

Aspergillomarsine from *Aspergillus versicolor* reduces antibiotic resistance in bacteria

Antibiotic resistance of Gram-negative bacteria is an issue of increasing international concern (McKenna 2013), and has been considered to pose a “catastrophic threat” by the UK’s Chief Medical Officer Sally Davies (Anon. 2013). Enzymes, β -lactamases, have emerged in bacteria which are able to hydrolyse the β -lactam ring that is essential for the antibacterial activity of cephalosporins, penicillins, carbapenems, and monobactams.

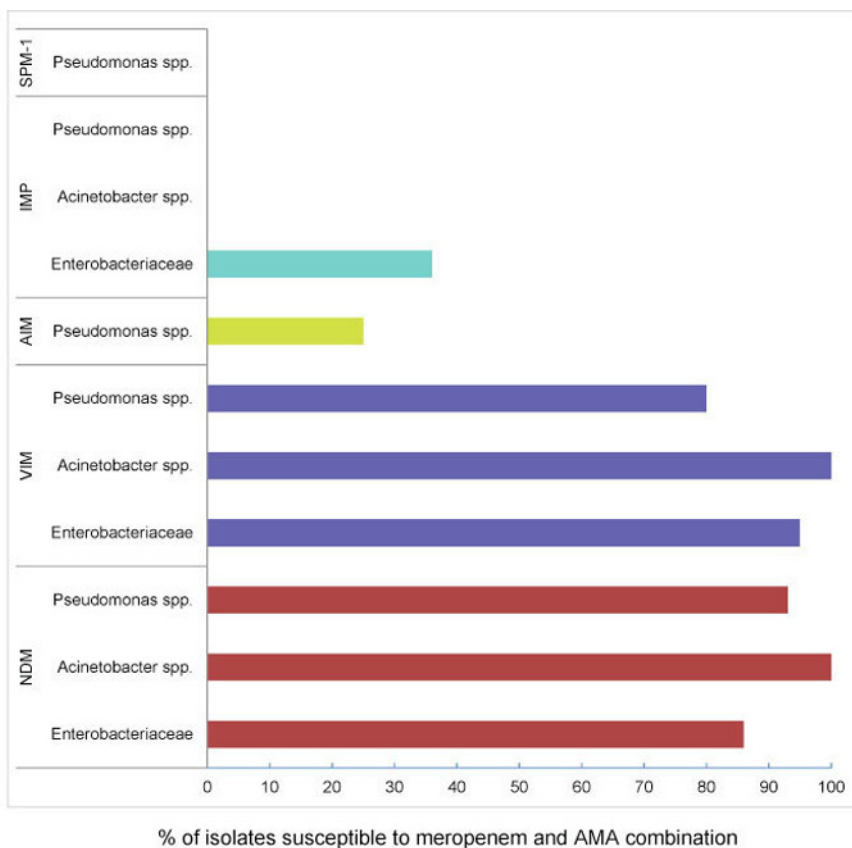
Now a fungal product of *Aspergillus versicolor*, aspergillomarsine A, promises to provide a way of inhibiting the activity of metallo- β -lactamases so that antibiotic activity is restored. Researchers at McMaster

University, the University of British Columbia, and Cardiff University (King *et al.* 2014) used mixtures of aspergillomarsine and meropenem (a carbapenem β -lactam) against a range of Gram-negative bacteria, including ones in *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae*, and found that the antibiotic activity could be restored to the extent that in some cases 80–100 % of the bacterial isolates tested were susceptible. The aspergillomarsine acts by interacting with the zinc ions in NDM-1 metallo- β -lactamases, so that meropenem can break down the cell wall of the target bacteria; this is illustrated diagrammatically by Meziane-Cherif & Courvalin (2014). This

particular compound achieved high levels of success in bacteria expressing VIM- and NDM- , but the mixture was not or less effective in bacteria expressing AIM-, IMP- and SPM-¹.

The restoration of meropenem activity found in the laboratory was successfully demonstrated in mice infected with NDM-1 expressing *K. pneumoniae*. While this is not a panacea to the problem as a whole, as it is becoming clear that different compounds able to inhibit the various metallo- β -lactamases are required, it does show that fungi are a source in which natural products can be searched for and assessed for activity against these antibiotic-inhibiting enzymes.

Antibiotic resistance is not just an issue in the case of harmful bacteria, as the pathogenic *Mucor circinelloides* has been found to develop spontaneous resistance to the antifungal drug tacrolimus. The basis of that resistance is only now starting to be elucidated and is quite different and involves epimutations (Calo *et al.* 2014), but it may also be of value to see if some other fungal product may also be of value in such cases.



Susceptibility of Gram-negative carbapenem-resistant bacteria to combined effect of meropenem and aspergillomarsine. Courtesy Gerard D. Wright.

Anon. (2013) The antibiotic alarm. *Nature* 495: 141.

Calo S, Shertz-Wall C, Lee S, Bastidas RJ, Nicolás FE, *et al.* (2014) Antifungal drug resistance evoked via RHAi-dependent epimutations. *Nature* 513: 555–558.

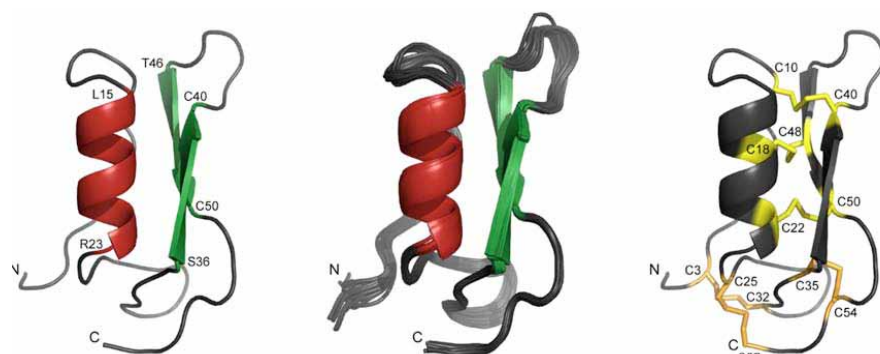
King AM, Reid-Yu SA, Wang W, King DT, de Pascale G, *et al.* (2014) Aspergillomarsine A overcomes metallo- β -lactamase antibiotic resistance. *Nature* 510: 503–506.

McKenna M (2013) The last resort. *Nature* 499: 394–396.

Meziane-Cherif D, Courvalin P (2014) To the rescue of old drugs. *Nature* 510: 477–478.

¹These acronyms refer to types of metallo- β -lactamases.

Copsin from *Coprinosopsis cinerea* inhibits cell-wall synthesis in bacteria



The structure of copsin as determined by NMR spectroscopy: highlighting the secondary structure (*left*), showing a bundle of conformers after energy refinement (*centre*), and with Cys residues (yellow and orange; *right*). See Essig *et al.* (2014) for further details. Courtesy Markus Aebi.

Agaricomycetes are not usually thought of as sources of antibiotics. Essig *et al.* (2014), however, have discovered a novel peptide of pharmaceutical interest in *Coprinosopsis cinerea*, which they named copsin. This discovery was made by Markus Aebi's group in the Swiss Federal Institute of Technology (ETH-Zurich), which is primarily concerned with the ecological interactions of fungi with bacteria (Stanley *et al.* 2014) and predators; in this case they collaborated with medical microbiologists in Bonn.

The compound was tested against various bacteria by inoculating Petri dishes, filled with a layer of glass beads, by plugs of the fungus; the fungus grew around

the beads in a liquid medium, and was co-cultivated with bacterial suspensions. It was found to be active against various Gram-positive, including the human pathogens *Enterococcus faecium* and *Listeria monocytogenes*. In contrast, *C. cinerea* was inhibited by Gram-negative bacteria it was challenged with, notably *Escherichia coli* and *Pseudomonas aeruginosa*.

The active compound was extracted and studied by mass spectrometry. The coding sequence was determined and the PCR product cloned and recombined into the yeast *Pichia pastoris* in order to obtain sufficient material for the elucidation of the 3-D structure by NMR. At present copsin

does not appear to have been searched for in other fungi, but might be expected at least in other coprophilous species of *Coprinosopsis* and other members of the family *Psyathyrellaceae*.

It seems that other agarics may also merit attention from drug discovery companies, especially as the same research group has found that the lectin tecton 2 from *Laccaria bicolor* is able to agglutinate Gram negative bacteria, and further is toxic to the nematode *Caenorhabditis elegans* (Wohlschlager *et al.* 2014). These discoveries also indicate the importance of allowing researchers freedom to work on topics that fascinate them, as it is impossible to know when such potentially exploitable side-products will emerge.

Essig A, Hofmann D, Münch D, Gayathri S, Künzler M, *et al.* (2014) Copsin, a novel peptide-based fungal antibiotic interfering with the peptidoglycan synthesis. *Journal of Biological Chemistry* 289: 34953–34964.

Stanley CE, Stöckli M, van Sway D, Sabotić J, Kallio PT, *et al.* (2014) Probing bacterial–fungal interactions at the single cell level. *Integrative Biology* 6: 935–945.

Wohlschlager T, Butschi A, Grassi P, Sutov G, Gauss R, *et al.* (2014) Methylated glycans as conserved targets of animal and fungal innate defense. *Proceedings of the National Academy of Sciences, USA* 111: E2787–E2796.

New light on the global diversity of soil fungi revealed by pyrosequencing

The limited availability of molecular data from soil samples has made it difficult to extrapolate from studied sites to a global scale. In order to address this constraint, Tendersoo *et al.* (2014) collected 40 soil cores from each of 365 sites distributed over the Earth. The project involved some 58 mycologists (listed as co-authors) from around 22 countries, and provides yet another fine example of international collaboration being used to address major issues in mycology.

Any overlying litter was removed and samples taken to a depth of 5 cm, and often contained fine roots. DNA was extracted from 2 g samples and PCR

carried out with forward and reverse ITS primers prior to 454 pyrosequencing. A massive 1, 019,514 quality sequences were obtained, representing 94,255 species level OTUs (Operational Taxonomic Units) of which 80,486 were *Fungi*. These were compared with sequences deposited in molecular databases, including UNITE, and 10,801 OTUs found to exhibit > 98 % similarity to sequences in existing databases. Intriguingly, about 6 % of the fungal OTUs could not be assigned to any known class of fungi, indicating that there may well be some extremely unusual fungi still to be isolated and described from soil.

Data were also obtained on mean annual temperatures and precipitation, and soil carbon from the WorldClim database. Sites were categorized into biogeographic regions and biomes, and the OTUs also by their biology. These and various other correlations were tested, and the proportions of different fungal classes presented as pie-charts for each biome (*see figure*). Overall species richness increased towards the equator, ectomycorrhizal fungi peaking at mid-latitudes (temperate forests and Mediterranean), while saprobes and pathogens increased at low latitudes. Inventories of the plants growing at the precise sites sampled were not made, but

data were extracted from a world vascular plant data set. Using that plant data, the plant:fungal species ratio was found to decrease with increasing latitude, and most markedly towards the poles, which the authors consider “calls into question present global fungal richness estimates”. In order to have confidence in such a conclusion, it would, of course, have been necessary not only to have site inventories of the plants, but further to conduct an inventory of the fungi in and on the aerial parts of plants, litter, in and on insects and other animals, and further lichens in all habitats, fungi growing on them, etc. There is also an issue over the level of similarity assumed to be

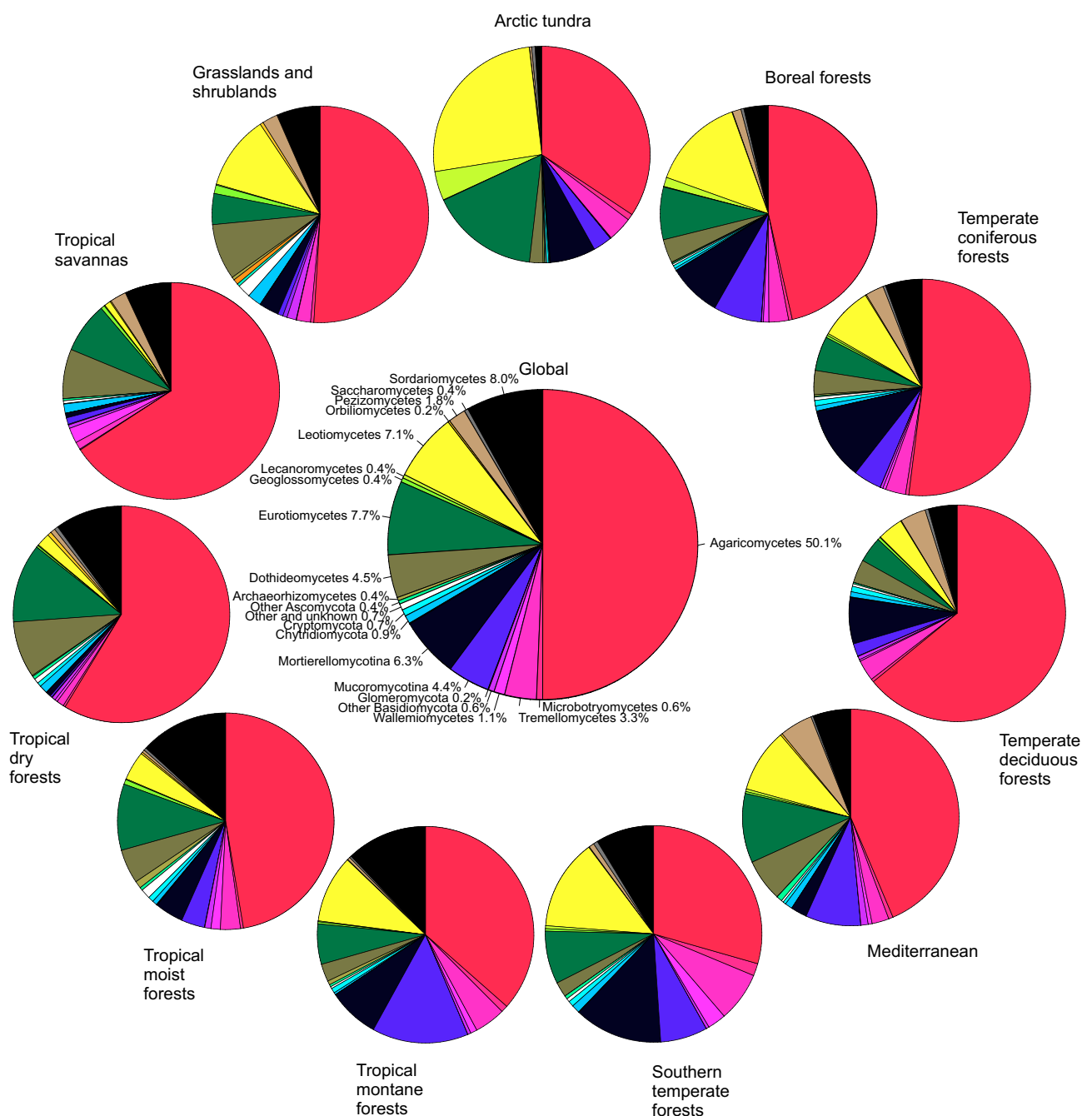
equivalent to species, and it would have been interesting to see what species numbers resulted if, for example, >98.5 or >99 % similarity were taken as cut-offs rather than the 98 % employed (Kõljalg *et al.* 2013). In addition, there seems to have been an assumption that all soil fungi present at the sites were recovered, and it may be that sequences represented by single occurrences at a site should not have simply been excluded prior to performing the analyses.

A pioneering study of this scale could not be expected to allow for all possible contributory factors that could influence the results, and it is a major first step. The wealth of information and generalizations made in

relation to the representation of fungi at the class level in soil in the different biomes is of particular interest, and it will be of interest to see how the other generalizations hold as more in-depth studies are carried out on particular sites – especially ones which also consider fungi not found in the soil.

Kõljalg U, Nilsson RH, Abarenkov K, Tendersoo L, Taylor AFS, *et al.* (2013) Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22: 5271–5277.

Tendersoo L, Bahram M, Põme S, Kõljalg U, Yoron NS, *et al.* (2014) Global diversity and geography of soil fungi. *Science* 346: DOI: 10.1126/science.1256688.



Relative proportion of fungal sequences assigned to major taxonomic groups in different biomes. Courtesy Leho Tendersoo.

Feedback loops with ectomycorrhizal fungi may maintain boreal forests

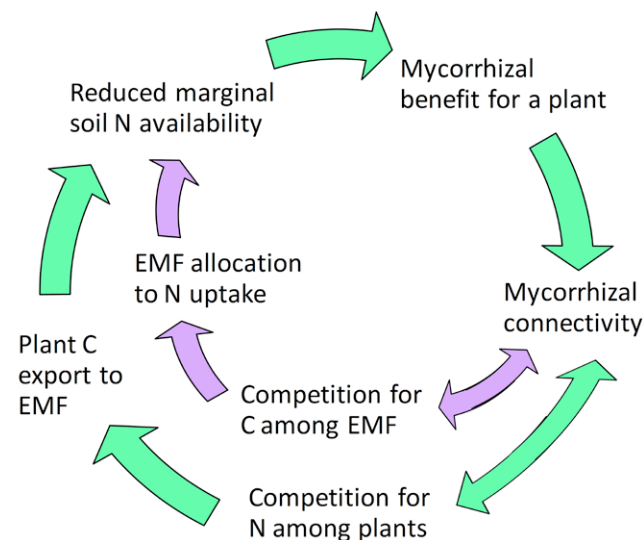
Ectomycorrhizal (ECM) associations are generally recognized as crucial to the maintenance of ecological processes in boreal forests, but the operational complexity has not been well-understood as experimental

studies have given inconsistent results with respect to any “benefit” to trees and other plants. Now, Franklin *et al.* (2014) have developed a model demonstrating how mycorrhizal networks between the roots of

different plants are related to carbon and nitrogen fluxes. A proportion of the carbon exported from the plants to the ECM fungi is used in the facilitation of nitrogen uptake. Where there are between different host plants and they are connected with a greater number of ECM fungi, the system appears to be more resilient. Further, the model predicts that plants would switch from a mixed mycorrhizal/non-mycorrhizal root strategy to an exclusively mycorrhizal one as the availability of soil nitrogen declines. This result is in agreement with actual data sets, and indicates that the communities tend to stabilize as a result of nitrogen depletion feedback. The authors also suggest that the feedback mechanism serves to maintain the nitrogen limitation situation for plant growth in these forests, and further that it may limit the ability of the forests to benefit from future rising atmospheric CO₂ levels.

Although field ecologists (including mycologists) are sometimes sceptical of models, and often rightly so, in this case the authors have based them on such a substantial experimental and field based body of research, that the conclusions cannot be ignored. Further, they provide an explanation, through the proposed feedback mechanism, of the long-term stability of the boreal forest ecosystems and the nutrient status of its soils.

Franklin O, Näsholm T, Högberg P, Högberg MN (2014) Forests trapped in nitrogen limitation – an ecological market perspective on ectomycorrhizal symbiosis. *New Phytologist* 203: 657–666.



Feedback loops stabilizing the mycorrhizal strategy, showing the interrelation between effects of ectomycorrhizal fungi (EMF; purple arrows) and plants (green arrows). See Franklin *et al.* (2014) for further explanation. Courtesy Oskar Franklin.

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Digested truffle spores as mycorrhizal inocula

Truffle-eating mammals, including marsupials, have tended to be seen as something of a curiosity until recent decades. It is now clear, especially from the studies of Andrew W. Claridge and James

(“Jim”) Trappe, that not only can truffles constitute a large proportion of the diet of some of these small mammals, they can be crucial to their survival in the immediate aftermath of forest fires as a food source.

They can even have a role as ecosystem engineers, as holes dug while searching for truffles, and reduce rainwater run-off on forested slopes. In one case a desire for truffles by badgers even contributed to a criminal conviction for badger-baiting in the UK (Hawksworth & Wiltshire 2011). For more information and more instances of the ecological roles of truffles, including as ectomycorrhizas, see the delightful book by Maser *et al.* (2010).

When truffles are eaten, an elegant study by Piattoni *et al.* (2014) has shed new light on what happens to the viability of spores of *Tuber aestivum* when they have passed through the gut of *Sus scrofa* (pig²). An individually housed piglet was fed on a mixture of boiled potato and fresh

²This binomial was used by the authors, but the domesticated pig is *Sus scrofa domesticus*; *Sus scrofa scrofa* is the wild boar, the ancestor of the domesticated pig.



Tuber aestivum. Photo: Todd Elliot.

truffles, and spores extracted from the faeces. The defecated spores were examined by sophisticated light and atomic force microscopy (AFM), and also tested for their ability to form mycorrhizas with *Quercus robur* seedlings in an otherwise sterile substrate in a growth chamber.

The microscopic studies showed that in comparison to undigested spores, the digested spores lost most of their surface ornamentation and the surface became uneven, with many small peaks and troughs; a change spectacularly visualized through AFM imagery. In the mycorrhization experiments, it was then

found that the seedlings inoculated with digested ascospores had 66 % colonization compared with 49 % with undigested ascospores. The study consequently suggests that in nature passage of truffle spores through the gut may enhance the chance of establishing mycorrhizas, as the same digestive processes can be expected to occur in wild boars and perhaps other truffle-eating mammals. The discovery may also contribute to improvements in the increasingly popular and commercialized artificial culture of truffles with trees (Hall *et al.* 2007).

- Hall IR, Brown GT, Zambonelli A (2007) *Taming the Truffle: the history, love, and science of the ultimate mushroom*. Portland: Timber Press.
- Hawksworth DL, Wiltshire PEJ (2011) Forensic mycology: the use of fungi in criminal investigations. *Forensic Science International* **206**: 1–11.
- Maser C, Claridge AW, Trappe JM (2010) *Trees, Truffles, and Beasts: how forests function*. New Brunswick: Rutgers University Press.
- Piattoni F, Amicucci A, Iotti M, Ori F, Stocchi V, Zambonelli A (2014) Viability and morphology of *Tuber aestivum* spores after passage through the gut of *Sus scrofa*. *Fungal Ecology* **9**: 52–56.