The biology of habitat dominance; can microbes behave as weeds?


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Minireview

The biology of habitat dominance; can microbes behave as weeds?

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Summary

Competition between microbial species is a product of, yet can lead to a reduction in, the microbial diversity of specific habitats. Microbial habitats can resemble ecological battlefields where microbial cells struggle to dominate and/or annihilate each other and we explore the hypothesis that (like plant weeds) some microbes are genetically hard-wired to behave in a vigorous and ecologically aggressive manner. These ‘microbial weeds’ are able to dominate the communities that develop in fertile but uncolonized – or at least partially vacant – habitats via traits enabling them to out-grow competitors; robust tolerances to habitat-relevant stress parameters and highly efficient energy-generation systems; avoidance of or resistance to viral infection, predation and grazers; potent antimicrobial systems; and exceptional abilities to sequester and store resources. In addition, those associated with nutritionally complex habitats are extraordinarily versatile in their utilization of diverse substrates. Weed species typically deploy multiple types of antimicrobial including toxins; volatile organic compounds that act as either hydrophobic or highly chaotropic stressors; biosurfactants; organic acids; and moderately chaotropic solutes that are produced in bulk quantities (e.g. acetone, ethanol). Whereas ability to dominate communities is habitat-specific we suggest that some microbial species are archetypal weeds including generalists such as: Pichia anomala, Acinetobacter spp. and Pseudomonas putida; specialists such as Dunaliella salina, Saccharomyces cerevisiae, Lactobacillus spp. and other lactic acid bacteria; freshwater autotrophs Gonyostomum semen and Microcystis aeruginosa; obligate anaerobes such as Clostridium acetobutylicum; facultative pathogens such as Rhdotorula mucilaginosa, Pantoaea ananatis and Pseudomonas aeruginosa; and other extremotolerant and extremophilic microbes such as Aspergillus spp., Salinibacter ruber and Haloquadratum walsbyi. Some microbes, such as Escherichia coli, Mycobacterium smegmatis and Pseudoxylaria spp., exhibit characteristics of both weed and non-weed species. We propose that the concept of nonweeds represents a ‘dustbin’ group that includes species such as Synodraopsis spp., Polypaecilum piscis, Metschnikowia orientalis, Salmonella spp., and Caulobacter crescentus. We show that microbial weeds are conceptually distinct from plant weeds, microbial copiotrophs, r-strategists, and other ecophysiological groups of microorganism. Microbial weed species are unlikely to emerge from stationary-phase or other types of closed communities; it is open habitats that select for weed phenotypes. Specific characteristics that are common to diverse types of open habitat are identified, and implications of weed biology and open-habitat ecology are discussed in the context of further studies needed in the fields of environmental and applied microbiology.

Introduction

As the collective metabolism and ecological activities of microorganisms determine the health and sustainability of life on Earth it is essential to understand the function of...
both individual microbes and in their communities. The cellular systems of an insignificant portion of microbial species have been intensively characterized at the levels of biochemistry, cell biology, genomics and systems biology; and a substantial body of (top-down) studies has been published in the field of microbial ecology in relation to species interactions, community succession and environmental metagenomics. There are, however, some fundamental questions that remain unanswered in relation to the behaviour of microbial species within communities: what type of biology, for instance, enables microbes to dominate entire communities and their habitats and thereby determine levels of biodiversity in specific environments?

Plant species that primarily inhabit freshly disturbed habitats – known as weeds – are characterized by vigorous growth; tolerance to multiple stresses; exceptional reproductive, dispersal and survival mechanisms; a lack of specific environmental requirements; production of phytotoxic chemicals; and/or other competitive strategies (Table 1). The biology of plant weeds, including their phenotypic and genetic traits, has been the subject of extensive study over the past 100 years and is now relatively well characterized (Table 1; Long, 1932; Anderson, 1952; Salisbury, 1961; Hill, 1977; Mack et al., 2000; Schnitzler and Bailey, 2008; Bakker et al., 2009; Chou, 2010; Wu et al., 2011; Liberman et al., 2012). We hypothesize that weed biology is also pertinent to microbial species, but that the defining characteristic of microbial weeds is their ability to dominate their respective communities. In the field of plant ecology it is open habitats (such as freshly exposed fertile soil) that facilitate the emergence of weed species both within specific ecosystems and across evolutionary timescales. We propose that weed behaviour is equally prevalent in some microbial habitats, that open microbial habitats promote the emergence of microbial weed species, and that microbial weed biology represents a potent ecological and evolutionary mechanism of change for some microbial species and their communities.

This article focuses on the following questions: (i) what types of substrate or environment facilitate the emergence of dominant microbial species, (ii) are there archetypal weed species that can consistently dominate microbial communities, (iii) what are the stress biology, nutritional strategies, energy-generating capabilities, antimicrobial activities and other competitive strategies of microbial weeds, (iv) which components and characteristics of microbial cells form the mechanics of weed biology, (v) what are the properties of open (microbial) habitats that promote the emergence of weed species, and (vi) how are microbial weeds conceptually distinct from plant weeds, and other ecophysiological groups of microorganism?

**Dominance within microbial communities**

The microbial diversity of specific habitats, as well as that of Earth’s entire biosphere, is an area of intensive scientific interest that has implications within the fundamental sciences and for drug discovery, biotechnology and environmental sustainability. Biodiversity provides the material basis for competition between microbial taxa, species succession in communities, and other aspects of microbial ecology that collectively impact the evolution of microbial species (Cordero et al., 2012). We believe that certain microbes are genetically, and thus, metabolically hard-wired to dominate communities (monopolizing available resources and space) and thereby reduce biodiversity within the habitat. Microbial communities can reach a stationary-phase or climax condition with a relatively balanced species composition (McArthur, 2006). However, whether tens or thousands of microbial species are initially present (Newton et al., 2006) the community can become dominated by a single – or two to three – species (Fig. 1; Table S1; Randazzo et al., 2010; Cabrol et al., 2012). For example the indigenous microflora of olives is comprised of significant quantities of phylogenetically diverse species such as *Aureobasidium pullulans, Candida spp., Debaryomyces hansenii, Metschnikowia pulcherrima, Pichia guilliermondii, Pichia manshurica, Rhodotorula mucilaginosa* and *Zygosaccaromyces* (Fig. 1Bi; Nisiotou et al., 2010). Nisiotou and colleagues (2010) found that species succession and the stationary-phase community during olive fermentations are determined by one dominating genus, *Pichia*, three species of which account for almost 100% of the community by 35 days (see Fig. 1Bii). The most prevalent of these, *Pichia anomala*, is a microbial generalist that is not only able to inhabit ecologically and nutritionally distinct environments (e.g. palm sugar, cereals, silage, oil-contaminated soils, insects, skin, various marine habitats; see Walker, 2011) but can also dominate the communities that develop in diverse habitat types (Table S1; Tamang, 2010; Walker, 2011). Studies of Pecorino Crotonese cheese fermentations reveal that, even though the curd contains > 300 bacterial strains, the community is eventually dominated by *Lactobacillus rhamnosus* (Randazzo et al., 2010).

Species that are able to dominate communities that develop in open habitats are not restricted to copiotrophs, generalists or any established ecophysiological grouping of microorganism (Table S1). Furthermore dominating species span the Kingdoms of life and, collectively, can be observed in most segments of the biosphere; from bioaerosols to solar salters, oceans and freshwater to sediments and soils, the phyllosphere and rhizosphere, to those with molar concentrations of salts or sugars (see Table S1). The phenomenon can also be observed during food-production, food-spoilage and waste-decomposition.
<table>
<thead>
<tr>
<th>Definition</th>
<th>Plant weeds</th>
<th>Microbial weeds</th>
<th>References (plants/microbes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ability to rapidly occupy available space</td>
<td>Important trait</td>
<td>Essential trait</td>
<td>Hill, 1977/current article</td>
</tr>
<tr>
<td>Overall vigour and competitive ability</td>
<td>Typically vigorous; competitive ability usually superior to that of crop plants</td>
<td>Exceptional vigour and competitive ability</td>
<td>Hill, 1977/Figs 1 and 2; Tables S1 and 2–5</td>
</tr>
<tr>
<td>Primary competitors</td>
<td>Intra- and inter-specific competition are likely to be stronger if dominance results from efficient resource acquisition or antimicrobial substances respectively</td>
<td>Intra- and inter-specific competition</td>
<td>Hill, 1977/current article</td>
</tr>
<tr>
<td>Resource acquisition and storage</td>
<td>Acquisition and/or storage of water, light, nutrients etc. typically excellent</td>
<td>Acquisition and/or storage of nutrients, water and/or light typically excellent</td>
<td>Hill, 1977; Oatham, 1997; Sadeghi et al., 2007/Table 5</td>
</tr>
<tr>
<td>Reproduction and dispersal</td>
<td>Exceptional</td>
<td>May not differ from non-weed species</td>
<td>Hill, 1977/current article</td>
</tr>
<tr>
<td>Obligatory associations or interactions with other organisms</td>
<td>Uncommon/atypical</td>
<td>Uncommon/typical</td>
<td>Hill, 1977/current article</td>
</tr>
<tr>
<td>Ubiquity</td>
<td>Many species are environmentally ubiquitous</td>
<td>Not necessarily widespread outside the habitat which they dominate</td>
<td>Hill, 1977/current article</td>
</tr>
<tr>
<td>Germination</td>
<td>No special requirements; high tolerance to diverse stresses (see below)</td>
<td>No special requirements; high tolerance to diverse stresses (see below)</td>
<td>Hill, 1977/current article</td>
</tr>
<tr>
<td>Longevity of dormant propagules</td>
<td>Exceptional</td>
<td>May not differ from non-weeds</td>
<td>Hill, 1977/current article</td>
</tr>
<tr>
<td>Able to regenerate from fragments</td>
<td>Yes, especially root fragments</td>
<td>Not relevant</td>
<td>Hill, 1977/current article</td>
</tr>
<tr>
<td>Maintenance of growth rate during nutrient limitation</td>
<td>Some species, e.g. black-grass (Alopecurus myurosides) on low-potassium soils</td>
<td>Many (photosynthetic as well as some heterotrophic) species</td>
<td>Hill, 1977/Table 5; Benning, 1998</td>
</tr>
<tr>
<td>Capable of creating a zone-of-inhibition</td>
<td>Not known</td>
<td>Yes, in some instances</td>
<td>[Not known]/Fleming, 1929</td>
</tr>
<tr>
<td>Secretion of toxic substances</td>
<td>Some species produce phytoxic inhibitors (exuded from leaves, roots, and/or necromass)</td>
<td>All species produce antimicrobial toxins (secreted into the extracellular environment)</td>
<td>Chou, 2010/Table 6</td>
</tr>
<tr>
<td>Production of volatile organic compounds (VOCs) that act as stressors</td>
<td>Some hydrophobic and highly chaotropic compounds act as phytoxic stressors (e.g. β-caryophellene)</td>
<td>Many species produce a spectrum of VOCs that can inhibit metabolic activity at concentrations in the nanomolar or low millimolar range</td>
<td>Wang et al., 2010; Inderjit et al., 2011; Tsubo et al., 2012/Table 6</td>
</tr>
<tr>
<td>Secretion of biosurfactants that act as stressors</td>
<td>Not known</td>
<td>Some species produce biosurfactants; substances that can solubilize hydrophobic substrates and act as cellular stressors</td>
<td>[Not known]/Tables 3 and 5; Nitschke et al., 2011</td>
</tr>
<tr>
<td>Bulk production, and secretion, of moderately chaotropic substances</td>
<td>Not known</td>
<td>Some species produce ethanol, butanol, acetone etc. that are potent cellular stressors at millimolar and molar concentrations</td>
<td>[Not known]/Table 6</td>
</tr>
<tr>
<td>Saprotrophic activity resulting in toxic/stressful breakdown products</td>
<td>Not known</td>
<td>Some species degrade macromolecules to release inhibitory catabolic products (e.g. chaotropic stressors such as phenol, catechol and vanillin)</td>
<td>[Not known]/Park et al., 2004; 2007; Zuroff and Curtis, 2012</td>
</tr>
<tr>
<td>Acidification of habitat</td>
<td>Not known</td>
<td>Some species, via bulk production of organic acids</td>
<td>[Not known]/Table 6</td>
</tr>
<tr>
<td>Secretion of aggressive enzymes</td>
<td>Unlikely</td>
<td>Some species</td>
<td>[Not known]/Table 6; Walker, 2011</td>
</tr>
<tr>
<td>Tolerance to environmental stresses</td>
<td>Tolerant to multiple, habitat-relevant stress parameters</td>
<td>Tolerant to multiple, habitat-relevant stress parameters</td>
<td>Hill, 1977/Fig. 2; Tables 2–4 and 6</td>
</tr>
<tr>
<td>Resistance to toxins and/or stressors of biotic origin</td>
<td>Likely</td>
<td>Yes, most species</td>
<td>Hill, 1977/Fig. 2; Tables 2–4 and 6</td>
</tr>
</tbody>
</table>

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Antimicrobial substances are used for various applications; as biofuels, biocides, pharmaceuticals, flavour compounds, food preservatives, etc.

Avoiding use of soaps or detergents (Capone et al. 1996; Bron et al., 2011). This has given rise to the use of probiotics (Molly et al., 1996; Bron et al., 2012) and a practice of preserving the skin microflora in babies and infants by natural ecosystems (Table S1; Senthilkumar et al., 1993; Nisiotou et al., 2007; Reynisson et al., 2009; Rajala et al., 2011). Microbial weed species are able to dominate open habitats that progress to a closed condition (e.g. fermenting fruit juice) as well as those that remain in a more or less perpetually open state (e.g. the surface-fluid film of

Table 1. cont.

<table>
<thead>
<tr>
<th>Phenotypic plasticity and intra-specific variation</th>
<th>Plant weeds</th>
<th>Microbial weeds</th>
<th>References (plants/microbes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greater than that of crop plants</td>
<td>Likely to be above average (e.g. in relation to stress biology)</td>
<td>Hill, 1977/current article; see references in Todd et al., 2004</td>
<td></td>
</tr>
<tr>
<td>Resistance to viruses, predators, and/or grazers</td>
<td>Some species can tolerate, resist, and/or repel viruses, sucking insects and grazing animals; and may contain poisonous substances – or have morphological adaptations such as spines – that minimize losses through grazing</td>
<td>Some strains/species can tolerate, resist, repel and/or avoid viruses, grazing plankton and arthropods etc; may contain toxic metabolites or have behavioural adaptations – e.g. formation of large microcolonies – that minimize losses through grazing/predation; and may inhabit environments that are too extreme for predatory species</td>
<td>Thurston et al., 2001; Raloffs, 2002; Shukurov et al., 2012/Table 6</td>
</tr>
</tbody>
</table>

Karyotype | Commonly of hybrid origin, polypliod or aneuploid | Frequency of hybrid origin, polypliody and aneuploidy not yet tested; may contain plasmids that contribute to weedingess | Anderson, 1952/Table 3 and 5 |

Proportion of total species | Minute (several hundred species) | Not yet established (likely to be minute) | Hill, 1977/current article |

Association with human activity | Evolution and ecology have been strongly favoured by land disturbance that creates and open (plant) habitats | Evolution is likely to have occurred primarily in nature; but habitat dominance is commonplace in both natural and manmade environments | Anderson, 1952; Hill, 1977/Fig. 2; Tables S1 and 7 |

Problematic for human endeavours | Yes, due to loss of crop-plant yields; toxicity/harm to livestock; role as hosts for crop pests and diseases, etc. | Yes, due to spoilage of foods, drinks; some species are pathogens of crop plants, livestock or humans; may emerge to take over industrial fermentations and other processes | Hill, 1977/Table S1 |

Value to humankind | Disproportionately important (origin of many crop-plant species; plant weeds have food value for humans or livestock; model organisms for research) | Disproportionately important (key sources of bulk chemicals and antimicrobials; for food and drinks production, and other biotechnological applications) | Hill, 1977; Koomneef and Meinke, 2010/Table S1; see Concluding remarks |

a. Typical traits.
b. Plant weeds may not be the dominating plant species.
c. Not necessarily the primary habitat of the microbial weed species.
d. Vigour can result from a combination of traits such as fast growth, stress tolerance, and immunity to toxins and diseases (see Tables 2–6).
e. Plant weeds typically have a high output of seeds under both favourable and poor growth conditions, and can produce seed early (prior to maturity of the parent plant; Hill, 1977).
f. Each microbial weed species may secrete diverse antimicrobial substances which can be the primary factor in habitat dominance (see Table 7; Hallsworth, 1998; Walker, 2011). For examples of the concentrations at which moderately chaotropic stressors, potent chaotropes and hydrophobic stressors can inhibit cellular systems see Bhaganna and colleagues (2010). Toxins such as antibiotics, and VOCs, biosurfactants, and other stressors can have additional key roles in the cellular biology and ecology of microbes.
g. See Hallsworth and colleagues (2003a); Bhaganna and colleagues (2010).
h. Some stressors of biotic origin are structurally identical to and/or exert the same stress mechanisms as stressors of abiotic origin (e.g. see Fig. 2; Tables 2–4 and 6).
i. Agricultural practices and crop-plant life-cycle can also select for early seed production and other weed traits (Hill, 1977).
j. Crop-yield losses caused by plant weeds are equivalent to those caused by plant pests or plant diseases (Cramer, 1967).
k. Open (microbial) habitats are not only the starting point for food and drinks fermentations, but many foodstuffs themselves represent open habitats and it is frequently microbial weed species that cause spoilage and therefore determine shelf-life. Open habitats located on and within host organisms can be readily invaded by microbial weed species including those with pathogenic activities (Cerdeño-Tárraga, et al., 2005). This has given rise to the use of probiotics (Molly et al., 1996; Bron et al., 2012) and a practice of preserving the skin microflora in babies and infants by avoiding use of soaps or detergents (Capone et al., 2011).
l. Antimicrobial substances are used for various applications; as biofuels, biocides, pharmaceuticals, flavour compounds, food preservatives, etc.
sphagnum moss and rhizosphere of plants growing in moist soils); see Table S1. Weeds emerge in habitats where competing cells are in close proximity (such as biofilms; Table S1; Rao et al., 2005) as well as those where there is limited mechanical contact between cells, such as the aqueous habitats of planktonic species (Table S1; Collado-Fabbri et al., 2011; Trigal et al., 2011; Leão et al., 2012); and in both extreme and non-extreme environments (Table S1). Some microbes emerge as a/the dominating species in diverse types of habitat including specialists such as *Lactobacillus* (that do so, in part, by acidifying their environment) and generalists such as *Aspergillus*, *Pichia* and *Pseudomonas* species (Table S1; Steinkraus, 1983; Tamang, 2010). For weed species such as *Saccharomyces cerevisiae* (high-sugar environments), *Haloquadratum walsbyi* (aerobic, hypersaline brines), and *Gonyostomum semen* and *Microcystis aeruginosa* (eutrophic freshwater) ability to dominate can be restricted to a specific type of habitat. Most of these microbes do not populate their respective habitat as a one-off or chance event; they can consistently dominate their microbial communities and do so regardless of their initial cell number (Table S1; for *P. anomala* see Fig. 1; for *S. cerevisiae* see Pretorius, 2000; Renouf et al., 2005; Raspor et al., 2006; for *L. rhamnosus* see Randazzo et al., 2010). It is therefore intriguing to consider what combination(s) of traits can give rise to a weed phenotype.

**Cellular biology of microbial weeds**

*Stress tolerance and energy generation*

During habitat colonization, even in apparently stable, homogenous environments such as sugar-based
solutions, there are dynamic/drastic changes in stressor concentrations, antimicrobials and stress parameters (D’Amore et al., 1990; Hallsworth, 1998; Eshkol et al., 2009; Fialho et al., 2011; see also below). As a result of these fluctuations microbes are exposed to multiple stresses some of which present potentially lethal challenges to the cell. In soil and sediments, for example the water activity can oscillate from low to high and high to low; these substrates typically cycle between desiccation and rehydration and are subjected to changes in solar radiation, temperature, and even freezing and thawing. Soils and sediments can be physically heterogeneous such that water availability, solute concentrations and other stress parameters exhibit profound spatial fluctuations. In addition, intracellular metabolites and extracellular substances (of both biotic and abiotic origin) impose stresses due to ionic, osmotic, chaotropic, hydrophobic and other activities of solutes (see Brown, 1990; Hallsworth et al., 2005; Bhaganna et al., 2007; Lo Nostro et al., 2005; Hallsworth et al., 2010; Cray et al., 2013). On the one hand resilience to stress undoubtedly contributes to competitive ability but on the other a vigorous growth phenotype and reinforced stress biology also require extraordinary levels of energy generation. Furthermore, microbes that can achieve a greater net gain in energy than competing species will have an ecological advantage (Pfeiffer et al., 2001). Here we suggest that species able to dominate microbial communities are exceptionally well equipped to resist the effects of multiple stress parameters, and that many have high-efficiency energy-generation systems.

We tested the hypothesis that microbial weeds are exceptionally tolerant to habitat-relevant stresses for two species; the soil bacterium Pseudomonas putida (Table 2) and the specialist yeast S. cerevisiae (see below). Environments in which P. putida is a major ecological player (Table S1) typically contain an array of chemically diverse aromatics and hydrocarbons that induce chaotropicity-mediated stresses (Hallsworth et al., 2003a; Bhaganna et al., 2010; Cray et al., 2013). This bacterium is not only renowned for its metabolic versatility, but is also known for its tolerance to specific solutes and solvents (Hallsworth et al., 2003a; Domínguez-Cuevas et al., 2006; Bhaganna et al., 2010). We compared the biotic windows for growth of P. putida for eight mechanistically distinct classes of stress using 37 habitat-relevant substances (Table 2). This bacterium was able to tolerate all substances within each group of stressor; this is the first data set to demonstrate that a mesophilic bacterium can tolerate the following combination of stresses: up to 4–11 mM hexane, toluene and benzene; 8–115 mM concentrations of aromatic, chaotropic solutes; 300–825 mM chaotropic salts; 1300–2160 mM sugars or polyols; 200–750 mM kosmotropic salts; and considerable levels of matric stress (Brown, 1990) generated by kosmotropic polysaccharides (Table 2). The P. putida tolerance limits to highly chaotropic substances and hydrophobic compounds were superior to those of other microbes (including extremophiles and polyextremophiles; Table 2; Hallsworth et al., 2007; Bhaganna et al., 2010). Whereas prokaryotes are generally less tolerant to solute stress than eukaryotes (Brown, 1990), the resilience to this array of mechanistically diverse solute stresses (Table 2) has neither been equalled nor been surpassed by any microbial species to our knowledge.

Pseudomonas putida is able to avoid contact with or prevent accumulation of stressors by using solvent-efflux pumps and synthesizing extracellular polymeric substances to form a protective barrier (Table 3). This bacterium also utilizes highly efficient macromolecule-protection systems that are upregulated in response to multiple environmental stresses including between 10 and 20 protein-stabilization proteins, oxidative-stress responses, and upregulation of energy generation and overall protein synthesis (Table 3). Furthermore, P. putida can synthesize an array of compatible solutes including betaine, proline, N-acetylglutaminylglutamine amide, glyceral, mannitol and trehalose (Table 3). Individual compatible solutes are unique in their physicochemical properties, interactions with macromolecular systems and functional efficacy for specific roles within the cell (e.g. see Chirife et al., 1984; Crowe et al., 1984; Brown, 1990; Hallsworth and Magan, 1994a; 1995; Yancey, 2005; Bhaganna et al., 2010; Bell et al., 2013). Microbes that are able to selectively utilize/destroy any substance(s) from a range of diverse compatible solutes have enhanced tolerance to diverse stresses (e.g. Hallsworth and Magan, 1995; Bhaganna et al., 2010). The range of compatible solutes utilized by P. putida may be unique for a bacterium (see Brown, 1990) and can confer tolerance to diverse stresses including those imposed by chaotropes and hydrocarbons (Bhaganna et al., 2010).

The exceptionally wide growth-windows of Aspergillus species in relation to a number of stress parameters correlate with their ability to dominate communities in both extreme and non-extreme (but physicochemically dynamic) environments including soils, plant necromass, saline and very high-sugar habitats (Table S1). At least 20 species of Aspergillus and the closely related genus Eurotium are xerotolerant (Pitt, 1975; Brown, 1990). Aspergillus penicillioides is capable of hyphal growth and conidial germination down to water activities of 0.647 and 0.68 respectively (Pitt and Hocking, 1977; Williams and Hallsworth, 2009); Aspergillus echinulatus can germinate down to 0.62 (Snow, 1949). Halotolerant species such as Aspergillus wentii can grow on the highly chaotropic salt guanidine-HCl at a higher concentration than any other microbe; up to 930 mM (= 26.5 kJ g⁻¹ chaotropic activity; Hallsworth et al., 2007) and, along with Aspergillus
Table 2. Growth windows for *Pseudomonas putida* under habitat-relevant stresses.*

<table>
<thead>
<tr>
<th>Type of stressor</th>
<th>Specific examples</th>
<th>Stressor concentration (mM) causing <em>P. putida</em> growth-rate inhibition of:</th>
<th>Notes and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic compounds</td>
<td>1,2,3-Trichlorobenzene&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>γ-Hexachlorocyclohexane&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.00034</td>
<td>0.00072</td>
</tr>
<tr>
<td></td>
<td>2,5-Dichlorophenol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.70</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>n-hexane&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.45</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Toluene&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.0</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Benzene&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.4</td>
<td>11.0</td>
</tr>
<tr>
<td>Surfactants and aromatic solutes (highly chaotropic)</td>
<td>Tween® 80&lt;sup&gt;g&lt;/sup&gt;</td>
<td>108</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>Ethanol&lt;sup&gt;h&lt;/sup&gt;</td>
<td>9.0</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>o-Cresol&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.82</td>
<td>8.10</td>
</tr>
<tr>
<td></td>
<td>Benzyl alcohol&lt;sup&gt;h&lt;/sup&gt;</td>
<td>20.0</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td>Sodium benzoate&lt;sup&gt;f&lt;/sup&gt;</td>
<td>69.2</td>
<td>115</td>
</tr>
<tr>
<td>Chaotropic salts</td>
<td>CaCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>210</td>
<td>302</td>
</tr>
<tr>
<td></td>
<td>MgCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>182</td>
<td>311</td>
</tr>
<tr>
<td></td>
<td>Guanidine-HCl&lt;sup&gt;i&lt;/sup&gt;</td>
<td>650</td>
<td>950</td>
</tr>
<tr>
<td></td>
<td>LiCl</td>
<td>595</td>
<td>825</td>
</tr>
<tr>
<td>Alcohols and amides</td>
<td>Butanol&lt;sup&gt;h&lt;/sup&gt;</td>
<td>158</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>Ethanol&lt;sup&gt;h&lt;/sup&gt;</td>
<td>600</td>
<td>993</td>
</tr>
<tr>
<td></td>
<td>Urea&lt;sup&gt;f&lt;/sup&gt;</td>
<td>650</td>
<td>950</td>
</tr>
<tr>
<td></td>
<td>Dimethyl sulfoxide&lt;sup&gt;i&lt;/sup&gt;</td>
<td>168</td>
<td>1080</td>
</tr>
<tr>
<td></td>
<td>Methanol&lt;sup&gt;h&lt;/sup&gt;</td>
<td>970</td>
<td>1440</td>
</tr>
<tr>
<td></td>
<td>Ethylene glycol&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1300</td>
<td>2160</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>840</td>
<td>1400</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>770</td>
<td>2120</td>
</tr>
<tr>
<td></td>
<td>Maltose&lt;sup&gt;f&lt;/sup&gt;</td>
<td>326</td>
<td>1300</td>
</tr>
<tr>
<td>Kosmotropic solutes</td>
<td>Proline&lt;sup&gt;f&lt;/sup&gt;</td>
<td>800</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>Sorbitol&lt;sup&gt;f&lt;/sup&gt;</td>
<td>503</td>
<td>1200</td>
</tr>
<tr>
<td></td>
<td>Dimethyl sulfoxide&lt;sup&gt;i&lt;/sup&gt;</td>
<td>353</td>
<td>1300</td>
</tr>
<tr>
<td></td>
<td>Trehalose&lt;sup&gt;f&lt;/sup&gt;</td>
<td>474</td>
<td>771</td>
</tr>
<tr>
<td></td>
<td>Glycine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>99</td>
<td>370</td>
</tr>
<tr>
<td></td>
<td>Betaine&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1570</td>
<td>2960</td>
</tr>
<tr>
<td>Kosmotropic salts</td>
<td>NaCl</td>
<td>650</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td>KH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>118</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Ammonium sulfate&lt;sup&gt;f&lt;/sup&gt;</td>
<td>198</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>Sodium citrate&lt;sup&gt;f&lt;/sup&gt;</td>
<td>53</td>
<td>500</td>
</tr>
<tr>
<td>Polyasacharides (kosmotropic)</td>
<td>Dextran 40000&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Polyethylene glycol 3350&lt;sup&gt;f&lt;/sup&gt;</td>
<td>43</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Polyethylene glycol 6000&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

**a.** Pertinent to the phyllosphere, rhizosphere, hydrocarbon-polluted environments, and other habitats (see Table S1), including catabolic products of hydrocarbon degradation, antimicrobial substances (see Table 6), compatible solutes, and other *P. putida* metabolites. At sufficient concentrations solutes such as glucose, maltose, proline, sorbitol, glycine, betaine, NaCl and KH<sub>2</sub>PO<sub>4</sub> induce osmotic stress; whereas dextran and high M polyethylene glycols induce matrix stress (see Brown, 1990); hydrophobic substances typically induce a chaotropically-mediated hydrocarbon-induced water stress (Bhaganna et al., 2010; Cray et al., 2013); and other aromatics, alcohols, amides and specific salts cause chaotrope-induced water stress (Hallsworth et al., 2003a; Cray et al., 2013). **b.** Relative to no-added stressor controls (see Hallsworth et al., 2003a). **c.** Log P > 1.95 (see Bhaganna et al., 2010; Cray et al., 2013). **d.** These data were taken from Bhaganna and colleagues (2010); *P. putida* KT2440 (DSMZ 6125) was grown in a minimal mineral-salt broth (with glucose and NH<sub>4</sub>Cl as the sole carbon and nitrogen substrates respectively; see Hartmans et al., 1989; Bhaganna et al., 2010) at 30°C. The media for control treatments had no stressors added. Some *P. putida* strains can tolerate benzene at up to 20 mM (Volkers et al., 2010). **e.** The pesticide γ-hexachlorocyclohexane (γ-HCH) is also known as lindane. **f.** *Pseudomonas putida* KT2440 was grown in modified Luria–Bertani broth at 30°C; stressors were incorporated into media prior to inoculation, and growth rates calculated, as described by Hallsworth and colleagues (2003a). The media for control treatments had no stressors added. **g.** Tween® 80 was used as a model substitute for biosurfactants (Cray et al., 2013). **h.** These data were taken from Hallsworth and colleagues (2003a); *P. putida* KT2440 was grown in modified Luria–Bertani broth at 30°C as described in footnote (f). **i.** Extrapolated values. **j.** Glycerol is a notable exception (Williams and Hallsworth, 2009).
Table 3. Stress tolerance and energy generation: cellular characteristics that contribute to weed-like behaviour.

<table>
<thead>
<tr>
<th>Metabolites, proteins, molecular characteristics, and other traits</th>
<th>Function(s)</th>
<th>Phenotypic traits</th>
<th>Notes and references</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tolerances to multiple environmental stresses</strong></td>
<td>Specific compatible solutes can protect against osmotic stress, temperature extremes, freezing, dehydration and rehydration, chaotropy, and/or hydrophobic stressors (Crowe et al., 1984; Brown, 1990; Hallsworth et al., 2003b; Bhaganna et al., 2010; Chin et al., 2010; Bell et al., 2013)</td>
<td>Ability to maintain functionality of macromolecular systems and/or cell turgor under diverse stresses</td>
<td>Some microbes utilize a wide array of compatible solutes depending on the type and severity of stress; e.g. <em>Pseudomonas putida</em> is exceptional among bacteria in its ability to synthesize and accumulate protectants as diverse as betaine, proline, N-acetylglutaminylglutamine amide, glycerol, mannitol and trehalose (D’Souza-Ault et al., 1993; Kets et al., 1996; Bhaganna et al., 2010); Saccharomyces cerevisiae can accumulate proline, glycerol and trehalose (see Table 6; Kaino and Takagi, 2008). Like other fungi, <em>Aspergillus</em> species utilize a range of polyols as well as trehalose (Hallsworth and Magan, 1994c; Hallsworth et al., 2003b) but their exceptional ability to generate energy under stress (see below) may aid glycerol retention and thereby extending the growth window at low water activity (Hocking, 1993; Williams and Hallsworth, 2009).</td>
</tr>
<tr>
<td>Upregulation of protein-stabilization proteins in combinations tailored to specific stressors</td>
<td>In addition to stressors (including those of biotic origin; Table 6), protein-stabilization proteins also protect against perturbations induced by stresses such as temperature extremes and desiccation (Arsène et al., 2000; Ferrer et al., 2003)</td>
<td>Maintenance of protein structure and function during environmental challenges</td>
<td>Several studies show highly efficient responses of <em>P. putida</em> to chaotropic and hydrophobic stressors via upregulation of between 10 and 20 protein-stabilization proteins (Hallsworth et al., 2003a; Santos et al., 2004; Segura et al., 2005; Domínguez-Cuevas et al., 2006; Tsirogianni et al., 2006; Volkers et al., 2006; Ballerstedt et al., 2007). The PaP protein in <em>Aspergillus nidulans</em> induces a rapid upregulation of protein-stabilization proteins in response to extreme pH (Freitas et al., 2011); high production rates of protein-stabilization proteins enhance tolerance to chaotropic salts and alcohols in <em>S. cerevisiae</em> and <em>Lactobacillus plantarum</em>, to hydrophobic stressors in <em>S. cerevisiae</em> and <em>Escherichia coli</em> (for references, see Bhaganna et al., 2010), and low temperature, salt and other stresses in <em>Rhodobulbus mucilaginosus</em> (Lahav et al., 2004).</td>
</tr>
<tr>
<td>Intracellular accumulation of ions for osmotic adjustment; intracellular proteins structurally adapted to high ionic strength</td>
<td>The utilization of ions for osmotic adjustment avoids the energy-expensive synthesis of organic compatible solutes (Kushner, 1978; Oren, 1999)</td>
<td>Ability to maintain metabolic activity at high extra- and intracellular ionic strength and high tolerance to chaotropic and hydrophobic solvent stressors</td>
<td>Due to its exceptional solvent tolerance <em>P. putida</em> is the organism of choice for many industrial applications including biotransformation in two-phase systems and bioremediation (Timmis, 2002).</td>
</tr>
<tr>
<td>Diverse proteins and pathways conferring tolerance to solvents, chaotropic solutes and hydrophobic stressors</td>
<td>Solvent-tolerant species such as <em>P. putida</em> can function at high concentrations of chaotropic and hydrophobic stressors due to the collective activities of solvent pumps that expel solvent molecules, pathways that catabolize the solvent, membrane-stabilization proteins (see below), compatible solutes (see above), and additional chaotrope- and solvent-stress responses (Ramos et al., 2002; Timmis, 2002; Hallsworth et al., 2003a; Volkers et al., 2006; Bhaganna et al., 2010; Fillet et al., 2012)</td>
<td>Ability to maintain metabolic activity at high extra- and intracellular ionic strength and high tolerance to chaotropic and hydrophobic solvent stressors</td>
<td>Haloquadratum walsbyi, and <em>Salinibacter ruber</em> (unusually for a bacterium), utilize the so-called ‘salt-in’ strategy (Oren et al., 2002; Bardavid and Oren, 2012; Saum et al., 2012)</td>
</tr>
<tr>
<td>Membrane-stabilization proteins; e.g. cis/ trans-isomerase (CTI)</td>
<td>Membrane stabilization under stress</td>
<td>Enhanced tolerance to stresses that impact membrane structure</td>
<td>Several proteins are involved in maintaining bilayer rigidity in <em>P. putida</em> during exposure to toluene and other solvents or hydrophobic stressors (Bernali et al., 2007)</td>
</tr>
<tr>
<td>Proteins involved in the moderation of membrane fluidity</td>
<td>Maintenance of membrane fluidity and membrane-associated processes under conditions such as low temperature (Turk et al., 2011)</td>
<td>Enhanced tolerance to diverse, physicochemical stress parameters</td>
<td>For example <em>Aureobasidium pullulans</em>, <em>Cryptococcus liquescentis</em> and <em>R. mucilaginosus</em> (Turk et al., 2011)</td>
</tr>
<tr>
<td>Promoter(s) that simultaneously control multiple stress-response genes, e.g. stress-response elements (STREs)</td>
<td>Activation of multiple cellular stress-responses</td>
<td>Enhanced tolerance to multiple stress-parameters</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Proteins involved in DNA protection under stress; e.g. DNA-binding protein (Dps)</td>
<td>Formation of a protein/chromosome complex that protects DNA</td>
<td>Enhanced DNA stability and reduced risk of mutation in relation to multi-various stresses</td>
<td></td>
</tr>
<tr>
<td>Pathways for synthesis of carotenoids (e.g. β-carotene in Dunaliella salina; torularhodin in Rhodotorula spp.)</td>
<td>Carotenoids absorb light and thereby protect cellular macromolecules and organelles from exposure to ultraviolet</td>
<td>Tolerance of exposure to ultraviolet irradiation</td>
<td></td>
</tr>
<tr>
<td>Preferential utilization of a chaotropic compatible solute at high NaCl concentration and/or low temperature</td>
<td>Rigidification of macromolecular structures at high NaCl and low temperature can induce metabolic inhibition (Brown, 1990; Ferrer et al., 2003; Chin et al., 2010) that can be compensated for by glycerol and/or fructose (Brown, 1990; Chin et al., 2010)</td>
<td>Enhanced tolerance to extreme conditions that rigidify macromolecular systems</td>
<td></td>
</tr>
<tr>
<td>Efficient system to eliminate free radicals (see above)</td>
<td>Elimination of reactive oxygen species (see also Table 5)</td>
<td>Various substances and stress parameters can induce oxidative stress</td>
<td></td>
</tr>
<tr>
<td>Proteins associated with regulation of cytosolic pH; e.g. the lysine decarboxylase system (coded for by the cadA operon); the Na+/H+ antiporter</td>
<td>Conversion and export of lysine/cadaverine that results in increased pH of the cytosol in bacteria (Álvarez-Ordóñez et al., 2011); the Na+/H+ antiporter Nha1 decreases intracellular hydrogen ion concentration</td>
<td>Tolerance of low-pH environments</td>
<td></td>
</tr>
<tr>
<td>Multidrug efflux pumps (for details see Table 6)</td>
<td>Production and secretion of polymeric substances</td>
<td>Enhanced tolerance to multiple stressors and stress parameters</td>
<td></td>
</tr>
<tr>
<td>Enzymes for synthesis of extracellular polymeric substances</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uprogulation of proteins involved in protein synthesis under stress</td>
<td>To enhance or maintain the quantity, type, and functionality of cellular proteins under stress</td>
<td>Maintenance of the cellular system and its metabolic activity under stress</td>
<td></td>
</tr>
<tr>
<td>Polyplody, aneuploidy, association with plasmids, and/or hybridization that enhance(s) stress tolerance</td>
<td>Whereas hybridization, polyplody, aneuploidy and association with plasmids can be ecologically disadvantageous, such traits can enhance the vigour, stress tolerance and competitive ability of some microbes such as P. chia spp., S. cerevisiae, P. putida and S. ruber (Table 1; Rainies et al., 1999; Naumov et al., 2001; Mongodin et al., 2005; Tark et al., 2005; Dhar et al., 2011; Chen et al., 2012)</td>
<td>Enhanced stress tolerance</td>
<td></td>
</tr>
</tbody>
</table>

The transcription factors (Msn2p and Msn4p) involved in STRE-mediated stress responses in S. cerevisiae accumulate in the nucleus upon heat-, ethanol- (chaotrope), and osmotically induced stresses, and during nutrient limitation. The STRE is upstream of genes for protein-stabilization proteins (see above) metabolic enzymes, and cellular organization proteins (Gómer et al., 1998; Moskvina et al., 1998).

The role of Dps has been well-characterized in E. coli in relation to diverse environmental challenges including reactive oxygen species, iron and copper toxicity, thermal stress and extremes of pH (Nair and Finkel, 2004).

Synthesis of β-carotene is upregulated in response to light in D. salina (Chen and Jiang, 2009); carotenoids are concentrated in the plasma membrane of S. ruber (Molina et al., 2010); studies of R. mucilaginosa show that torularhodin enhances tolerance to ultraviolet B exposure (Maliné et al., 2010).

The high intracellular concentrations of glycerol in D. salina under salt stress and glycerol and/or fructose in and Aspergillus and Eurotium spp. (1998) diversely environmental challenges including reactive oxygen species, iron and copper toxicity, thermal stress and extremes of pH (Nair and Finkel, 2004). Tolerance of exposure to ultraviolet irradiation.

Enhanced DNA stability and reduced risk of mutation in relation to multi-various stresses.

Synthesis of β-carotene is upregulated in response to light in D. salina (Chen and Jiang, 2009); carotenoids are concentrated in the plasma membrane of S. ruber (Molina et al., 2010); studies of R. mucilaginosa show that torularhodin enhances tolerance to ultraviolet B exposure (Maliné et al., 2010).

Efficient system to eliminate free radicals (see above) such as hydrogen peroxide, superoxide radicals, and lipid peroxides. Elimination of reactive oxygen species (see also Table 5) by these mechanisms can induce oxidative stress.

Diverse environmental substances and parameters induce oxidative stress, either directly or indirectly (e.g. via lipid peroxidation). For example, chaotropic stressors and heat shock have been associated with the induction of oxidative stress responses in S. cerevisiae and P. putida (Costa et al., 1997; Hallsworth et al., 2003a; Hallsworth et al., 2003b).

Decarboxylase systems that increase intracellular pH also exist for other amino acids (Álvarez-Ordóñez et al., 2011); Nha1 greatly enhances viability of Saccharomyces boulardii at pH 2 (dos Santos Sant’Ana et al., 2009).

Extracellular polymeric substances have been shown to confer protection to E. coli cells exposed to high temperature, low pH, salt and oxidative stress (Chen et al., 2004), and are associated with stress protection in Clostridium spp., Pantoaea anaerolactica, P. putida, and other Pseudomonas spp. (Chang et al., 2007; Moroshoshi et al., 2011; see also Table 6).

Can be upregulated in response to stress induced by high temperature, chaotropic solutes, hydrophobic stressors and other stress parameters (e.g. P. putida; Hallsworth et al., 2003a; Volkers et al., 2008).

Aneuploid cells of S. cerevisiae have enhanced tolerance to oxidative stress and diverse inhibitors of biochemical processes (Chen et al., 2012); polyplody can enhance vigour and stress tolerance in some species (see also Tables 1 and 5); the stress tolerance of some microbial weeds is enhanced by their association with plasmids (e.g. the P. putida tol-plasmid that enhances solvent tolerance; Tark et al., 2005; Domínguez-Cuevas et al., 2006; the S. ruber plasmid contains a gene involved in ultraviolet protection; Mongodin et al., 2005).
Table 3. cont.

<table>
<thead>
<tr>
<th>Metabolites, proteins, molecular characteristics, and other traits</th>
<th>Function(s)</th>
<th>Phenotypic traits</th>
<th>Notes and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyextremophilic growth-phenotype</td>
<td>Ability to grow optimally under multiple, extreme conditions; e.g. extremely acid and alkaline (pH 2–10), high-salt (2.5 M NaCl), and low-temperature conditions (Lahav et al., 2002; Lilkind and Sampaio, 2010)</td>
<td>Polyextremophile</td>
<td>Rhodotorula mucilaginosa is capable of growth over an incredible range of physicochemical conditions (including almost the entire pH range for microbial life) and is able to colonize a correspondingly wide diversity of environments (for examples see main text)</td>
</tr>
<tr>
<td>Genes associated with salt stress</td>
<td>Sugar catabolism (e.g. RmPGM2, RmGPA2, RmAACK1, RmMCP1 and RmPET19), protein glycosylation (e.g. RmSEC53), aromatic amino acid synthesis (e.g. RmARO4), regulation of protein synthesis (e.g. RmANB1)</td>
<td>Maintenance of protein synthesis and energy generation under salt stress</td>
<td>Between 10 and 20 genes are involved in increased tolerance to NaCl and/or LiCl in R. mucilaginosa (Lahav et al., 2004; Gostincˇar et al., 2012)</td>
</tr>
<tr>
<td>Metabolites associated with resistance to antimicrobial plant metabolites and survival in phyllosphere habitats</td>
<td>Metabolites involved in quorum sensing may enhance competitive ability on the leaf surface</td>
<td>Enhanced survival and competitive ability</td>
<td>By contrast to other phyllosphere microbes, R. ananatis is resistant to the inhibitory effects of plant alkaloids and this correlates with the production of metabolites involved in quorum sensing such as N-acyl-homoserine lactone (Enya et al., 2004)</td>
</tr>
<tr>
<td>High-efficiency energy-generation systems</td>
<td>Rapid catabolism of carbon substrates leading to enhanced synthesis of ATP</td>
<td>Efficient energy-generation</td>
<td>Xanthorhodopsin, found in S. ruber, is a proton pump made up of two chromophores; a light-absorbing carotenoid and a protein (Balashov et al., 2005); a light-activated proton pump was also recently described in H. walsbyi (Lobasso et al., 2012)</td>
</tr>
<tr>
<td>Enhanced energy-efficiency at low nutrient concentrations</td>
<td>Maintenance of energy generation close to the point of system failure under hostile conditions</td>
<td>Efficient energy generation during potentially lethal challenges</td>
<td>The exceptional ability of P. putida to respond and adapt to chaotropic, solvent and hydrocarbon stressors has, in part, been attributed to considerates increases in energy- and NAD(P)H-generating systems</td>
</tr>
<tr>
<td>Upregulation of proteins involved in energy generation under chaotropic and solvent-induced (hydrocarbon) stresses</td>
<td>Enhanced primary metabolism and energy-generation systems</td>
<td>Enhanced vigour and energy production</td>
<td>Aspergillus species have numerous genome modifications, such as gene duplications for pyruvate dehydrogenase, citrate synthase, aconitase and malate dehydrogenase that enhance primary metabolism including glycolysis and the TCA cycle (Filippini et al., 2009)</td>
</tr>
<tr>
<td>Idiosyncratic genome modifications associated with reinforced energy metabolism</td>
<td>Catabolism of stored substances to boost growth, stress tolerance, production of secondary metabolites, motility and/or other key cellular functions under stress</td>
<td>Ability to maintain energy production during nutrient limitation and under hostile conditions</td>
<td>Efficient storage (see Table 5) and utilization of energy-generating substances such as tetrodotoxin, glycogen and polyhydroxylalkanoates confer a competitive advantage in S. cerevisiae and other species (Costrol et al., 1999; Kadosi et al., 2005)</td>
</tr>
<tr>
<td>Efficient utilization of intracellular reserves for energy generation</td>
<td>Concomitant energy generation and synthesis of antimicrobials with no loss of ATP-generation efficiency</td>
<td>Energy generation linked to synthesis of an antimicrobial</td>
<td>Each glucose molecule fermented by S. cerevisiae can simultaneously generate energy while synthesizing the chaotropic stressor ethanol (see Table 6)</td>
</tr>
</tbody>
</table>

a. The current table provides examples of proteins, genes or other characteristics that can be associated with and give rise to weedyenes; the categories of traits are not mutually exclusive. Individual traits may not be unique to microbial weeds; however, weeds species are likely to have a number of these types of characteristics.

b. For example chaperonins, heat-shock proteins and cold-shock proteins.

c. That code for fructose-1,6-biphosphate aldolase, pyruvate kinase and ATP synthase subunits F1 a and F1 b respectively.
oryzae, up to ~ 4 M NaCl or KCl (Hallsworth et al., 1998). *Aspergillus* (and *Eurotium*) strains are capable of reasonable growth rates close to 0°C (Chin et al., 2010), and some reports suggests that the biotic windows for some *Aspergillus* species ultimately fail at ≤−18°C (Vallentyne, 1963; Siegel and Speitel, 1976). Whereas a number of comparable fungal genera show moderate levels of stress tolerance, *Aspergillus* species generally out-perform their competitors and top numerous league tables for ascomycete stress tolerance (see Pitt, 1975; Wheeler and Hocking, 1993; Williams and Hallsworth, 2009).

Studies of seven *Aspergillus* species have revealed a number of genome modifications within this genus that reinforce both primary metabolism and energy generation (Table 3; Flipphi et al., 2009). These include gene duplications for key enzymes that control metabolic flux, such as pyruvate dehydrogenase, citrate synthase, aconitate, and malate dehydrogenase that enhance glycolysis and the TCA cycle (Flipphi et al., 2009). There is evidence that, at the low water activities (high osmotic pressures) associated with very high-salt and high-sugar habitats, the biotic window of xerophilic fungi fail due to the prohibitive energy expenditure required to prevent the high levels of intracellular glycerol from leaking out of the membrane (Hocking, 1993). The adaptability and competitive ability of *Aspergillus* species (most of which can also proliferate under non-extreme conditions) may be attributed, in part, from enhanced energy-generating capability. *Aspergillus* species (like many fungi) can utilize a range of polyols – as well as trehalose – as compatible solutes based on their individual strengths as protectants against osmotic stress, desiccation rehydration, chaotropes etc. (Crowe et al., 1984; Brown, 1990; Hallsworth et al., 2003b). *Aspergillus* and *Eurotium* strains can also accumulate chaotropic sugars from the medium, or preferentially synthesize and accumulate chaotropic glycerol, in order to enhance growth at temperatures below ~ 10°C (Chin et al., 2010; see also below).

*Salinibacter ruber* is a halophilic bacterium found in high numbers at molar salt concentrations in aerobic aquatic habitats that are otherwise more densely populated by archaeal than bacterial species. Unusually for a bacterium, *S. ruber* takes in ions to use as compatible solutes and thereby avoids synthesis of organic compatible solutes that would be energetically unfavourable in environments of > 1 M NaCl (Oren, 1999) and has evolved intracellular proteins that are both structurally stable and catalytically functional in the cytosol at high ionic strength (Table 3). *Salinibacter ruber* (as well as the alga *Dunaliella salina* and yeast *R. mucilaginosa*) accumulates carotenoids in response to light which can protect against oxidative stress that is a potential hazard in salt-  

ems exposed to high levels of ultraviolet (Table 3). *Haloquadratum walsbyi*, an Archaeae that is also a dominant player in saltern communities (Table S1), also uses the ‘salt-in’ compatible-solute strategy, and – along with *S. ruber* – has light-activated protein pumps that generate proton-motive force and thereby boost energy generation (Table 3). In addition, *H. walsbyi* is unusually tolerant to the chaotropic salt MgCl₂ that can reach high concentrations in evaporate ponds (Hallsworth et al., 2007; Bardavid et al., 2008). *Dunaliella salina* is also highly prevalent in saline habitats that (unusually for an alga) can successfully compete with the multitude of halophilic Archaea found in 5 M NaCl environments (see later; Table S1). This alga preferentially utilizes glycerol as a compatible solute, is able to accumulate glycerol to molar concentrations (6–7 M; see Bardavid et al., 2008), and has low membrane permeability to this compatible-solute thus aiding retention (Bardavid et al., 2008).

Glycerol is unique in its ability to maintain the flexibility of macromolecular structures under conditions that would otherwise cause excessive rigidification, e.g. molar concentrations of NaCl, or low temperature (Back et al., 1979; Hallsworth et al., 2007; Williams and Hallsworth, 2009; Chin et al., 2010). Species able to grow at low or subzero temperatures (e.g. *Aspergillus*, *Cryptococcus*, *Rhodotorula* species, including *R. mucilaginosa*) either primarily use glycerol as a compatible solute or preferentially accumulate this chaotrope at low temperature (Table 3; Lahav et al., 2004; Chin et al., 2010). *Rhodotorula mucilaginosa* is able to colonize – and frequently dominates – diverse substrates/environments including saline and non-saline soils, aquatic habitats (both liquid- and ice-associated), the acidic surface-film of sphagnum moss (as well as other plant tissues) and various surfaces within the human body and other animals (Table S1; Egan et al., 2002; Trindade et al., 2002; Vital et al., 2002; Lahav et al., 2002; 2004; Lisichkina et al., 2003; Buzzini et al., 2005; Perniola et al., 2006; Goyal et al., 2008; Turk et al., 2011; Kachalkin and Yurkov, 2012). This ability is coupled with an exceptional level of tolerance to multiple stresses: *R. mucilaginosa* is (highly unusual for a yeast) a polyextremophile that is psychrophilic, highly salt-tolerant, and can grow over the majority of the pH range known to support microbial life (i.e. < 2 and > 10, see Table 3; Lahav et al., 2002).

Some microbial habitats such as fruits that have been disconnected from the vascular system (and immune responses) of the parent plant have a favourable water activity and high nutrient availability and are therefore potentially habitable by a wide range of microbes (Ragaert et al., 2006). As the microbial community develops on a detached fruit (or in apple or grape musts; see Table S1) there can be changes in the composition and concentration of sugars, osmolality, water activity, ethanol concentration (and that of other antimicrobials), pH and other environmental factors that are severe enough to
inhibit or eliminate most members of the original micro-flora (see Brown, 1990; Hallsworth, 1998; de Pina and Hogg, 1999; Pretorius, 2000; Bhaganna et al., 2010). The yeast S. cerevisiae that is relatively scarce in natural habitats (and even when present forms a quantitatively insignificant component of the natural community; Renouf et al., 2005; Rasper et al., 2006) is unsurpassed in its ability to emerge to dominate high-sugar habitats at water activities above 0.9 (Table S1; Hallsworth, 1998; Pretorius, 2000). We therefore compared its tolerance towards a range of habitat-relevant stressors with those of 12 other yeasts that occur at the insect–flower interface, on plant tissues, and/or are environmentally widespread (Fig. 2; Table 4). This experiment revealed that S. cerevisiae has a more vigorous growth phenotype and more robust stress tolerance than the other species tested with the exception of two species of Pichia, another genus characterized by weed-like traits (see Fig. 2; Table 4).

Stress phenotypes of the yeasts assayed were compared on the basis of stressor type and mechanistically distinct stress-parameters in order to assess resilience to both the degree and diversity of environmental challenges (Table 4). Under the assay conditions it was the three S. cerevisiae strains, Pichia (Kodamaea) ohmeri and Pichia sydowiorum that exhibited the greatest tolerance to the widest range of solute-imposed stresses (Table 4). Other species studied were slow growers, have specific environmental requirements (e.g. the halotolerant D. hansenii and Hortaea werneckii), and/or otherwise lacked the capacity for vigorous growth on the high-sugar substrate at 30°C (see Fig. 2). Generally S. cerevisiae strains were fast-growing, xerotolerant, and highly resistant to chaotrope-induced, osmotic, matric and kosmotrope-induced stresses and are known to be acidotolerant and capable of growth over a wide temperature range, from ~0°C to 45°C (Fig. 2A and B; Table 4). Although Pichia species can produce ethanol (P. anomala can produce up to 0.36 M ethanol; Djelal et al., 2005) when growing side by side in high-sugar habitats with S. cerevisiae, Pichia species are unable to tolerate the molar ethanol concentrations that S. cerevisiae can produce (Lee et al., 2011).

Saccharomyces cerevisiae is metabolically wired to concomitantly synthesize an antimicrobial chaotrope (ethanol), accumulate a compatible solute (glycerol) that affords protection against this chaotrope (see below), and at the same time generate energy without any loss of ATP-generation efficiency (Table 3): a remarkable phenotype that is coupled with the deployment of other compatible solutes under specific conditions (trehalose and proline). Trehalose, which can reach concentrations of up to 13% dry weight in S. cerevisiae (Aranda et al., 2004), is highly effective at stabilizing macromolecular structures that are exposed to chaotropic substances (e.g. ethanol, 2-phenylethanol) and enabling cells to survive desiccation–rehydration cycles (Crowe et al., 1984; Mansure et al., 1994). The highest reported tolerance to ethanol stress was in S. cerevisiae cells capable of growth and metabolism up to 28% v/v (~20% w/v or 4.3 M; chaotropic activity ~25.3 KJ g⁻¹; Hallsworth, 1998; Hallsworth et al., 2007) when the medium contained high concentrations of a compatible solute, proline (Thomas et al., 1993); furthermore of the seven microbes reported to have the highest resistance to other chaotropic substances (including urea, phenol) the majority are weed species including A. wentii, S. cerevisiae, Escherichia coli and P. putida (Hallsworth et al., 2007).

The stress mechanisms for numerous types of cellular stress operate at the level of water:macromolecule interactions (Crowe et al., 1984; Brown, 1990; Sikkema et al., 1995; Casadei et al., 2002; Ferrer et al., 2003; Hallsworth et al., 2003a; 2007; McCamlick et al., 2009; Bhaganna et al., 2010; Chin et al., 2010). As front-line stress protectants, compatible solutes (within limits) can therefore mollify stress the inhibitory effects of an indefinite number of stress parameters (see Crowe et al., 1984; Brown, 1990; Hallsworth et al., 2003b; Bhaganna et al., 2010; Chin et al., 2010; Bell et al., 2013) but cells also upregulate other protection systems (see also above; Table 3). In S. cerevisiae, an extensive number of genes that code for
Table 4. Stress phenotypes and ecophysiological profiles of *Saccharomyces cerevisiae* relative to those of other yeast species.

<table>
<thead>
<tr>
<th>Yeast speciesa</th>
<th>Tolerance to diverse stressorsb</th>
<th>Tolerance to distinct stress mechanismsb</th>
<th>Additional notes on stress and growth phenotypesb</th>
<th>Typical habitat</th>
<th>Ecophysiological descriptionb</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CY19-4-13</td>
<td>Higher</td>
<td>Higher</td>
<td>$T_{\text{opt}} 30–35^\circ \mathrm{C}$; $T_{\text{min}} -0.5^\circ \mathrm{C}$; $T_{\text{max}} 45^\circ \mathrm{C}$; $pH_{\text{opt}} 3–7$; fast grower</td>
<td>Fruits; other filamentous tissues; nectar</td>
<td>Multiple stress tolerances including sugar and ethanol, stress phenotype of a xerotolerant, acidotolerant, vigorous weed</td>
</tr>
<tr>
<td>Alcotac 8</td>
<td>Higher</td>
<td>Higher</td>
<td>As above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBS 6412</td>
<td>Higher</td>
<td>Higher</td>
<td>As above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other species (strain)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida etchelisi (UWOPS 01–168.3)</td>
<td>Lower</td>
<td>Higher</td>
<td>Slow grower</td>
<td>Pollinating insects; flowers; fermented foods</td>
<td>Intolerant to specific stressors; slow-growing</td>
</tr>
<tr>
<td>Cryptococcus terreaus (P94)</td>
<td>Mid</td>
<td>Mid</td>
<td>$T_{\text{opt}} 10^\circ \mathrm{C}$; $T_{\text{min}} 1^\circ \mathrm{C}$; slow grower</td>
<td>Soils and sediments; subsurface environments</td>
<td>A slow-growing psychrophile</td>
</tr>
<tr>
<td>Debaryomyces Hansenii (UWOPS 05–230.3)</td>
<td>Mid</td>
<td>Mid</td>
<td>$T_{\text{opt}} 30–32^\circ \mathrm{C}$; $pH_{\text{opt}} 5.5$; fast grower</td>
<td>High-salt environments and foods</td>
<td>Halotolerant; a fast-growing high-salt specialist</td>
</tr>
<tr>
<td>Hansenula (Ogataea) polyplora (CBS 4722)</td>
<td>Mid</td>
<td>Mid</td>
<td>$T_{\text{opt}} 37–43^\circ \mathrm{C}$; $pH_{\text{opt}} 4.5–5.5$; fast grower</td>
<td>Soil, insect gut, fruit juice</td>
<td>Neither fast-growing nor highly stress tolerant; a high-temperature specialist</td>
</tr>
<tr>
<td>Hirtaei wernckii (MZK1 B736)</td>
<td>Mid</td>
<td>Mid</td>
<td>$T_{\text{opt}} 30–40^\circ \mathrm{C}$; $T_{\text{max}} 65^\circ \mathrm{C}$; pH opt 5–7, fast grower</td>
<td>Salty environments; animal and plant surfaces</td>
<td>Intolerance to matric stress; a slow-growing halophile</td>
</tr>
<tr>
<td>Kluyveromyces marxianus (CBS 712)</td>
<td>Higher</td>
<td>Lower</td>
<td>$T_{\text{opt}} 37^\circ \mathrm{C}$; $T_{\text{min}} 5^\circ \mathrm{C}$; slow grower</td>
<td>Milk; plant surfaces; natural fermentations</td>
<td>Thermotolerant, solute-intolerant and xero-intolerant</td>
</tr>
<tr>
<td>Pichia (Kodamaea) climeri (UWOPS 05–228.2)</td>
<td>Higher</td>
<td>Higher</td>
<td>$pH_{\text{opt}} 2.2$; not fast-growing</td>
<td>Beetles; fruits and other plant</td>
<td>Multiple stress tolerances including ethanol and sugar, stress phenotype of a xerotolerant, vigorous weed</td>
</tr>
<tr>
<td>Pichia (Komagatella) pastoris (CBS 704)</td>
<td>MD</td>
<td>Mid</td>
<td>$pH_{\text{opt}} 6–6.5$; fast grower</td>
<td>Polluted environments; waste water</td>
<td>Intolerant to osmotic stress, glycerol and sugar; a xero-intolerant yeast</td>
</tr>
<tr>
<td>Pichia sydowiorum (UWOPS 03–414.2)</td>
<td>MD</td>
<td>Mid</td>
<td>$T_{\text{opt}} 10^\circ \mathrm{C}$; $T_{\text{min}} -1^\circ \mathrm{C}$; slow grower</td>
<td>Subsurface; soil</td>
<td>Intolerant to acids, alcoholic; xerotolerant a psychrophile slow grower</td>
</tr>
<tr>
<td>Rhodotorula creativiva (PB7)</td>
<td>MD</td>
<td>MD</td>
<td>$pH_{\text{opt}} 2$; slow grower</td>
<td>Grapes; plant tissues and fermentations</td>
<td>Intolerant to glycerol and NaCl; tolerant to sugars; an osmophile</td>
</tr>
<tr>
<td>Saccharomyces ludwigii (UWOPS 92–218.4)</td>
<td>MD</td>
<td>MD</td>
<td>$pH_{\text{opt}} 3–7$, $pH_{\text{min}} 1.5–2$; slow grower</td>
<td>Fruits and other high-sugar habitats</td>
<td>Intolerant to NaCl; tolerant to glycerol and sugars; an osmophile</td>
</tr>
<tr>
<td>Zygopichia gymnocepa (CBS 713)</td>
<td>MD</td>
<td>Higher</td>
<td>Mixed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Sources of strains are given in Fig. 2.
b. Based on data for growth on MYPA at 30°C (see Fig. 2). Alcohols were ethanol and methanol; sugars were fructose and sucrose; salts were NaCl, MgCl₂ and ammonium sulfate, the other stressors were proline, xantham gum and PEG 8000 (Fig. 2). Chaotropic stress is induced by ethanol, fructose, glycerol and MgCl₂; osmotic stress by sucrose, NaCl and proline; matric stress by xantham gum and PEG 8000; and kosmotropic stress by ammonium sulfate (Fig. 2; Brown, 1990; Hallsworth, 1998; Hallsworth et al., 2003a; Bhaganna et al., 2010; Chin et al., 2010; Cray et al., 2013). Stress-tolerance assessments (higher, mid-range, lower) are relative to the range of those of the other species assayed, and based on values in Fig. 2.
c. Temperature optima, minima and/or maxima ($T_{\text{opt}}$, $T_{\text{min}}$, $T_{\text{max}}$) and pH optima, minima and/or maxima ($pH_{\text{opt}}$, $pH_{\text{min}}$, $pH_{\text{max}}$) for growth were obtained from cited publications. Extreme growth phenotypes (fast or slow growers) were listed according to growth rates on MYPA at 30°C (see also Fig. 2). Species designated as slow growers did not have a vigorous growth phenotype under these conditions; i.e., all strains listed are able to grow rapidly within the assay period on/at their favored media/temperature. The designation slow growing did not relate to an extended lag phase (data not shown).
d. Primarily based on data presented in the current table as well as Fig. 2 (unless otherwise stated) so is most pertinent to high-sugar habitats at ~30°C. Habitat dominance will ultimately be influenced by antimicrobial activities of species present within a community; see Table 6. Ecophysiological descriptions of weed-like species are underlined in bold.
e. Values for temperature- and pH-tolerances were taken from: (for *S. cerevisiae*) Phapailong and Fleet (1997), Yalcin and Ozbas (2008) and Salvadó and colleagues (2011); (for *C. etchelisi*) Kralish and colleagues (2006); (for P. ohmeri) Zhu and colleagues (2010); (for P. pastoris) Chinavati and colleagues (1998), (for P. sydowiorum) Kanekar and colleagues (2009); (for *R. creativiva*) Klis and colleagues (2006); (for S. ludwigii) Stratford (2006); and (for Z. rouxii) Praphailong and Fleet (1997). Tolerance windows for *Candida etchelisi* are yet to be established.
f. Under some conditions strains of *S. cerevisiae* can tolerate up to 28% (v/v) ethanol (see Hallsworth, 1998).
g. See also Kralish and colleagues (2006).
h. In high-salt habitats, *H. werneckii* can grow at molar concentrations of NaCl (see Gunde-Cimerman et al., 2000) but was less salt tolerant under the conditions of this assay.
i. This species appears to have a requirement for high water activity.

j. Habitat dominance also depend on factors such as production of antimicrobials (see also Table 6). Pichia ohmeri can dominate some habitats, Pichia anomala may be more vigorous as a weed species (see Rosa et al., 1990; Lee et al., 2011).
protein-stabilizing proteins, metabolic enzymes and cellular-organization proteins are regulated by a single promoter – the stress response element (STRE) – which is activated under cellular stress. This regulatory system enables the coordination of an array of stress responses in an energy-efficient manner (Table 3). Numerous studies show that the \textit{S. cerevisiae} responses to osmotic stress [via the high-osmolarity glycerol-response (HOG) pathway], oxidative stress, and challenges to protein and membrane stability are exceptionally efficient (Table 3; Brown, 1990; Costa \textit{et al.}, 1997; Hallsworth, 1998; Rodriguez-Peña \textit{et al.}, 2010; Szopinska and Morosomme, 2010). In addition to these stress responses, there is evidence that the dynamics of polyphosphate and glyco-gen metabolism in \textit{S. cerevisiae} are wired in such a way to boost ATP production (Castrol \textit{et al.}, 1999), and that the ploidy levels of many strains enhance tolerance towards oxidative and other stresses (Table 3). The energy-generation and energy-conservation strategies employed by \textit{S. cerevisiae} have apparent parallels in other weed species; for example, analyses of synonymous codon bias in the genomes of fast-growing bacteria including \textit{E. coli} and \textit{Bacillus subtilis} suggest that genes associated with energy generation are highly expressed (Karlin \textit{et al.}, 2001; Table 5; see also above). The enhanced energy efficiency that is apparent in some weed species not only underpins their robust stress biology, but is required to sustain a vigorous growth phenotype and/or to out-grow competitors.

\textit{Growth, nutritional versatility, and resource acquisition and storage}

Efficient cell division and an ability to out-grow competitors are implicit to the emergence of weed species. Species that grow relatively slowly or remain quiescent, and those with specific requirements for a host, a symbiotic partner, or obligate interactions with other microbial species are disadvantaged during community development in open habitats: dominance ultimately results from vigour and – in many habitats – a degree of nutritional versatility (Tables S1 and 5). Ability to out-grow competitors is equally important (possibly even more so) for weed species in nutrient-poor and physicochemically extreme habitats that can both select for slow-growing species and limit overall biomass development. Many traits that give rise to an efficient growth phenotype can be found in weed species (Table 5): multiple copies of ribosomal DNA operons that can enhance rates of protein synthesis; transport proteins to scavenge macromolecules thus negating the need for \textit{de novo} synthesis; high-efficiency DNA polymerase for rapid genome replication; efficient systems to eliminate reactive oxygen species that are a by-product of rapid growth; and polyploidy, aneuploidy and plasmids that can be associated with enhanced stress tolerance, higher growth-rates and increased overall vigour (Table 5). We believe that another key aspect of weed biology for some microbial species is their ability to sequester and store available nutrient and substrate molecules with a high efficiency as an auxiliary energy reserve (see below), to be able to supply daughter cells with nutrients, and simultaneously deprive other microbes of nutritional resources (e.g. Duiff \textit{et al.}, 1993; Castrol \textit{et al.}, 1999; Loaces \textit{et al.}, 2011; Renshaw \textit{et al.}, 2002; Jahid \textit{et al.}, 2006). Microbial weeds are highly efficient at nutrient-sequestration and substrate utilization, as well as intracellular storage of these substances (Table 5). For example, fungal siderophores that remove iron from the habitat have been shown to suppress growth of other microorganisms due to iron limitation (Renshaw \textit{et al.}, 2002).

Intermittent nutrient availability and low nutrient concentrations are characteristic of many aquatic environments so the ability to scavenge and store, circumvent the requirement for, or strategically utilize scarce and potentially limiting nutrients can greatly enhance the competitive ability of planktonic species. Under phosphate-limiting conditions some aquatic species, including \textit{S. ruber}, are able to use sulfur-containing glycolipids in place of phospholipids during low phosphate availability (Table 5). The cyanobacterium \textit{M. aeruginosa} has high-efficiency phosphate-transport systems and accumulates intracellular polyphosphate which is used to maintain activity once extracellular phosphate is depleted (Table 5; Shi \textit{et al.}, 2003). It is likely that this trait contributes to its weed-like phenotype in Lake Taihu (China) that is a well-studied, phosphate-limited habitat frequently dominated by \textit{M. aeruginosa} (Shi \textit{et al.}, 2003). Iron is another potentially limiting nutrient in aquatic habitats. \textit{Dunaliella salina} utilizes a highly efficient iron-binding protein to take up iron which is then stored in vacuoles (Paz \textit{et al.}, 2007). Species such as \textit{D. salina} and \textit{H. walsbyi} cope with oligotrophic conditions by hyperaccumulating starch and/or lipids such as polyhydroxybutyrate especially when nutrients other than carbon are limiting (Table 5; Bardavid \textit{et al.}, 2008). One weed species with adaptations to endure low-nitrogen conditions is \textit{Mycobacterium smegmatis}. This species (as well as other mycobacteria) which can be prevalent in relatively low-nutrient aquatic and non-aquatic habitats (Koch, 2001; Kazda and Falkingham III, 2009) have biochemical adaptations to enable growth and metabolic activity even when nitrogen concentrations are an order of magnitude lower than those usually required for structural growth (i.e. a C : N ratio of 8:1; Roels, 1983; Behrends \textit{et al.}, 2012). In addition to nitrogen oligotrophy, \textit{M. smegmatis} has a DNA polymerase capable of nucleotide processing rates and generation times that
<table>
<thead>
<tr>
<th>Ability to out-grow competitors</th>
<th>Function(s)</th>
<th>Phenotypic traits</th>
<th>Notes and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Key roles of di-oligopeptide transport protein (dtp) and oligopeptide transport ATP-binding protein (Opp)</td>
<td>Enhanced production of ribosomes</td>
<td>Rapid protein synthesis</td>
<td>Soil bacteria able to grow rapidly on high-nutrient media have an average of ~ 5.5 copies of ribosomal DNA operons (compared with ~ 1.4 copies for slow-growing species; Klappenbach et al., 2000)</td>
</tr>
<tr>
<td>High-efficiency DNA polymerase</td>
<td>Facilitate the synthesis of proteins that enable the utilization of hydrocarbons (including polyol aromatic hydrocarbons)</td>
<td>Ability to utilize diverse hydrocarbons as a carbon and/or energy source</td>
<td>Such compounds can be utilized as a sole carbon source by a small number of metabolically versatile microbes such as Pantoea spp., Pseudomonas putida and Pichia anomala (Timmis, 2002; Pan et al., 2004; Vaislav-kova and Gaskenha, 2007) but are also known to exert cellular stress even at low concentrations (Bhaganna et al., 2010). Catabolism of hydrocarbons will therefore alleviate stress at the same time as providing for energy generation and growth (Lim et al., 2002; Dominguez-Echevaria et al., 2006)</td>
</tr>
<tr>
<td>Overlapping replication cycles</td>
<td>Advanced system to regulate utilization of diverse substrates</td>
<td>Energy-efficient growth in nutritionally complex environments</td>
<td>A cAMP-independent catabolite-repression system has been identified in P. putida that enables the ordered assimilation of carbon, nitrogen and sulfur substrates (most energy-favourable first; Daniels et al., 2010)in vitro studies of Pantoea and P. aeruginosa demonstrate that biosurfactants enhance the uptake of hydrophobic substrates (Al-Tahhan et al., 2000; Vaislav-kova and Gaskenha, 2007; Reis et al., 2011) and thereby facilitate colonization of hydrocarbon-containing habitats (see Table S1). Aspergillus species can produce copious amounts of biosurfactant (Desai and Banat, 1997; Kiran et al., 2003)</td>
</tr>
<tr>
<td>Traits for high-efficiency energy generation (see above)</td>
<td>Solubilization of hydrophobic molecules (and antimicrobial activity; see Table 6)</td>
<td>Solubilization of hydrophobic substrates</td>
<td>Saprotrophic enzymes from Aspergillus species can function over a wide range of environmental stresses (Hong et al., 2001)</td>
</tr>
<tr>
<td>Nutritional and metabolic versatility[d]</td>
<td>Extracellular degradation of polymeric organic molecules for use as a carbon and energy source</td>
<td>Ability to grow saprophytically</td>
<td>Saprotrophic enzymes from Aspergillus species can function over a wide range of nutritional substrates (Oum et al., 2007)</td>
</tr>
<tr>
<td>Ability to adapt metabolism for growth at high- or low-nutrient concentrations</td>
<td>Glutamate dehydrogenase plays key roles in nitrogen assimilation and metabolism</td>
<td>Ability to adapt metabolism for growth at high- or low-nutrient concentrations</td>
<td>Versatility in the utilization of nitrogen substrates can enable proliferation in open habitats by, and opportunistic pathogenicity for, Aspergillus, Pseudomonas putida and other weed species (Kaspman and Braus, 2005; Daniels et al., 2010; see also Table S1). Rhodotorula spp., including metabolically versatile weed species, can grow on D-amino acids as a sole carbon and nitrogen source (Simonetta et al., 1989; Pollegioni et al., 2007)</td>
</tr>
</tbody>
</table>

Note: See Table S1 for additional information.
<table>
<thead>
<tr>
<th>Enzyme systems that enable metabolism under nutrient-limiting conditions by avoiding dependence on specific cofactors</th>
<th>Utilization of substitute cofactor, or replacement of enzyme system(s) that would require a cofactor that is environmentally scarce</th>
<th>Maintenance of essential enzyme functions at low nutrient concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathways to synthesize phosphate-free membrane lipids</td>
<td>Maintenance of membrane functions under phosphate-limited conditions</td>
<td>Ability to remain active, and grow, in phosphate-depleted environments</td>
</tr>
<tr>
<td>High glucosylglycerate under low-nitrogen conditions</td>
<td>Control of nitrogen homeostasis to enable growth under nitrogen-limiting conditions</td>
<td>Nitrogen oligotrophy</td>
</tr>
</tbody>
</table>

### Efficient acquisition and storage of resources

**Transport proteins to scavenge peptides and/or other macromolecules**

Efficient scavenging of biomacromolecules avoiding the need for de novo synthesis

**Biosurfactants to access hydrophilic substrates** (see above)

Efficient degradation of complex extracellular substances

**High-affinity hexose-transport proteins, e.g., Hxt2p (HXT gene family)**

Highly efficient uptake of diverse carbon substrates

**Phytases, inorganic phosphate transport proteins and polyphosphate kinase**

Extracellular degradation of organic phosphate and uptake and intracellular polymerization of inorganic phosphate

**Production and secretion of siderophores**

To scavenge iron from the extracellular milieu which is then removed once the siderophores have been transported back into the cell

**Efficient scavenging of iron**

To scavenge iron from the extracellular milieu which is then removed once the siderophores have been transported back into the cell

**High-affinity hexose-transport proteins**

Ability to accumulate iron under iron-limiting conditions

**Enhanced efficiency of iron-binding proteins (e.g., transferrin-like protein, Tff1, in *D. salina*)**

Binding and uptake of extracellular iron

**Arginine biosynthesis proteins**

Bulk synthesis of arginine as a nitrogen substrate store

**Trehalose-synthesis proteins (e.g., TPS, TDP and glycogen synthase (e.g., GSY1 and GSY2))**

Synthesis of disaccharide- and polysaccharide-storage compounds (e.g., trehalose and glycogen respectively)

**Polyhydroxybutyrate-synthesis proteins (and those producing other storage lipids)**

Synthesis of lipid stores (see also Table 3)

**Optimized light capture, and high oxygen concentrations**

Light cell shape gives a high surface-to-volume ratio; gas vesicles help to maintain the horizontal orientation of planktonic cells

**Efficient chlorophyll a and chloroplast arrangement**

Optimization of light harvesting and high photosynthetic rate

**Genes related to gas synthesis and gas vesicle formation (e.g., gvp family in *M. aeruginosa*)**

Gas vesicles provide buoyancy to optimize access to light and high oxygen concentrations (close to the water/air interface of aquatic habitats)

### The Remarkable Morphology of H. Walsby Cells

The remarkable morphology of *H. Walsby* cells optimizes acquisition of resources in highly competitive high-salt habitats (Bothnus et al., 2006; Bardavid et al., 2008). Habitat dominance by *Gonyostomum semen* can be attributed, in part, to having a higher photosynthetic rate than other autotrophic species (Coleman and Heywood, 1981; Pettomaa and Oja, 2010). Twelve genes involved in gas synthesis and storage have been identified in *M. aeruginosa* (Molukw et al., 2004); models suggest regulation of buoyancy in *M. aeruginosa* has a key role in habitat dominance (Bonnet and Poulin, 2002).

**For example arginase in *S. cerevisiae* (Middelhoven et al., 1969) and phospholipase C in *P. aeruginosa* (Stinson and Hayden, 1979)**

**Pseudomonas spp. can synthesize membrane glycolipids as an alternative to phospholipids under low-phosphate conditions (Minnikin et al., 1974).**

**S. cerevisiae**

**Salinobacter ruber**

**High glucosylglycerate under low-nitrogen conditions**

**Efficient scavenging of iron**

**High-affinity hexose-transport proteins**

**Enhanced efficiency of iron-binding proteins (e.g., transferrin-like protein, Tff1, in *D. salina*)**

**Arginine biosynthesis proteins**

**Trehalose-synthesis proteins (e.g., TPS, TDP and glycogen synthase (e.g., GSY1 and GSY2))**

**Polyhydroxybutyrate-synthesis proteins (and those producing other storage lipids)**

**Optimized light capture, and high oxygen concentrations**

**Efficient chlorophyll a and chloroplast arrangement**

**Genes related to gas synthesis and gas vesicle formation (e.g., gvp family in *M. aeruginosa*)**

**For example arginase in *S. cerevisiae* (Middelhoven et al., 1969) and phospholipase C in *P. aeruginosa* (Stinson and Hayden, 1979)**

**Pseudomonas spp. can synthesize membrane glycolipids as an alternative to phospholipids under low-phosphate conditions (Minnikin et al., 1974).**

**S. cerevisiae**

**Salinobacter ruber**
are an order of magnitude faster than those of comparable Mycobacterium species (Table 5). Light and/or oxygen are key resources for many planktonic species; 12 genes have been identified that are involved in gas synthesis and storage in species such as M. aeruginosa and H. walsbyi (Table 5). Modelling approaches indicate that buoyancy, which enables the cell to access the upper (well-illuminated, well-oxygenated) section of the water column, plays a key role in habitat dominance by M. aeruginosa (Bonnet and Poulin, 2002). Studies of H. walsbyi suggest that its peculiar, flat cell-shape optimizes the uptake of nutrients and oxygen and its intracellular gas vesicles help to maintain a horizontal orientation to capture light (Table 5). In freshwater habitats dominance of algal blooms by G. semen has been attributed in part to a high photosynthetic rate that results from a combination of its high-efficiency chlorophyll a and a highly efficient chloroplast arrangement (Table 5).

In open habitats that are nutritionally dynamic and/or complex (Table S1) metabolic versatility in nutrient utilization can enhance the competitive ability of microbes (for examples see Table 5). Pseudomonas putida and related species are remarkable in their range of nutritional strategies (Table 5; Timmis, 2002; 2009): they are able to catabolize an indeterminate number of hydrocarbons (e.g. decanoate, toluene) and other organic molecules (e.g. amino acids, and organic acids such as acetic, citric and lactic acid) for use as carbon and energy sources; amino acids, dipeptides, ammonium chloride, urea, ammonium nitrate, and other substances as nitrogen sources; amino acids, organic acids, sodium sulfite, thiouracil, agmatine sulfate, etc. as sulfur sources; as well as accumulating diverse substances as intracellular reserves (Chakrabarty and Roy, 1964; Daniels et al., 2010; Table 5) enabling a switch to oligotrophic growth at low nutrient concentration (Table 5; Timmis, 2002). In addition, P. putida and closely related species have evolved a complex, cAMP-independent catabolite repression system which incorporates five different signals and enables an ordered assimilation of carbon, nitrogen and sulfur substrates from the most to least energetically favourable (Daniels et al., 2010). This may contribute to the ability of many pseudomonads to sustain vigorous growth in nutritionally complex habitats. Pseudomonads, and closely related bacteria such as Acinetobacter, are not only environmentally ubiquitous but can dominate microbial communities in numerous types of open habitat including the plant phyllosphere and rhizosphere, bioaerosols, oil sands, and other hydrocarbon-containing environments (see Table S1; Ogino et al., 2001; Röling et al., 2004; Louvel et al., 2011). Pseudomonas species are able to enhance the availability of hydrophobic substrates by secreting biosurfactants and, at the same time, tolerate the stress induced by the latter (see below). Despite their copiotrophic phenotype, pseudomonads utilize a number of strategies to maintain growth and metabolism under phosphate-, iron- or nitrogen-depleted conditions (Table 5), can synthesize glutamate via glutamate dehydrogenase rather than glutamate synthase (thereby consuming 20% less ATP; Syn et al., 2004), and have even been observed growing in distilled water (Favero et al., 1971; Hirsch, 1986). Specific enzymes of Pseudomonas aeruginosa are catalytically active even when cofactor nutrients are scarce, and some strains have a manganese-dependent superoxide dismutase which does not require iron and is upregulated at low iron concentrations (Table 5).

Weeds such as lactic acid bacteria and S. cerevisiae that lack saprotrophic capabilities can dominate communities in habitats with high nutrient concentrations (Tables S1 and 5); they possess high-affinity transport proteins that enable rapid and efficient uptake of substrate molecules to support their vigorous growth phenotypes (see Table 5). Cells of S. cerevisiae are not only highly efficient at storing carbohydrates (combined trehalose and glycogen may reach 25% dry weight; Aranda et al., 2004; Guillou et al., 2004), but they can also accumulate high concentrations of arginine and polyphosphate and have adaptations to retain activity under low-nitrogen conditions (Table 5). Plant weeds that excel in open habitats have distinct genetic properties; they are often of hybrid origin and/or polyploid, traits that give rise to increased growth rates and stress tolerance, and have specific genes that confer their vigorous phenotype (Anderson, 1952; Hill, 1977; Schnitzler and Bailey, 2008; Bakker et al., 2009). There is also evidence that the multiple copies of genomic material found in vigorous microbes that enhanced vigour have arisen via hybridization and/or increased ploidy levels in bacterial weed species, as well as Pichia and Saccharomyces (Table 5; Cox, 2004). A recent study by Timberlake and colleagues (2011) reported vigorous growth for the majority of F1 hybrids produced by crossing 16 wild-type S. cerevisiae strains (although hybridization and/or polyploidy may not always be advantageous; see Table 5). Many microbial weed species are phenotypically variable (like their plant counterparts; Table 1; Hill, 1977) and may form part of a species complex due to polyploidization and hybridization (e.g. Acinetobacter, Pseudomonas, Mycobacterium, Saccharomyces sensu stricto; Table S1; Rainieri et al., 1999; Todd et al., 2004; Ballerstedt et al., 2007; Parkinson et al., 2011; Sicard and Legras, 2011); such factors may contribute to the vigour and weediness of such species.

High-M, macromolecules are the primary microbial substrates available in many habitats: e.g. hydrocarbons (in bioaerosols, oil-containing environments, and the rhizosphere), and polysaccharides (within or derived from plant necromass such as cellulose, lignin and humic...
substances; see Table S1). In order to out-compete other species in such habitats, microbes must utilize these macromolecular substrates that typically have a low bioavailability, require specialist enzymes to degrade, can be toxic, and/or are energy-expensive to catabolize. Facultatively saprotrophic microbes such as *Aspergillus*, *Pichia*, *Rhodotorula* and *Acinetobacter* species are able to access and catabolize recalcitrant polysaccharides; some weed species can produce exceptional amounts, or high-affinity versions, of inducible saprotrophic enzymes, such as xylanases (Table 5). Several saprotrophic enzymes produced by *Aspergillus* species (that can dominate physicochemically diverse habitats; Table S1) can retain activity over a wide range of environmental stresses (Hong *et al.*, 2001); and *Aspergillus* and *Rhodotorula* spp. are able to utilize a broad range of nitrogen substrates thereby avoiding the need for *de novo* synthesis (Table 5). Whereas nutritionally complex habitats can select for metabolic versatility (see below) and thereby favour the growth of a great number of generalist species, only a minute fraction of such microbes are able to dominate what were (initially) phylogenetically diverse microbial communities (Table S1).

**Antimicrobial strategies and trophic interactions**

Grazing, predation and viral infections adversely impact the growth dynamics of and/or incur massive losses on microbial populations and so exert a powerful selective pressure, and this can be well illustrated by the ecologies of many planktonic species in aquatic habitats (Matz *et al.*, 2004; Jütter *et al.*, 2010; Berdjeb *et al.*, 2011; Thomas *et al.*, 2011; Yang and Kong, 2012). The competitive ability of some aquatic bacteria and eukaryotes (both marine and freshwater species) is associated with their ability to resist or tolerate virus infection (see Table 6). Species with a halophilic growth phenotype, especially those which have high growth rates in salt-saturated environments such as *S. ruber* and *H. walsbyi* minimize losses to predators because these salt concentrations are hostile to protozoa due to their low water activity, high ionic strength, and/or (for high-MgCl₂ and high-CaCl₂ brines) high chaotropicity (Table 6; Hallsworth *et al.*, 2007). *Gonyostomum semen* has evolved vertical migration patterns through the water column that are out of phase with those of grazing species and thereby minimizes losses (Table 6). Other weeds, including *P. aeruginosa* and *M. aeruginosa*, are capable of forming multicellular structures – microcolonies – that are sufficiently large to reduce losses by many species of grazers and predators (Table 6). Species such as *M. aeruginosa* accumulate toxic metabolites, and *M. aeruginosa* and *Aspergillus* spp. are known to synthesize secondary metabolites that repel to grazing plankton and arthropods respectively (Table 6).

A number of agricultural (plant) weeds are able to dominate their habitats due largely to the production of phytoxic inhibitors that can be exuded from leaves, roots and plant necromass and which inhibit the germination and/or growth of other plant species (Hill, 1977; Xuan *et al.*, 2006; Alsaadawi and Salih, 2009; Chou, 2010; Mekawat and Pornprom, 2010; Inderjit *et al.*, 2011). It is likely that most microbial weed species also secure their competitive advantage – in part at least – via the deployment of antimicrobial substances. Assuming that the examples listed in Table S1 are representative of the microbial biosphere then the proportion of habitats dominated by eukaryotic species is approximately 40%. If a short generation time was sufficient to facilitate habitat dominance then prokaryotic species would populate almost all these habitats. The fact that species of algae, fungi and yeast are also able to do so underscores the importance of factors other than generation time in the biology of habitat dominance. *Saccharomyces cerevisiae* provides an illustration of how one weed species combines the synthesis of a potent antimicrobial, energy generation, and coordinated synthesis of stress protectants in a single process, i.e. aerobic production of the chaotropic alcohol, ethanol (see above; Table 6). Ethanol can be produced and secreted at concentrations > 4 M that cause a reduction of water activity (to 0.896) and exert an exceptionally high chaotropic activity (see above) sufficient to prevent the growth or metabolism of any living system (Hallsworth, 1998; Hallsworth and Nomura, 1999; Hallsworth *et al.*, 2007); *S. cerevisiae* simultaneously produces glycerol (which can reduce ethanol stress in yeast and fungi; Hallsworth, 1998; Hallsworth *et al.*, 2003b) during ethanol fermentation and is metabolically wired to synthesize high concentrations of the protectant trehalose at the end of its growth cycle when ethanol concentration is highest (see Table 6).

We believe that the bulk production and secretion of chaotropic solutes and organic acids (that can reach molar concentrations) is unique to microbial weed species (Tables 1 and 6). Whereas chaotropic substances can promote growth and expand the biotic windows of microbes under specific conditions (Hallsworth and Magan, 1994b; 1995; Williams and Hallsworth, 2009; Bhaganna *et al.*, 2010; Chin *et al.*, 2010), in other circumstances chaotropicity can be highly inhibitory or even lethal (Hallsworth, 1998; Hallsworth *et al.*, 2003a; 2007; Duda *et al.*, 2004; Bhaganna *et al.*, 2010). For plant weeds it is the early stages of habitat colonization that are key to their success (Hill, 1977), and is may be that the efficient synthesis of antimicrobials is frequently as important as much as the type of substance produced (Hallsworth, 1998; Pretorius, 2000; Eshkol *et al.*, 2009; Fialho *et al.*, 2011). Unlike plant weeds, where phytoxic inhibitors tend to have the greatest impact on neighbouring plants, microbes can also produce volatile
Table 6. Antimicrobial activities and trophic interactions: cellular characteristics that contribute to weed-like behaviour. 

<table>
<thead>
<tr>
<th>Metabolites, proteins, molecular characteristics, and other traits</th>
<th>Function(s)</th>
<th>Phenotypic traits</th>
<th>Notes and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avoidance or resistance to viral infection, predators, and grazers</td>
<td>Secondary metabolites produced by Aspergillus spp. repel (and can reduce the fecundity of) grazing arthropods such as Folsomia candida; those produced by Microcystis aeruginosa act as a repellent to planktonic grazers</td>
<td>Ability to repel grazers and predators</td>
<td>Aspergillus nidulans mutants lacking the last gene, a global regulator of secondary metabolite synthesis, are consumed by F. candida in preference to the wild-type strain (Rothfuss et al., 2007). Aldehydes produced by plankton repel crustacean grazers (Jüttner, 2005); β-cyclocitral from M. aeruginosa has been shown to repel Daphnia magna (Jüttner et al., 2010)</td>
</tr>
<tr>
<td>Synthesis of extracellular polymeric substances (e.g. M. aeruginosa), or use of flagella (e.g. Pseudomonas aeruginosa), that facilitate microcolony formation</td>
<td>Formation of multicellular structures that are sufficiently large to minimize grazing and predation by single-celled protozoa, zooplankton etc.</td>
<td>Microcolony formation</td>
<td>The role of polymeric substances in colony formation has been established using M. aeruginosa as a model organism (Yang and Kong, 2012). Functional analysis of P. aeruginosa mutants that were unable to form flagella, or were alginate-deficient, demonstrated an inability to form microcolonies that can confer resistance to grazing (Matz et al., 2004)</td>
</tr>
<tr>
<td>Biochemical adaptations to resist viral infection</td>
<td>Diverse mechanisms including modification of cell surface to prevent binding, intracellular suppression or viral genome replication, and degradation of viral DNA by restriction endonucleases</td>
<td>Resistance to or tolerance of viral infection</td>
<td>There are a number of mechanisms by which dominating species of bacteria and eukaryotic plankton (e.g. P. aeruginosa, Ostreococcus tauri; Bathycoccus plankton (e.g. Micromonas spp.) may synthesize of extracellular polymeric substances (e.g. M. aeruginosa) can be induced by exposure to zooplankton; concentrations of the toxin in cells exposed to D. magna, Daphnia pulex and Moina macrocopa were up to fivefold those found in control cells (Jang et al., 2003)</td>
</tr>
<tr>
<td>Toxic metabolites (e.g. microcystin in M. aeruginosa)</td>
<td>Toxicity against grazing or predatory zooplankton</td>
<td>Ability to protect against excessive grazing</td>
<td>Microcystin synthesis in M. aeruginosa can be induced by exposure to zooplankton; concentrations of the toxin in cells exposed to D. magna, Daphnia pulex and Moina macrocopa were up to fivefold those found in control cells (Jang et al., 2003)</td>
</tr>
<tr>
<td>Halophilic growth phenotype</td>
<td>Colonization of salt-saturated habitats that are hostile to predatory protozoa and minimizes losses from grazing and predation</td>
<td>Ability to inhabit environments hostile to predators and grazers</td>
<td>Halophiles that dominate high-salt habitats (e.g. D. salina, S. ruber) minimize interactions with grazers and predators most of which cannot tolerate molar salt concentrations (Bardavid et al., 2008)</td>
</tr>
<tr>
<td>Biochemical and cellular infrastructure that give rise to idiosyncratic migration patterns in the water column</td>
<td>Vertical migration within the water column that minimizes loss through grazing</td>
<td>Avoidance of predators and grazers</td>
<td>The predominance of Gonostomum semen in eutrophic habitats has been associated in part with migration patterns that minimize loss through grazing (Salonen and Rosenberg, 2000)</td>
</tr>
<tr>
<td>Bulk production of highly soluble, moderately chaotropic stressors&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Efficient conversion of acetoin to ethanol; in contrast, low-K&lt;sub&gt;m&lt;/sub&gt; Adh typically converts ethanol to acetoldehyde under in vivo conditions (Pikkar et al., 2006)</td>
<td>Production and secretion of ethanol up to molar concentrations</td>
<td>Ethanol, secreted in quantity by species such as S. cerevisiae and Zymomonas mobilis, is a moderately chaotropic substance that is nevertheless a potent stressor at millimolar to molar concentrations (Hallsworth, 1998; Bhaganna et al., 2010; Gray et al., 2013)</td>
</tr>
<tr>
<td>Enzymes for acetone, isopropanol and butanol synthesis</td>
<td>Synthesis of chaotropic substances in the anaerobic bacterium Clostridium acetobutylicum</td>
<td>Bulk production, and secretion, of multiple chaotropic stressors</td>
<td>Acetone, isopropanol and butanol that are produced by C. acetobutylicum (George et al., 1983) are more chaotropic ethanol – when compared on a molar basis – and are therefore more potent in their inhibitory activity as cellular stressors (Bhaganna et al., 2010; Gray et al., 2013; Bell et al., 2013)</td>
</tr>
<tr>
<td>Production of high concentrations of urea and ammonia</td>
<td>Synthesis of low-M, nitrogenous chaotropes</td>
<td>Production and secretion of urea and ammonia at inhibitory concentrations</td>
<td>Urea and ammonia are produced by phylogenetically diverse bacteria such as Pseudomonas stutzeri and Clostridium amnionphilum respectively (Theikidsen et al., 1997; Watanabe et al., 1998; Anderson et al., 2010)</td>
</tr>
<tr>
<td>Release of VOCs as low-solubility antimicrobial stressors</td>
<td>Inhibition of microbial metabolism; typically via hydrocarbon-induced water stress (Bhaganna et al., 2010)</td>
<td>Production and release of volatile antimicrobials</td>
<td>Examples are: isoamyl acetate (produced by Pseudomonas putida and other Pseudomonas species (Morales et al., 2006)</td>
</tr>
<tr>
<td>Highly volatile, strongly chaotropic metabolites (log P typically in the range 1–1.9)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Inhibition of microbial metabolism via chaotrope-induced water stress (Hallsworth, 1998; Hallsworth et al., 2003a; Bhaganna et al., 2010)</td>
<td>Production and release of volatile antimicrobials</td>
<td>Examples are: ethyl acetate (produced by Pseudomonas putida and other Pseudomonas species (Morales et al., 2006)</td>
</tr>
</tbody>
</table>

<sup>a</sup>See above.

<sup>b</sup>Bhaganna et al., 2005; Ozaki et al., 2006; Buzzini et al., 2006; Watanabe et al., 2008; Fialho et al., 2008; Anderson et al., 2003.

<sup>c</sup>See above.

<sup>d</sup>Examples are: isovaleryl butyric acid (produced by Pseudomonas putida and other Pseudomonas species (Morales et al., 2006)
Production and release of volatile antimicrobials

Inhibition of microbial metabolism typically via chaotropes or hydrocarbon-induced water stress (Hallsworth et al., 2003a; Bhagania et al., 2010)

Acidification of the extracellular environment

Intracellular conversion of pyruvate to lactate

Production and secretion of lactic acid

Conversion of acetyl-CoA to acetate; and butyryl-CoA to butyrate

Production/release of acetic and butyric acid synthesis (e.g. phosphotransacetylase and acetate kinase, and phosphate butyryltransferase and butyrate kinase respectively)

Production of biosurfactants, enzymes, and/or toxins with antimicrobial activities

Solubilization of hydrophobic substrates; inhibition of the growth of many microbial competitors

Production of inhibitory biosurfactants

Hydrolysis of glucans such as laminarin

Antimicrobial and cytotoxic activity via degradation of cell wall components

Panmycocin is produced by P. ananatis and is inhibitory or lethal to many species of yeasts and fungi including S. cerevisiae (Dziugi et al., 2005)

Inhibition of other microbial species/strains

Secretion of antimicrobials

Lipopeptidases from Pseudomonas sp. DFF1 were shown to inhibit Sclerotinia sclerotiorum (Lund et al., 2010); purified biosurfactants from Pseudomonas fluorescens inhibit growth of Botrytis cinerea and Rhizoctonia solani at low concentrations (25 μg mL−1) and can lyse zoospores of Phytophthora capsici (Kotyk et al., 1999; Magan et al., 1999). Studies of P. aeruginosa mammolipid suggest a key role in competitive ability against other bacteria (Ott et al., 2011). Admetabolics biosurfactants can inhibit phylogenetically diverse microbes (Kotyk et al., 2010; Boynton et al., 2010; Bhagania et al., 2010).

Antifungal peptide (Anat; in Aspergillus niger)

Mode-of-action not yet resolved

Secretion of an antifungal peptide

Antifungal activity

For example L. plantarum, Lactobacillus acidophilus

Prepared production of antibacterial peptides

Production of inhibitory biosurfactants

Pyoluteorin-producing P. putida strains have potential as biocontrol agents of pathogens such as Glomerella cucumerinica (sugar beet red rot; Nowak-Thompson et al., 1999; Hassan et al., 2011)

Pyoluteorin (in P. putida)

Inhibitory to oomycetes and fungi; mode-of-action not yet resolved

Antifungal activity

For example L. plantarum, Lactobacillus acidophilus and Pseudomonas spp.

Phenazine and phenazine-derived antimicrobials

Probable antimicrobial activity

Antimicrobial activity

Produced by species such as M. aeruginosa and P. aeruginosa (Mayrov et al., 2006), Lits et al., and Carmeli, 2012)

Mechanisms to induce cell lysis

Elimination of potential competitors

Algalicidal activity

Gonyostomum semen is able to lyse mycelial cells (e.g. Rhodotorula lacacea) on contact; mechanism not yet resolved (Rengel et al., 2008)

Resistance to antimicrobial stressors and toxins

Coordination of production of compatible solutes and ethanol

Protection against ethanol stress

Resistance to ethanol

The synthesis of glycerol (known to afford some protection against ethanol stress; Hallsworth, 1998; Hallsworth et al., 2003a) and ethanol production are coupled in S. cerevisiae; accumulation of trehalose (an effective protectant against ethanol; Mansure et al., 1994) coincides with the growth phase when ethanol concentrations are highest
Table 6. cont.

<table>
<thead>
<tr>
<th>Metabolites, proteins, molecular characteristics, and other traits</th>
<th>Function(s)</th>
<th>Phenotypic traits</th>
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</tr>
</thead>
</table>
| Compensable-solute accumulation in response to solvent and hydrophobic stressors (see above) | Protection of macromolecular systems challenged by chaotropic and hydrophobic stressors (see studies of A. nidulans, S. cerevisiae and P. putida; Mansure et al., 1994; Hallsworth et al., 2003b; Park et al., 2007; Bhagania et al., 2010; Bell et al., 2013) | Ability to maintain functionality of macromolecular systems | Kosmotropic compatible-solutes and other kosmotropic substances can reduce stress induced by chaotropic solutes such as MgCl₂, glycerol² and ethanol (Hallsworth, 1998; Hallsworth et al., 2007; Williams and Hallsworth, 2009). Trypsinogen and trypsinase are upregulated in cells of both eukaryotic and bacterial species in response to ethanol (Pamou et al., 1997; Punvis et al., 2000; Hisasawa et al., 2007; Horiouchi et al., 2010). S. cerevisiae could tolerate 28% v/v ethanol when protected by the kosmotropic compatible-solute proline (Thomas et al., 1993; Hallsworth, 1998).
| Upregulation of protein-stabilization proteins (see Table 3) | Protection of macromolecular systems from perturbations induced by antimicrobial stressors | Maintenance of protein structure and function during stressor-induced challenges | Saccharomyces cerevisiae upregulates synthesis of up to 10 heat-shock proteins under ethanol stress (Ma and Liu, 2010). Pseudomonas putida synthesizes diverse protein-stabilization proteins in response to chaotropic and hydrophobic stressors (see above; Hallsworth et al., 2003b; Bhagania et al., 2010)
| Membrane-stabilization proteins; solvent pumps (see Table 3) | Expulsion of chemically diverse stressors and toxins | Tolerance to a wide array of stressors and toxins | Including chaotropic solutes (e.g. phenol, benzyl alcohol), hydrophobic stressors (e.g. n-hexane, pyrene) and antibiotics (e.g. chloramphenicol, tetracycline, novobiocin) by AcrAB-ToIC; fluorescent dyes (e.g. daunorubicin, rhodamine), drugs (e.g. tamoxifen) and ionophoric peptides (e.g. nigericin) by Pdr5p (Kolaczkowski et al., 1996); and antibiofilm cle备用ing enzymes and echinocandin B by the AUMR gene product (Tobin et al., 1997; Nishino et al., 2002).
| Membrane-stabilization proteins; solvent pumps (see Table 3) | Removal of bacteriocins from the cell | Resistance to bacteriocins | LmrB is an ATP-binding cassette-type transporter in Lactococcus lactis (Gajic et al., 2003); BacA is a membrane-associated protein that in E. coli for (El Ghachi et al., 2004)
| Enzymes that inactivate antimicrobial substances | Detoxification and/or catabolism of inhibitory stressors and toxins | Ability to detoxify or degrade inhibitory antimicrobials | Examples include degradation of ß-lactam antibiotics in Acinetobacter spp. and P. aeruginosa (Langaire et al., 2000; Sinha et al., 2007) and degradation of volatile stressors such as dichloromethane (e.g. Bacillus spp.; Wu et al., 2009).
| Enhanced tolerance to a chaotropic volatile | Resistance to 2-phenylethanol | Resistance to VOCs | One strain of S. cerevisiae was found to be capable of growth at up to ~ 24 mM 2-phenylethanol (Eshkol et al., 2009). For a chaotropic stressor, log P 1.52, almost as chaotropic as phenol, this is an exceptional level of tolerance (see Bhagania et al., 2010).
| Cell wall composition that confers resistance to specific antibiotics | Pathways targeted by specific antibiotics may be absent; cell wall can act as a barrier to antimicrobials | Resistance to antibiotics | The Haloquadratum walsbyi cell wall is not composed of peptidoglycan so this species has reduced sensitivity to ß-lactam antibiotics (Bardavid and Den, 2008); the M. smegmatis cell envelope contains mycolic acids that reduce permeability, and therefore enhance resistance, to stressors and antibiotics (Liu and Nikaido, 1999).
| Synthesis of extracellular polymeric substances (see Table 3) | Production and secretion of polymeric substances | Resistance to diverse antimicrobials | For example production of alginate by P. aeruginosa increases resistance to antibiotics such as tobramycin (Hentzer et al., 2001). |
| a. The current table provides examples of proteins, genes or other characteristics that can be associated with and give rise to weediness; the categories of traits are not mutually exclusive. Individual traits may not be unique to microbial weeds; however, weeds species are likely to have a number of these types of characteristics. |
| b. Chaotropic activity of > 5 and < 40 kJ kg⁻¹ mole⁻¹ (Cray et al., 2013). |
| c. Volatile organic compounds are unlikely to exceed micromolar concentrations in the cytosol of the weed cell. |
| d. Glycerol is a phylogenetically ubiquitous as a compatible solute for eukaryotic microbes, yet at molar concentrations it acts as a chaotropic stressor (Williams and Hallsworth, 2009; Cray et al., 2013). |
organic compounds (VOCs) that inhibit the growth of both proximal and distal cells via their dispersion in both aqueous and gaseous milieu (Table 6).

Volatile organic compounds – including aldehydes, furones, terpenes and many alcohols – can be powerful inhibitors of microbial metabolism and growth (Table 6; Jorge and Livingston, 1999; Passoth et al., 2006; Minerdi et al., 2009; Fialho et al., 2011; Corcuff et al., 2011). Despite their volatility they are to some degree also water-soluble, and are sufficiently inhibitory to prevent metabolic activity and growth, even in aqueous solution at subsaturated solute-concentrations (Bhaganna et al., 2010). VOCs with a log $P_{\text{octanol-water}} < 1.95$ typically partition into the aqueous phase of cellular macromolecules and can act as potent chaotropic stressors (Table 6; Hallsworth et al., 2003a; Bhaganna et al., 2010; Cray et al., 2013). Those stressors with a log P of $> 1.95$ preferentially partition into the hydrophobic domains of membranes and other macromolecular systems, and thereby act as hydrophobic stressors that induce a distinct type of chaotropicity-mediated water stress (Bhaganna et al., 2010). We believe that the primary ecophysiological value of VOCs for microbial cells pertains to competition, species succession and (for weed species) habitat dominance. *Saccharomyces cerevisiae* synthesizes both hydrophobic and chaotropic volatiles that have antimicrobial activities, including ethyl octanoate and isoamyl acetate, and ethyl acetate and 2-phenylethanol (respectively, see Table 6; Fialho et al., 2011). Ethanol and 2-phenylethanol are synergistic in their antimicrobial activity (Ingram and Buttke, 1984; Seward et al., 1996), and this is consistent with their common chaotropicity-mediated mode-of-action. The more potent chaotrope of the two (when compared on a molar basis) is 2-phenylethanol which prevents growth of microbes in the range 10–40 mM (Berrah and Konetzka, 1962; Eshkol et al., 2009), values consistent with a chaotropic mode-of-action for a stressor with a log P of 1.36 (see Bhaganna et al., 2010). *Saccharomyces cerevisiae* like *P. anomala*, *Rhodotorula mucilaginosa* produces an array of volatile chaotropic and hydrophobic stressors as well as killer toxins (Table 6). Many fungi also produce mycotoxins; for example Aspergillus ochraceus growing on maize grain can produce ochratoxins at up to 1.4 mg g$^{-1}$ (Magan et al., 2003). Indeed a number of *Aspergillus* species are notorious for the potent activity of their ochratoxins against other fungal strains and as well as bacteria (Table 6).

Like *S. cerevisiae*, some other microbial species are able to produce moderately chaotropic antimicrobials in bulk quantities: *Clostridium acetobutylicum* produces high concentrations of butanol, ethanol and acetone in anaerobic habitats, and *Zymomonas mobilis* produces high concentrations of ethanol (Tables S1 and 6). Butanol is a particularly potent chaotrope that is highly inhibitory at millimolar concentrations (Bhaganna et al., 2010; Ezeji et al., 2010; Cray et al., 2013). We suspect that *Clostridium* spp. that can dominate chaotropic environments such as ammonia-rich animal slurries (Table S1) have an unusually high resistance to the net chaotropicity of the ammonia, ethanol, butanol, acetone and other chaotropic substances in their habitat (a hypothesis that has not yet been investigated to our knowledge; Williams and Hallsworth, 2009; Cray et al., 2013). The chaotropicity of urea and ammonia is greater than that of ethanol when compared at equivalent molar concentrations (Hallsworth et al., 2003a; Williams and Hallsworth, 2009) and there is evidence that some bacteria that secrete these compounds, including *Clostridium aminophilum* and

Pseudomonas stutzeri, benefit from chaotropicity-induced inhibition of their competitors (Table 6). There is no absolute resistance to the inhibitory/lethal activities of chaotropic and hydrophobic stressors (Sikkema et al., 1995; Hallsworth, 1998; Hallsworth et al., 2003a; 2003b; 2007; Duda et al., 2004; Bhaganna et al., 2010; Bell et al., 2013) so the production of such antimicrobials coupled with reasonably effective self-protection mechanisms (see Tables S1 and 6) can sometimes be the ultimate means to achieve community dominance (see also Table S1).

Pseudomonas putida can generate and/or secrete diverse antimicrobial substances including catabolites produced from degradation of macromolecular substrates, VOCs and biosurfactants (Table 6). Biosurfactants that solubilize hydrocarbons and other hydrophobic substrates can also be highly inhibitory to other microbial species. Studies of Pseudomonas rhamnolipids suggest a key role in competitive ability (Wecke et al., 2011); surfactants not only disorder plasma membranes and solubilize lipids, but can be chaotropic even for macromolecules that lack hydrophobic domains (Table 2; Cray et al., 2013). The membrane-permeabilizing activities of rhamnolipids are particularly detrimental to Gram-positive bacteria, and are lethal to Phytophthora zoospores (Kruit et al., 2009, Wecke et al., 2011). Bacteriocins produced by pseudomonads, like those of lactic acid bacteria, modify cellular membranes increasing permeability, causing leakage, disrupting transport processes and causing a dissipation of proton-motive force (Table 6). Catabolites such as phenol, catechol, vanillin, biphenyl, and sodium benzoate that are produced from the degradation of high-M hydrocarbons have inhibitory activity as chaotropic and hydrophobic stressors (Table 6; Hallsworth et al., 2003a; Bhaganna et al., 2010). Pseudomonas putida and other pseudomonads produce a wide variety of VOCs that collectively impact microbial competitors (Table 6). As outlined above, P. putida has multiple layers of defence against the potential stressful impact of solvent, chaotropic, and hydrophobic stressors via a versatile compatible solute metabolism (e.g. trehalose; Park et al., 2007; Bhaganna et al., 2010), protein-stabilization proteins, and production of extracellular polymERIC substances etc. (Tables 3 and 6). In addition, and arguably the most important feature for stressor tolerance, P. putida cells are equipped with efflux pumps (for example the plasmid-encoded TtGHI) that are highly effective at expulsion of hydrocarbons such as toluene as well as many other stressors and toxins (Ramos et al., 2002; Fillet et al., 2012).

Whereas the aquatic habitats of planktonic species such as D. salina, G. semen and M. aeruginosa may serve to dilute secreted antimicrobials, there is evidence that VOCs produced by D. salina, M. aeruginosa, and other aquatic species (including compounds such as β-cyclocitral and dichloromethane) are inhibitory to competing species (Table 6). Gonyostomum semen is able to lyse the cells of competing algal species on contact although the mechanism by which it does so has yet to be resolved (Table 6); and M. aeruginosa synthesizes a diverse range of volatile antimicrobials including phenazines and phenazine derivatives (Table 6; Lifshits and Carmeli, 2012). Lactic acid bacteria, in addition to bacteriocins, produce significant quantities of organic acids and secrete a vast array of inhibitory VOCs (Table 6). The analysis of volatiles produced, primarily by L. rhamnosus, during the production of a ewe’s-milk cheese (Table S1) gives an insight into the complexity of the microbial warefare that takes place in open habitats: 57 compounds were detected including terpenes, alcohols, ketones and esters (Randazzo et al., 2010) Although it is plausible that some of these substances exert specific toxic effects, all of them have the properties of either chaotropic or hydrophobic stressors in microbial cells (Table 6; Bhaganna et al., 2010), and their roles in habitat dominance has rarely received attention.

Microbial weed ecology

Open habitats

The concept of an open habitat is a fundamental, well-established principle in the fields of plant and animal ecology. For microorganisms, open habitats can be defined according to the diversity, interactions and community succession of their inhabitants in contrast to other types of microbial habitat that are defined by physico-chemical characteristics, substrate type or nutrient concentration (Table 7). Hydrocarbons are the primary carbon substrate in approximately one-third of the open habitats listed in Table S1, and are generally derived from dense, non-aqueous phase liquids (e.g. oil) or plant metabolites secreted into the rhizosphere, phyllosphere or atmosphere (bioaerosols). A distinguishing feature of hydrocarbon-based systems is that they can remain perpetually open because, as dissolved hydrocarbons are assimilated, the substrate reservoir may be continually replenished by molecules from the oil entering the aqueous phase or (for the rhizosphere and phyllosphere) because the plant continues to produce and secrete hydrocarbons and other organics (Nunes et al., 2005; Kazda and Falkinham III, 2009; McCammick et al., 2009; Hunter et al., 2010; Chakalik and Yurkov, 2012). These habitats are commonly dominated by species such as Acinetobacter johnsonii, Alkanivorax borkumensis, Pantoea ananatis and P. putida. Whereas these organisms – all members of the gammaproteobacteria – may share many molecular and ecophysiological characteristics (see Tables S1 and 3–6) in most cases it is P. putida that emerges as the most prevalent species (Table S1).
et al microbial life (i.e. 0.61–0.71: Pitt and Christian, 1968; Brown, 1990; Grant, 2004; Williams and Hallsworth, 2009; Leong, 2011) typically supports a remarkably high microbial diversity and biomass (Antón et al., 2002; Daffonchio et al., 2006; Baati et al., 2011) – and whereas NaCl-saturated habitats are generally considered extreme, their water activity is > 0.75 – well above that which ultimately limits microbial life (i.e. 0.61–0.71: Pitt and Christian, 1968; Brown, 1990; Grant, 2004; Williams and Hallsworth, 2009; Leong et al., 2011) – and typically supports a remarkably high microbial diversity and biomass (Antón et al., 2002; Daffonchio et al., 2006; Baati et al., 2011; Bardavid et al., 2008; Khemakhem et al., 2010).

Table 7. Properties of open (microbial) habitats.

<table>
<thead>
<tr>
<th>Typical characteristic(s)</th>
<th>Value as a descriptor for an open habitat*</th>
<th>Notes and examples</th>
<th>For open habitats</th>
<th>For habitats that are not open</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbial ecology</strong></td>
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<tr>
<td>Open to population by a</td>
<td>Primary</td>
<td>Potentially habitable by an indefinite number of species which may be from diverse Kingdoms of life (e.g. solar salterns see Table S1)</td>
<td>The fracture water of a South-African goldmine (2.8 km deep) was found to contain a single-species ecosystem (Chivian et al., 2008)</td>
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<td>multitude of microbial</td>
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<tr>
<td>species</td>
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<td></td>
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<tr>
<td>Promote intense competition between microbial species</td>
<td>Primary</td>
<td>Conditions favour strong growth of many species and may select for ability to produce antimicrobial substances (see below)</td>
<td>A climax community, single-species habitat, and symbiotic partnership are characterized by low levels of inter-species competition</td>
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<tr>
<td>Can facilitate dominance of the developing community by one or more species (though this may not always take place)</td>
<td>Primary</td>
<td>Diverse types of substrate/environment can accommodate community progression towards dominance by one or more microbial species (see Table S1)</td>
<td>The antithesis of an open habitat is one that is occupied by a climax community that is no longer capable of further evolution (e.g. Paul et al., 2006)</td>
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<tr>
<td>Transient and self-terminating; unless maintained in an ‘open’ condition</td>
<td>Primary</td>
<td>Frequently leading to the development of a closed community dominated by a single species (see Fig. 1; Table S1); unless ‘re-opened’ due to grazing for example, or replenished (e.g. by fresh inputs of nutrients)</td>
<td>Closed habitats typically lack conditions that facilitate biomass formation and species succession</td>
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<td><strong>Resident microbes</strong></td>
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<td>Strong selection for</td>
<td>Primary</td>
<td>For example, habitats rich in nutrients may favour species of bacterial copiotroph which may merge as dominating species if they possess other weed traits</td>
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<tr>
<td>microbes able to out-grow competitors</td>
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<tr>
<td>Open to manipulation by</td>
<td>Primary</td>
<td>Dynamic biomass formation makes open habitats available for/vulnerable to manipulation by species that can introduce growth-limiting substances (Table 6; Cordero et al., 2012)</td>
<td>In closed habitats the community is already restricted in terms of space, nutrients and/or physicochemical constraints etc.</td>
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<td>species able to modify</td>
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<td>environmental conditions</td>
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<tr>
<td>and/or secrete</td>
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<td>antimicrobials</td>
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<tr>
<td>Select for microbes that</td>
<td>Secondary</td>
<td>Microbes such as S. cerevisiae and Lactobacillus spp. typically grow without idiosyncratic requirements (such as a requirement for interactions with other species)</td>
<td>Specialist microbes are found in niche habitats, such as those that form lichens (Hoffland et al., 2004)</td>
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<td>lack specific requirements</td>
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<td>(such as rare trace</td>
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<td>elements, or a specific</td>
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<td>host)</td>
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<td><strong>Chemistry and properties of the cellular environment</strong></td>
<td>Primary</td>
<td>Open (microbial) habitats are, by definition, open to population by a large number and high diversity of microbial species so are typically not physically or chemically extreme⁹</td>
<td>Extreme habitats that are occupied by a small number of obligate extremophiles rarely support the high growth rates or promote the intense competition that typify open habitats⁶</td>
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<td>Not excessively stressful</td>
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<td>for resistant microbes</td>
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<td>in terms of water activity, chaotropicity, pH, temperature etc. b</td>
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<td>Have the potential to</td>
<td>Primary</td>
<td>Open habitats have sufficient space and resources to facilitate the ecosystem development (Table S1; Fig. 1)</td>
<td>Non-open habitats can be constrained by nutritional and/or physicochemical parameters (Chivian et al., 2008)</td>
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<td>support a substantial</td>
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<td>microbial biomass and so</td>
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<td>do not constrain</td>
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<td>ecosystem development</td>
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<tr>
<td>Not contaminated with</td>
<td>Secondary</td>
<td>Some polluted, hydrocarbon-rich environments may also support the development of microbial communities</td>
<td>High levels of toxic substances will prevent high growth rates and minimize diversity</td>
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<td>inhibitory substances</td>
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<td>such as heavy metals or</td>
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<td>other potent toxins</td>
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</table>

a. Those designated as descriptors of primary importance are considered essential to the definition of an open (microbial) habitat.
b. Not so extreme that there is limited diversity or possibility for biomass formation: i.e. typically > 0.75 water activity (see Pitt, 1975; Williams and Hallsworth, 2009), < 20–25 kJ kg⁻¹ mole⁻¹ chaotropic activity (Hallsworth et al., 2007), < pH 11 or > –15°C.
c. Whereas NaCl-saturated habitats are generally considered extreme, their water activity is ³ 0.75 – well above that which ultimately limits microbial life (i.e. 0.61–0.71: Pitt and Christian, 1968; Brown, 1990; Grant, 2004; Williams and Hallsworth, 2009; Leong et al., 2011) – and typically supports a remarkably high microbial diversity and biomass (Antón et al., 2002; Daffonchio et al., 2006; Baati et al., 2008; Bardavid et al., 2008; Khemakhem et al., 2010).
This is a product of its metabolic versatility, exceptional tolerance to hydrocarbons and other solutes, and antimicrobial activities (Tables 3, 5 and 6). Other weed species, however, have greater acid tolerance than the gammaproteobacteria: in the acidic surface film of the sphagnum moss grey layer it is Mycobacterium (including Mycobacterium terrae, M. smegmatis and Mycobacterium sphagni) that emerges as a dominant genus; and in the green layer at lower pH (2–2.5), R. mucilaginosa has the competitive advantage (Table S1). Phyllosphere, rhizosphere and other habitats can also remain continuously open if they are replenished with organic substances from ions released from soil particles, extracellular polymeric substances and compatible solutes from microbial cells, root diffusates, animal wastes, diverse sources of necromass, etc.

Habitats which facilitate weed emergence are typically not so extreme that there is a negligible microbial diversity or little capacity for community development/biomass formation; i.e. > 0.75 water activity, < 20–25 kJ kg⁻¹ mole⁻¹ chaotropic activity, < pH 11 or > −15°C (Table 7). Open habitats have available nutrients, electron acceptors etc and C : N ratios that favour microbial growth, and do not contain high levels of inhibitory substances such as heavy metals or toxic pollutants. They will therefore promote intense competition between species assuming that a diversity of microbial species is present (Tables S1 and 7). Whereas high-sugar habitats with a water activity below 0.9 are too stressful for the vast majority of microbes, including S. cerevisiae, there is still a considerable diversity of xerotolerant and xerophilic species that are highly active between water activities of 0.84 and 0.9 (Pitt, 1975; Brown, 1990). Similarly, crystallizer ponds or solar salt pans are known as extreme environments and only a small fraction of microbial species can survive and grow at saturated NaCl. Nevertheless, it is the limited solubility of NaCl (and not the limitations of salt-adapted macromolecular and cellular systems) that determines the limit for microbial growth in high-NaCl habitats: a number of obligate halophiles grow optimally at 35% w/v NaCl (0.755 water activity) and different types of NaCl-saturated environment are biodiverse, highly competitive, biomass-rich microbial habitats (Antón et al., 2002; Daffonchio et al., 2006; Baati et al., 2008; Bardavid et al., 2008; Khemakhem et al., 2010). Salters (and also algal mats) may under some conditions function as continually open habitats if nutrients are not limiting; D. salina for example can release sufficient glycerol into the system to satisfy the carbohydrate requirement of the wider community (Borowitzka, 1981; Bardavid et al., 2008).

Open habitats favours microbes with shorter generation-times and relatively vigorous growth-phenotypes and in this way may drive the evolution of faster-growing and more stress-tolerant species (as well as those with effective antimicrobial strategies; Tables 4, 6 and 7). That the ecology and evolution of plant weeds are intimately and inextricably linked with open habitats has been established for some time (Anderson, 1952); in contrast there is little information about open habitats of microorganisms (see Table 1; Hallsworth, 1998). Open habitats can be spontaneously created (e.g. due to removal of microbial biomass by predators and grazers; McArthur, 2006), dynamic (e.g. due to desiccation–rehydration cycles or nutrient limitation), and/or transient (Tables S1 and 7). As is true for open habitats of plants (Anderson, 1952), open habitats of microbes can also become closed if they becomes water-constrained or otherwise resource-depleted; and/or the microbial community reaches stationary phase (Paul et al., 2006): and these changes may be accompanied by – or even a consequence of – domination by weed species. Environments can even progress to become sterile; for example those with high ethanol concentrations (Hallsworth, 1998; Pretorius, 2000). However, a dualistic concept of open habitats on the one hand and closed habitats on the other would be simplistic; in reality there is a seamless continuum between the two extremes of fertile but uncolonized and hostile/uninhabitable environment. Habitats with a favourable water activity for most bacteria (> 0.94) and high concentrations of carbon and nitrogen substrates such as milk, cheese and crushed peanuts (Table S1) typically progress to a closed condition (and are dominated by lactic acid bacteria), as do most high-sugar habitats (Table S1). A number of the open habitats listed in Table S1 that are resource-rich, and stressful, temporally or spatially heterogenous, and/or require saprotrophic nutrition were dominated by Pichia (e.g. moist barley grains; salted fermenting olives) or Aspergillus species (e.g. decomposing leaf litter, sugarcane juice). The stress biology, energy-generating capabilities and antimicrobial activities of these species undoubtedly contribute to their success (Tables 3 and 6).

Ecophysiological classification

Microorganisms have been previously categorized based on the way in which their phenotype impacts ecological behaviour: as autotrophs and heterotrophs; generalists and specialists; r- and K-strategists, aerobes and anaerobes; copiotrophs and oligotrophs; fast and slow growers; and extremophiles and mesophiles (see Table 4 and S2). The definition of microbial weeds is qualitatively distinct from that of other microbial groups (see Table S2): only weeds are characterized by their ability to dominate communities and the associated/underlying biology (Tables S1, 2–6 and S2) and it is only weeds that are linked via their ecology and evolution with open habitats (Tables 1, S1, 3 and S2). The biology and ecology of microbial weed species cuts across established groupings of...
microorganisms (i.e. the former does not represent a parallel category; see Table S2). Some microbes have a considerable number of weed traits but only rarely emerge as dominant members of communities (e.g. Pseudoxylaria spp., E. coli and M. smegmatis; see Tables S1 and 3, 5 and 6). Such species serve as a reminder that weeds and non-weeds represent two extremes of an otherwise continuous scale. An insightful study of Termitomyces ecology, for example, discusses the biology of Pseudoxylaria; a fungus that is present in Termitomyces habitats but is typically inactive and/or present at negligible cell densities (Visser et al., 2011). Pseudoxylaria could be ecologically disadvantaged if present in greater numbers as termites, which can detect VOCs produced during interactions between Pseudoxylaria and Termitomyces, remove the unwanted fungus from their Termitomyces cultures. The extant Pseudoxylaria strains thereby appear to have been selected for/have evolved a low competitive ability (Visser et al., 2011). Nevertheless Pseudoxylaria can occasionally emerge to dominate the substrate (Visser et al., 2011), a fertile open habitat consisting of decomposing plant material (Table S1). Non-weed species make up a loose affiliation of species that are only linked by their poor ability to dominate communities in open habitats and may not share any specific metabolic or phenotypic traits (Table S2). We propose that this ‘dustbin’ group includes Synodropsis spp., Polypaecilum pisce, Metschnikowia orientalis, Caulobacter crescentus and Salmonella species (Table S2). Several ecophysiological classification systems that are based on microbial growth-phenotype and/or substrate utilization (Table S2). Microbial generalists can occupy a wide range of ecological niches, and typically achieve this via the ability to utilize a wide range of nutrients and substrates (Table S2). Conversely microbial specialists, such as S. cerevisiae, H. werneckii and many basidiomycetes have specific nutritional and/or physicochemical requirements (de Fine Licht et al., 2005; Kashangura et al., 2006). Obligate extremophiles commonly occupy, and are adapted to grow optimally in, niches that are hostile to the majority of microorganisms (Table S2). There have been relatively few studies (and is consequently inadequate terminology) to properly describe microbes that are extremo-intolerant such as xero-intolerant species (see Table 4), with the exception of mesophilic species that function optimally at non-extreme temperatures (though the term mesophiles can be used in a general sense to refer to non-extremophiles: Table S2; Gostincar et al., 2010). Non-extremophilic microbes grow optimally in physicochemical conditions which favour metabolic activity and growth of the majority of microbial species, and this grouping includes both weed and non-weed species (Table S2). While fast-growing microbes have short doubling times, the vast majority of these species do not have weed-like capabilities and are unable to out-grow their competitors (Tables 4 and S2). It may be that relatively few oligotrophic microbes or K-strategists are weed species, although some mycobacteria do have a weed phenotype (Tables S1, 3, 5 and 6). Pseudomonas putida is generally considered to be a model r-strategist (e.g. Margesin et al., 2003) yet P. putida has an extraordinary competitive ability (see Tables S1 and 4–6), and may not be the best example of an r-strategist (the latter are characterized by a weak competitive ability; Table S2). By contrast P. putida appears to be an exceptional example of a microbial weed both in terms of its cellular biology and its ability to dominate diverse types of open habitat.

Concluding remarks

It is irrefutable that a small minority of microbial species recurrently appear as the dominant members of microbial communities; and we suggest here that these microbes have shared phenotypic traits. This implies that open-habitat ecology has impacted the evolutionary trajectories of these microbes, and that the activities of weeds determine microbial diversity in many localities within the biosphere. Whereas microbial weed species are not always useful to humankind (see Table 1), they make up a substantial portion of the world’s biotechnology sector. Saccaromyces cerevisiae supports global industries in fermented products, biofuel, biocides (ethanol is also an important disinfectant; McDonnell and Russell, 1999), and other white biotechnologies; P. anomala produces a substantial catalogue of biotechnologically useful substances and is employed in production of foodstuffs (see Walker, 2011); lactic acid bacteria form the basis of food- and drinks-production processes where the antimicrobial volatiles and organic acids that they secrete act as flavour compounds and natural preservatives, and are used as probiotics, and for the production of fine chemicals (Rose, 1982; Steinkraus, 1983; Molly et al., 1996; Nomura et al., 1998; Tiwari et al., 2009; Randazzo et al., 2010; Bron et al., 2012); P. putida is used for the commercial production of bulk and fine chemicals, industrial biocatalysis, and as the basis of many other industrial and environmental biotechnologies (Kruit et al., 2009; Puchalka et al., 2008; Hassan et al., 2011; Pohlete-Castro et al., 2012); a number of weed species, including Aspergillus spp., P. putida and Clostridium spp. play primary roles in the treatment or decomposition of plant matter, oil and hydrocarbons, xenobiotics in polluted soils, sludge and slurry treatments, and other organic wastes (Tables S2 and 4; Narihiro and Sekiguchi, 2007; Timmis, 2009); and Aspergillus species are used for production of foods (Rose, 1982; Tamang, 2010), fine chemicals (e.g. citric acid, secondary metabolites) and extracellular enzymes.
(e.g. amylase, cellulase, pectinase and protease), for industrial biocatalysis, and have potential for bioremediation and biosorption applications (Ward et al., 2006). It is the metabolic activities of substances that suppress potential competitors which make \textit{S. cerevisiae}, \textit{Rhodotorula} spp., \textit{P. anomala}, \textit{P. putida}, lactic acid bacteria and other weed species invaluable as agents of biological control, for preventing spoilage of drinks, foodstuffs and animal feeds (see Calvente et al., 1999; Kruijt et al., 2009; Hassan et al., 2011; Olstorpe and Passoth, 2011; Walker, 2011).

Microbial weeds are also used as model systems for research, yet key aspects of their biology remain enigmatic. Many plant weeds have exceptional reproduction and dispersal systems (Table 1) and it remains unclear whether microbial weed species produce more propagules, have superior dispersal mechanisms, or evolved propagules with exceptional longevity, and/or the ability to germinate over a wider range of conditions than their non-weed comparators. Could it be that, like plant weeds, the competitive ability of microbial weeds is enhanced by an abnormally high phenotypic plasticity and intra-specific variability (see Table 1)? Out of the world’s plant flora it has been estimated that only several hundred plant species are weeds (Hill, 1977); what percentage of microbes might exhibit weed phenotypes and behaviour? Is there evidence preserved in rocks, sediments, peat, ice, or the hypersaline fluid inclusions of salt crystals that can shed light on the evolutionary trajectories of microbial weed species? Is there an ideal genome size for prokaryotic and eukaryotic weed species? What are the minimum/essential characteristics required to enable a microorganism to dominate a specific habitat; can we design and manufacture a microbial weed via a synthetic biology approach? Will hitherto uninvestigated microbial weeds prove to be rich sources of antimicrobial compounds for novel biotechnological and clinical applications? Can microbial weed ecology shed light on wound microbiology, lead to the prevention of the microbial colonization of prosthetic devices, or minimize the emergence of opportunistic pathogens? Could knowledge-based interventions in weed ecology enhance our ability to manipulate and manage microbes at various levels of food-production and food-spoilage, or lead to better management of environments that harbor potentially dangerous species?

Crop plants such as sunflowers, potatoes, wheat and barley are known to have weedy ancestors (Anderson, 1952) and it is thought that the evolution of both plant weeds and crop plants has been driven by anthropogenically opened habitats. We believe that open (microbial) habitats also select for the phenotypes, and therefore act as a potent driving force that determines the evolutionary trajectories, of microbial weeds and their communities: open-habitat ecology may therefore be worthy of recognition as a distinct scientific field that spans all organismal biology including microbiology. There is a maxim that everything is everywhere but that the environment selects; this implies that microbial species are everywhere on Earth but that the local environment determines which are able to grow (Baas Becking, 1934; de Wit and Bouvier, 2006). While not necessarily untrue, this idea implies a level of passivity on the part of the microbial cell. Yet microorganisms are inherently variable and dynamic systems with considerable genetic dexterity and phenotypic plasticity (able to both respond and adapt). Baas Becking (1934) acknowledged that cells are able to modify their extracellular environment, but microbes are also proactive in their manipulation of each other. It may be that microbial weeds are the most accomplished examples of this.

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**Conflict of interest**

None declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Examples of open habitats in which specific microbes attain dominance.

Table S2. Biology of microbial weeds compared with that of other ecophysiological groups.