]*Institute for Pharmaceutical Biology, Technical University of Braunschweig, 38124 Braunschweig, Germany*

Corresponding author Email: t_sury2002@yahoo.com

(Submitted on October 13, 2021; Accepted on November 22, 2021)

ABSTRACT

The urgent need for antibiotics of novel molecular architecture to manage the rapid spread of multidrug-resistant pathogens (MDR) cannot be overstressed. It is obvious that compared with mere modifications of existing antibiotics, compounds of molecular structures hitherto not experienced by the pathogens would be more effective and also reduce the rate of evolution of drug resistance among them. We argue that instead of the routine exercise of bioprospecting different classes of microbes, the chances of finding such novel antibiotics are more if MDR pathogens are made to interact with microbes, especially with fungi from less explored and extreme habitats (LEEH).

Keywords: Antibiotic resistance, Bioprospecting, Extreme habitats, Antibiosis

INTRODUCTION

The discovery of antibiotics was one of the critical factors which improved several facets of human life in the 20th century (Dasgupta, 2021). However, the evolution and rapid spread of antibiotic resistance among human pathogenic bacteria and fungi is pushing the world to the pre-antibiotic period thus posing a formidable global health threat. Recently, Dr. Tedros Adhanom Ghebreyesus, Director-General of WHO has declared, "Never has the threat of antimicrobial resistance been more immediate and the need for solutions more urgent" (https://www.who.int/news/item/17-01-2020-lack-of-new-antibiotics-threatens-global-efforts-to-contain-drug-resistant-infections). It is projected that by 2050 these multidrug-resistant (MDR), pan-drug-resistant (PDR) and extensively drug-resistant (XDR) microbes would cause 10 million deaths annually resulting in a global cost of 100 trillion USD (Trotter *et al.,* 2019). Although many infections by MDR pathogens are nosocomial, some of them are community-acquired. These pathogens possess antibiotic resistance trait that exist even when the antibiotic pressure is absent thus aiding in their extraordinary wide dispersal (van Duin and Paterson, 2016). To manage this global health problem, the World Health Organization (WHO) recommends that future research strategies should focus on the discovery and development of new antibiotics specifically active against MDR and XDR microbes (https://www.who. int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed).

THE WORSENING CRISIS OF ANTIBIOTIC RESISTANCE

Although evolution of antibiotic resistance among susceptible microbes is a natural process, the rapid evolution of MDR pathogens is ascribed to the indiscriminate and overuse of antibiotics (Ventola, 2015). This situation is compounded by the reluctance of pharmaceutical companies to produce new antibiotics mainly due to lack of economic viability (Plackett, 2020). The very low economic returns compared to the expenditure in trying to find new antibiotics, the failure of many potential antibiotics to perform at the preclinical trial stage, the policy of the governments of many countries to subsidize medicine costs, the strategy of the physicians in not prescribing new antibiotics to delay the evolution of resistance, and the almost total lack of knowledge about the economics of drug resistance are the main causes for pharmaceutical companies not investing in antibiotic research (Plackett, 2020). The reluctance in sharing negative research results among researchers also retards significantly the discovery of new antibiotics (Yu, 2021). Furthermore, the so-called novel antibiotics in use are only chemical modifications of those for which resistance has been already developed and hence their effective life is bound to be short (Renwick *et al.,* 2016). Only very few of the antibiotics approved in the last 40 years are new classes of molecules (Miethke *et al.,* 2021). The screening of synthetic chemical libraries and synthetic drug chemical approaches (Ortholand and Ganesan, 2004) have not yielded new antibiotics (Brown *et al.,* 2014). It is arguable that the methods advocated to control MDR such as chemical modifications of ineffective antibiotics, use of antibiotic resistant breakers and directed drug delivery systems (Laws *et al.,* 2019) are stable solutions. Although methods such as the use of bacteriocins, antibodies, phage therapy, nanotherapy (Vivas *et al.,* 2019) and deep learning approach are being tried (Stokes *et al.,* 2020), bioprospecting for natural antibiotics to manage MDR pathogens could still be worthwhile as this holds more promise for finding truly novel antibiotics.

FUNGI OF LEEH IN BATTLE WITH MDR PATHOGENS INCREASED CHANCES OF FINDING NOVEL ANTIBIOTICS

Although research on natural products compared to synthetic ones holds promise to deliver new antibiotics especially owing to their 'loose adherence' to Lipinski's rule of five (Miethke *et al.,* 2021), searching for effective antibiotics among natural products is fraught with a major drawback of the repeated discovery of the same molecules (Cox *et al.,* 2017). One reason for this could be that almost all the currently used antibiotics have been obtained from bacteria and fungi of only one ecological niche, the soil (Gogineni *et al.,* 2020). Thus, it would be prudent to bioprospect microbes of less explored and extreme habitats (LEEH) for different chemical classes of antibiotics (Marcolefas *et al.,* 2019; Schneider, 2021). Although a ratiocinative conclusion is that the need for antibiotics for microbes of such 'unusual' environments would be less due to the depauperate species abundance in such settings, several recent reports suggest otherwise. For instance, marine planctomycetes bacteria

appear to be an excellent source of many novel antibiotics (Wiegand *et al.,* 2020); similarly, marine actinobacteria elaborate novel antibiotics effective against drug resistant fungal (Zhang *et al.,* 2020a) and bacterial pathogens (Igarashi *et al.,* 2021). Microbes associated with sponges (Zhang *et al.,* 2020b) and corals produce novel antimicrobial compounds (Zhang *et al.,* 2020c).

Here, fungi seem to hold promise. Fungi possess diverse biosynthetic pathways (Kück *et al.,* 2014). In fungi, the genes encoding enzymes involved in synthesizing secondary metabolites are in continuous arrangement forming biosynthetic gene clusters (BGCs). A single fungus houses 30 to 70 BGCs; along with the information gleaned from the number of sequenced fungal genomes, this indicate that the secondary metabolite spectrum of fungi has hardly been explored (Keller, 2019). The fact that merely 8% of the estimated 2.2 to 3.8 million existing species of fungi are known currently (Hawksworth and Lücking, 2017) lends credence to the high expectation of obtaining novel drugs from fungi. Owing to their phenotypic plasticity, fungi survive in extreme habitats exhibiting desiccation (Barnard *et al.,* 2013), high and low temperatures (McKay *et al.,* 2003; Onofri *et al.,* 2007), acidic and alkaline pH (Gonzalez-Toril *et al.,* 2003; Gonsalves, 2012), or salinity, or radiation (Zhdanova *et al.,* 2004; Dadachova and Casadevall, 2008; Fendrihan *et al.,* 2009; Stan-Lotter and Fendrihan, 2013); they are known to tolerate radiation and dry heat (Suryanarayanan *et al.,* 2011) and survive even under simulated Mars conditions (Blachowicz *et al.,* 2019). Fungi from such extreme environments have hardly been addressed for their metabolite profile (Suryanarayanan and Hawksworth, 2005) though a few studies endorse that they produce several novel metabolites including those with potential antibiotic properties. Zhang *et al.* (2018) in their review have collated a total of 314 novel compounds from extremophilic fungi of which 161 are bioactive compounds. It is known that environmental factors such as temperature, pH, and carbon source and nitrogen source affect secondary metabolite production in fungi (Calvo *et al.,* 2002). As one of the adaptations to survive in harsh conditions, fungi could have evolved a metabolite spectrum different from those of normal environment. Thus, fungi from LEEH are a good source of novel metabolites (Chávez *et al.,* 2015).

To enhance the chances of finding novel antibiotics, we propose creating artificial microbial communities where MDR pathogen(s) are made to interact with fungi of LEEH. Such interactions could result in antibiosis as one of the mechanisms to overcome competition between the microbes for space and resource (Fig. 1). This could be more effective than a routine search of any natural environment including coculture method since habitat filtering selects microbes with suitable traits to survive in particular habitats (Feinstein and Blackwood, 2013; Crowther *et al.,* 2014). The three aspects of this proposal which could bolster the possibility of identifying new antibiotics are (1) the use of MDR assures the detection of , at least at the screening level, antibiotics acting against them since secondary metabolite gene clusters in fungi are activated by interactions and communications with bacteria (Netzker *et al.,* 2015), (2) fungi of LEEH have hardly been explored for their antibiotic spectrum, and (3) since extreme habitats harbor low species diversity of microbes sharing a common selective pressure, it would be easier to construct synthetic communities (Ponomarova *et al.,* 2017) for further exploration. MDR pathogens isolated from dressings, tissues and body fluid wastes of infected patients, catheters and disposable tissues could be grown in the laboratory as axenic cultures. They could then be challenged with fungi of LEEH to know if they produce antibiotics against the MDR pathogens. It is essential to subject this challenging setup to different grades of stress conditions since the physiology of some halophilic fungi is known to change as a response to the external salinity (Pérez-Llano *et al.,* 2020).

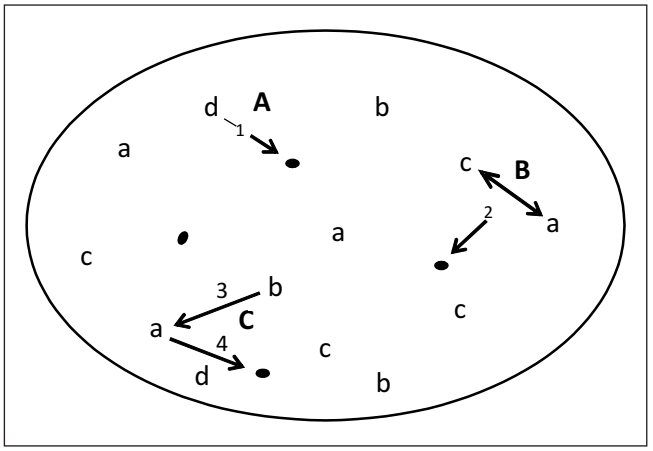

Fig. 1: Possible ways by which novel antibiotics could be produced when LEEH fungi are allowed to interact with MDR in a simulated harsh environment. A. A fungus (d), as a response to the harsh condition, produces a metabolite (1) which inhibits MDR. B. Interaction between two fungi (c) and (a), induces production of a metabolite (2) by one of them which inhibits MDR. C. Interaction of the fungi (b) and (a) induces production of a metabolite by one of them which is biotransformed by the fungus (d) which inhibits MDR.

a,b,c and d - LEEH fungal species, ● MDR pathogen.

SIMPLE METHODS TO SCREEN LEEH FOR ANTIBIOTICS

Some LEEH fungi such as thermotolerant, pH or salt-tolerant fungi are highly adaptable and grow both under harsh and normal environments. To screen such fungi, we propose a modified Rossi-Cholodny buried slide technique (Cholodny, 1930; Rossi, 1936). A mixture of LEEH fungi (spores and/or mycelial bits) isolated from an environment is added to a molten water agar medium, mixed and poured in a sterile beaker. After the medium has set, slots are made in it by inserting sterile microscope slides and removed. Sterile microscope slide coated with nutrient agar inoculated with a MDR pathogen is inserted in the slot and incubated in a moist chamber. The slide is removed periodically and plated on a nutritive agar medium to isolate the fungi attached to the

slide. Based on the principle that a natural filtering mechanism works in a competitive environment, it is likely that some of the fungi which have come in contact with the slide supporting the growth of the MDR pathogen could exhibit antibiosis. Hence, the fungi isolated from the slide can be tested by simple agar diffusion method for production of anti-MDR metabolites. The same procedure could be repeated for obligate extremophiles by simulating conditions of the harsh environments and after ensuring that the test MDR pathogen is not killed by the harsh environment. Solidified nanofibrous cellulose-containing media which withstand extreme conditions could be used for culturing acidophiles, alkaliphiles, thermophiles, acidothermophiles and alkalithermophiles (Tsudome *et al.,* 2009).

RAPID QUALITATIVE SCREENING FOR ANTIBIOTIC PRODUCTION

A LEEH fungus is cultured in suitable liquid medium (with and without extreme conditions) for 3-4 weeks. Its culture filtrate (secretome) is collected, extracted with a solvent and concentrated. The concentrated secretome (20-50 µL of each fungus) is spotted on a silica gel coated aluminium thin-layer chromatography (TLC) sheet. The TLC sheet is then sprayed with an MDR pathogen suspended in a growth medium and incubated for 3-5 days in moist chambers. Then the TLC plate is sprayed with tetrazolium salt such as MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Living MDR bacteria convert tetrazolium salt to purple formazan and hence the uncoloured spots on the TLC indicate dead MDR bacteria suggesting the production of antibiotic metabolites (Choma and Jesionek, 2015).

A major concern in bioprospecting LEEH microbes including fungi, is the non-cultivable nature of many of them. Thus, any effort involving the culturable microbes alone would capture only a part of their chemical diversity. Occurrence in very low numbers in an ecological niche, slow growth rates, specific growth requirements, low competitive ability, or dependence on chemicals produced by co-occurring microbes are among the major factors that are responsible for the inability of some microbes to grow in culture. Hence, different methods to encourage the growth of the unculturable LEEH fungi have to be tried to enhance the chances of the discovery of novel antibiotics. Some of these include the reduction of nutrients in the growth media, reduction of the diversity of microbes by dilution to culture the species occurring in very low numbers, long term incubation to encourage slow growers, and creating growth conditions mimicking natural habitats (Vartoukian *et al.,* 2010). Once a potential antibiotic producing fungus is identified, co-culturing methods, exposure to light, adjustment of nutrients, epigenetic manipulation and expression of its BGCs in heterologous systems could be tried for activation of its genes to produce effective antibiotics (Keller, 2019).

CONCLUSION

We conclude that creating a battlefield for MDR and LEEH and involving all the possible culture and molecular techniques would increase appreciably the chances of finding novel antibiotics to manage such pathogens than random bioprospecting. As stressed by Suryanarayanan and Hawksworth (2018), funding agencies should encourage exploration of fungi from unexplored habitats for the production of novel compounds active against MDR pathogens.

REFERENCES

Barnard, R.L., Osborne, C.A. and Firestone, M.K. 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *The ISME Journal* **7**: 2229-2241.

Blachowicz, A., Chiang, A.J., Elsaesser, A., Kalkum, M., Ehrenfreund, P., Stajich, J.E., Torok, T., Wang, C.C.C. and Venkateswaran, K. 2019. Proteomic and metabolomic characteristics of extremophilic fungi under simulated mars conditions. *Frontiers in Microbiology* **10**: 10-13.

Brown, D.G., May-Dracka, T.L., Gagnon, M.M. and Tommasi, R. 2014. Trends and exceptions of physical properties on antibacterial activity for gram-positive and gram-negative pathogens. *Journal of Medicinal Chemistry* **57**: 10144-10161.

Calvo, A.M., Wilson, R.A., Bok, J.W. and Keller, N.P. 2002. Relationship between secondary metabolism and fungal development. *Microbiology and Molecular Biology Reviews* **66**: 447-459.

Chávez, R., Fierro, F., García-Rico, R.O. and Vaca, I. 2015. Filamentous fungi from extreme environments as a promising source of novel bioactive secondary metabolites. *Frontiers in Microbiology* **6**: 903.

Cholodny Kiew, N. 1930. Über eine neue methode zur untersuchung der bodenmikroflora. *Archiv für Mikrobiologie* **1**: 620-652.

Choma, I.M. and Jesionek, W. 2015. TLC-Direct bio-autography as a high throughput method for detection of antimicrobials in plants. *Chromato-graphy* **2**: 225-238.

Cox, G., Sieron, A., King, A.M, De Pascale, G., Pawlowski, A.C., Koteva, K. and Wright, G.D. 2017. A common platform for antibiotic dereplication and adjuvant discovery. *Cell Chemical Biology* **24**: 98-109.

Crowther, T.W., Maynard, D.S., Crowther, T.R., Peccia, J., Smith, J.R. and Bradford, M.A. 2014. Untangling the fungal niche: the trait-based approach. *Frontiers in Microbiology* **5**: 579.

Dadachova, E. and Casadevall, A. 2008. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. *Current Opinion in Microbiology* **11**: 525-531.

Dasgupta, P. 2021. The economics of biodiversity: the Dasgupta review. HM Treasury, London.

Feinstein, L.M. and Blackwood, C.B. 2013. The spatial scaling of saprotrophic fungal beta diversity in decomposing leaves. *Molecular Ecology* **22**: 1171-1184.

Fendrihan, S., Bérces, A., Lammer, H., Musso, M., Rontó, G.,

Polacsek, T.K., Holzinger, A., Kolb, C. and Stan-Lotter, H. 2009. Investigating the effects of simulated martian ultraviolet radiation on Halococcus dombrowskii and other extremely halophilic archaebacteria. *Astrobiology* **9**: 104-112.

Gogineni, V., Chen, X., Hanna, G., Mayasari, D. and Hamann, M.T. 2020. Role of symbiosis in the discovery of novel antibiotics. *The Journal of Antibiotics* **73**: 490-503.

Gonsalves, V. 2012. Halophilic fungi in a polyhaline estuarine habitat. *Journal of Yeast and Fungal Research* **3**: 30-36.

Gonzalez-Toril, E., Llobet-Brossa, E., Casamayor, E.O., Amann, R., and Amils, R. 2003. Microbial ecology of an extreme acidic environment, the Tinto river. *Applied and Environmental Microbiology* **69**: 4853-4865.

Hawksworth, D.L. and Lücking, R. 2017. Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology Spectrum* **5 (4)**: FUNK-0052-2016.

Igarashi, M., Sawa, R., Umekita, M., Hatano, M., Arisaka, R., Hayashi, C., Ishizaki, Y., Suzuki, M. and Kato, C. 2021. Sealutomicins, new enediyne antibiotics from the deep-sea actinomycete *Nonomuraea* sp. MM565M-173N2. *The Journal of Antibiotics* **74**: 291-299.

Keller, N.P. 2019. Fungal secondary metabolism: regulation, function and drug discovery. *Nature Reviews Microbiology* **17**: 167-180.

Kück, U., Bloemendal, S. and Teichert, I. 2014. Putting fungi to work: harvesting a cornucopia of drugs, toxins, and antibiotics. *PLoS Pathol.* 10: e1003950; doi: org/10.1371/journal.ppat. 1003950.

Laws, M., Shaaban, A. and Rahman, K.M. 2019. Antibiotic resistance breakers: current approaches and future directions. *FEMS Microbiology Reviews* **43**: 490-516.

Marcolefas, E., Leung, T., Okshevsky, M., McKay, G., Hignett, E., Hamel, J., Aguirre, G., Blenner-Hassett, O., Boyle, B., Lévesque, R.C., Nguyen, D., Gruenheid, S. and Whyte L. 2019. Culture-dependent bioprospecting of bacterial isolates from the Canadian High Arctic displaying antibacterial activity. *Frontiers in Microbiology* **10**: 18-36.

McKay, C.P., Friedmann, E.I., Gómez-Silva, B., Cáceres-Villanueva, L., Andersen, D.T. and Landheim, R. 2003. Temperature and moisture conditions for life in the extreme arid region of the Atacama Desert: four years of observations including the El Niño of 1997-1998. *Astrobiology* **3**: 393-406.

Miethke, M., Pieroni, M., Weber, T. *et al.* 2021. Towards the sustainable discovery and development of new antibiotics. *Nature Reviews Chemistry* **19**: 1-24.

Netzker, T., Fischer, J., Weber, J., Mattern, D.J., König, C.C., Valiante, V., Schroeckh, V. and Brakhage, A.A. 2015. Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. *Frontiers in Microbiology* **6**: 299.

Onofri, S., Selbmann, L., de Hoog, G.S., Grube, M., Barreca, D., Ruisi, S. and Zucconi, L. 2007. Evolution and adaptation of fungi at boundaries of life. *Advances in Space Research* **40**: 1657-1664.

Ortholand, J.Y. and Ganesan, A. 2004. Natural products and combinatorial chemistry: back to the future. *Current Opinion in Chemical Biology* **8**: 271-280.

Pérez-Llano, Y., Rodríguez-Pupo, E.C., Druzhinina, I.S., Chenthamara, K., Cai, F., Gunde-Cimerman, N., Zalar, P., Gostinčar, C., Kostanjšek, R., Folch-Mallol, J.L., Batista-García, R.A. and Sánchez-Carbente, M.D.R. 2020. Stress reshapes the physiological response of halophile fungi to salinity. *Cells* **9**: 525.

Plackett, B. 2020. Why big pharma has abandoned antibiotics. *Nature* **586**: S50-S52.

Ponomarova, O., Gabrielli, N., Sévin, D.C., Mülleder, M., Zirngibl, K., Bulyha, K., Andrejev, S., Kafkia, E., Typas, A., Sauer, U., Ralser, M. and Patil, K.R. 2017. Yeast creates a niche for symbiotic lactic acid bacteria through nitrogen overflow. *Cell Systems* **5**: 345-357.

Renwick, M.J., Simpkin, V. and Mossialos, E. 2016. Targeting Innovation in Antibiotic Drug Discovery and Development: the Need for a One Health One Europe One World Framework. *European Observatory on Health Systems and Policies, Health Policy Series,* Study No. 45, WHO regional office for Europe, Copenhagen, Denmark pp. 1-126.

Rossi, G., Riccardo, S., Gesue, G., Stanganelli, M. and Wang, T.K. 1936. Direct microscopic and bacteriological examination of soil. *Soil Science* **41**: 53-66.

Schneider, Y.K. 2021. Bacterial natural product drug discovery for new antibiotics: strategies for tackling the problem of antibiotic resistance by efficient bioprospecting. *Antibiotics* **10**: 842.

Stan-Lotter, H. and Fendrihan, S. 2013. Survival Strategies of Halophilic Oligotrophic and Desiccation Resistant Prokaryotes. In: *Polyextremophiles* (Eds.:Seckbach, J., Oren, A. and Stan-Lotter, H.), Dordrecht: Springer, pp. 233-248.

Stokes, J.M., Yang, K., Swanson, K. *et al.* 2020. A deep learning approach to antibiotic discovery. *Cell* **180**: 688-702.

Suryanarayanan, T.S. and Hawksworth, D.L. 2005. Fungi from Little-explored and Extreme Habitats. In: *Biodiversity of Fungi: Their Role in Human Life* (Eds.: Deshmukh, S.K and Rai, M. K), Oxford & IBH Publishing, New Delhi, pp. 33-48.

Suryanarayanan, T.S., Govinda Rajulu, M.B, Thirumalai, E., Reddy, M.S. and Money, N.P. 2011. Agni's fungi: heat-resistant spores from the Western Ghats,

southern India. *Fungal Biology* **115**: 833-838.

Suryanarayanan, T.S. and Hawksworth, D.L. 2018. The war against MDR pathogens: move fungi to the front line. *Current Science* **115**: 2201-2205.

Trotter, A.J., Aydin, A., Strinden, M.J. and O'Grady, J. 2019. Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance. *Current Opinion in Microbiology* **51**: 39-45.

Tsudome, M., Deguchi, S., Tsujii, K., Ito, S. and Horikoshi, K. 2009. Versatile solidified nanofibrous cellulose-containing media for growth of extremophiles. *Applied and Environmental Microbiology* **75**: 4616-4619.

van Duin, D. and Paterson, D. L. 2016. Multidrug-resistant bacteria in the community: trends and lessons learned. *Infectious Disease Clinics of North America* **30**: 377-390.

Vartoukian, S.R., Palmer, R.M. and Wade, W.G. 2010. Strategies for culture of 'unculturable' bacteria. *FEMS Microbiology Letters* **309**: 1-7.

Ventola, C.L. 2015. The antibiotic resistance crisis. Part 1: causes and threats. *Pharmacology and Therapeutics* **40**: 277-283.

Vivas, R., Barbosa, A.A., Dolabela, S.S. and Jain, S. 2019. Multidrug-resistant bacteria and alternative methods to control them: an overview. *Microbial Drug Resistance* **25**: 890-908.

Wiegand, S., Jogler, M., Boedeker, C. *et al.* 2020. Cultivation and functional characterization of 79 planctomycetes uncovers their unique biology. *Nature Microbiology* **5**: 126-140.

Yu, H. 2021. Responsible use of negative research outcomes-accelerating the discovery and development of new antibiotics. *The Journal of Antibiotics* **74**: 543-546.

Zhang, F., Zhao, M., Braun, D.R. *et al.* 2020a. A marine microbiome antifungal targets urgent-threat drug-resistant fungi. *Science* **370**: 974-978.

Zhang, B., Zhang, T., Xu, J., Lu, J., Qiu, P., Wang, T. and Ding, L. 2020b. Marine sponge-associated fungi as potential novel bioactive natural product sources for drug discovery: a review. *Mini-Reviews in Medicinal Chemistry* **20**: 1966-2010.

Zhang, Z., Zhou, T., Harunari, E., Oku, N. and Igarashi, Y. 2020c. Iseolides AC, antifungal macrolides from a coral-derived actinomycete of the genus *Streptomyces*. *The Journal of Antibiotics* **73**: 534-541.

Zhang, X., Li, S.J., Li, J.J., Liang, Z.Z. and Zhao, C. Q. 2018. Novel natural products from extremophilic fungi. *Marine Drugs* **16**: 194.

Zhdanova, N.N., Tugay, T., Dighton, J., Zheltonozhsky, V. and Mcdermott, P. 2004. Ionizing radiation attracts soil fungi. *Mycological Research* **108**: 1089-1096.