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The prospect of superior yeast for winemaking: recent successes through bioprospecting

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This article provides a selective review of recent reports that describe developments in the pursuit of superior yeast for winemaking through bioprospecting. In recent years, the focus of researchers and starter-culture companies has broadened. No longer are the targets merely strains that are reliable and sensorily inoffensive. Rather, efforts have expanded to seek much greater precision in these age-old targets and/or strains that address aspects of sustainability by enabling reduced waste, fewer chemicals or less energy input.

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Introduction

Winemaking is conceptually straightforward and has been practised for eight thousand years [1]. One simply grows or purchases grapes, harvests and crushes them, and allows the associated microbes or those on the equipment to effect a conversion to an alcoholic beverage. However, the challenge is to make a quality product that consumers want and, increasingly, to do so sustainably with minimal inputs. This depends on high-quality grapes, the avoidance of chemical and microbial spoilage during all stages of fermentation, maturation and storage, and the desired chemical and biological degradation/modification/production of sensorily important compounds. Microbiology is critical in this process, and the understanding of the role of microbes in wine production has come a long way since the recognition of their active participation over 160 years ago [2].

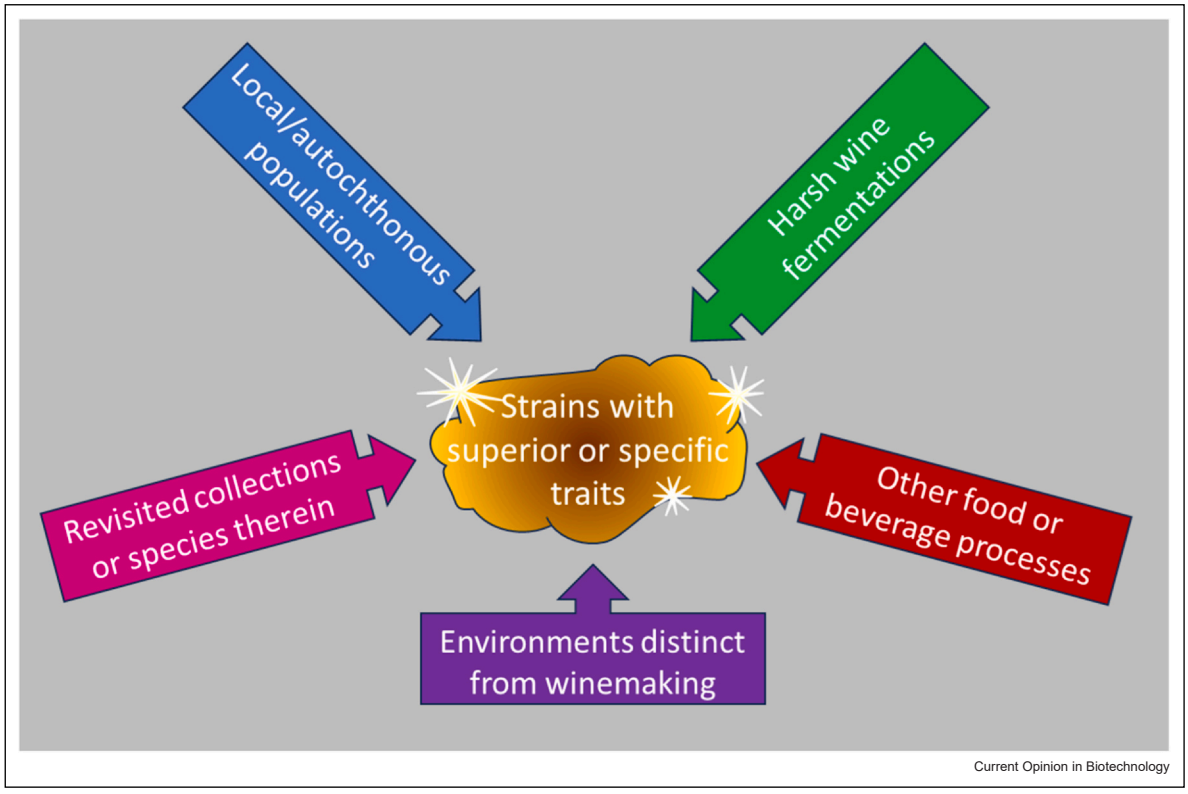
Given this recognition, the vast majority of wine fermentations are now deliberately inoculated with a starter culture of a selected yeast(s), particularly in New World wine-producing countries [••3]. But winemaking is not static. Consumer preferences evolve, the climate has and continues to change and thus so too are the demands placed on and the stresses experienced by wine microbes. For these reasons, the calls for new microbes with novel properties or those tailored to a particular process or product remain loud. In this regard, it is worth noting the growing interest in non-*Saccharomyces* yeasts, which are seen to inherently offer diverse sensory contributions beyond what is possible from *Saccharomyces*. Certainly, the research community is responding. A *Google Scholar* search for the terms 'wine' and 'non-*Saccharomyces*' returned 1630 items for the period 2009–2014 and almost four times this number (5890) a decade later (2019–2024).

Finding superior yeast, whether *Saccharomyces* or non-*Saccharomyces*, involves many approaches, ranging from discovery to modification, followed by characterisation, either as pure cultures or as mixed cultures, the latter being more reflective of the real-world scenario. Several recent reviews describe these approaches in detail [••3–5]; thus, many will not be presented here. Instead, only a selection of notable developments in the bioprospecting space will be covered.

The quest

In this context, bioprospecting involves the search for desired, superior microbial strains (i.e. the 'gold nuggets') from amongst a field of existing, suboptimal or perhaps worse strains (i.e. the worthless 'rocks'). The starting point for bioprospecting can be from targeted or untargeted parts of the environment, related niches or existing collections of strains, and is then followed by various stages of screening and evaluation. Isolation from the field is an important means of finding strains suited to a particular winemaking context. Many of the ~250 yeast strains that are available commercially to winemakers as starter cultures originated in this way, having been isolated from in and around vineyards and wineries from a select group of key wine-producing regions. Being an ethanol-tolerant, robust fermenter that persists through fermentation, *Saccharomyces cerevisiae* is well represented amongst these strains [••3–6]. Given this and the likelihood that it has been vectored between wine regions through the movement of vines, people and equipment [7], it is perhaps not surprising that there is

Figure 1



Origins that might be explored for opportunities to discover strains with distinct properties or better suited to a particular winemaking context.

redundancy and limited genetic and phenotypic diversity among these *S. cerevisiae* strains [6]. The desire for new and different strains therefore continues. Across the research community, several strategies are being pursued to find strains with novel attributes and/or those suited to a particular winemaking niche (Figure 1). And of course, as already mentioned, non-*Saccharomyces* yeasts have enjoyed a surge in interest in this regard.

New hunting grounds

It is likely that important new strains will be recovered from less intensely sampled parts of the world (Table 1). Yeast surveys have historically been undertaken in developed countries from a narrow set of niches, such as insects, plants, ephemeral flowers and soils [8]. Many entirely unexplored areas potentially containing new stains, let alone new species, remain to be examined, for example, many parts of northern China, Russia, Africa and Australia. To this end, we have sampled sugar-rich sites in remote locations, such as cider gums (*Eucalyptus gunnii*) in the central highlands of Tasmania or plants in the Torres Strait [9,10], revealing a plethora of yeasts. In addition to many known species, particularly of the genera *Kregervanrija*, *Hanseniaspora*, *Lachancea*, *Zygosaccharomyces*, *Candida* and *Pichia*, identification by internal transcribed spacer (ITS) amplicon sequencing revealed a large proportion of sequences that did not align with known fungal genomes [9], possibly representing new species. Culturable isolates of known species revealed several with tolerance to industrially relevant challenges (ethanol, SO_2, copper and so on) and the production of sensorially important metabolites (ethyl and acetate esters, volatile acids and so on) [•10]. Ongoing characterisation, including by methods described below, will reveal their usefulness in winemaking or other fermented beverages.

Where the recent focus of searches has been on wine-related settings, the less prominent and under-sampled wine regions have proven productive (Table 1). For example, Feng et al. [11] screened 52 *S. cerevisiae* strains isolated from five regions in northwest China to identify candidate strains better suited to the local winemaking conditions. Several strains were highlighted by performing well in high sugar (250–300 g/L) media, resistance to 250 mg/L SO_2, 16% (v/v) ethanol, and 0.5 mmol/L Cu^{2+}, and for producing low amounts of H_2S or more esters. A sensory panel of wine professionals also preferred the wines made with these strains. Similarly, Perrusquía-Luévano and co-workers [12] investigated high-sugar must fermentations from desert-grown wine grapes in the Chihuahuan Desert in Mexico. In this

Table 1

Recent examples of yeast strain screening efforts to identify isolates exhibiting either broad utility in winemaking or else attributes offering specific benefits to wine processing or composition.

Strain origin	Isolates examined	Properties assessed	Highlighted strains	Reference
General utility in winemaking				
Spontaneous wine fermentations, Ningxia, China	148[a]	Tolerance of glucose (≤28%), ethanol (≤8%), SO_2 (≤80 mg/L), plus combinations; Fermentation kinetics; volatile production; β-D-glucosidase, esterase	*H. uvarum* QTX-C10	[51]
Tasmania and Erub Island, Australia	94	Tolerance of ethanol (≤12%), SO_2 (13 mg/L free), Cu (0.2 mM), high glucose (≤40%), cold (12°C), anaerobiosis; protease, pectinase, β-lyase and β-glucosidase; sugar utilisation and metabolite production; volatile production	Numerous non-*Saccharomyces*	[•10]
Spontaneous fermentations, northwest China	52	Tolerance of ethanol (≤16%), SO_2 (≤250 mg/L), Cu (2 mmol/L), cold (12 & 15°C); fermentation rate; killer status; H_2S; foaming, flocculation; volatile production	*S. cerevisiae* XJ3, SAX2, SAX5, and SAX6	[11]
Fruit, Taiwan	42	Tolerance of temperature (19–50°C), SO_2 (≤100 mg/L), ethanol (≤18%); β-D-glucosidase; volatile production	*Hanseniaspora uvarum* Pi235, *H. guilliermondii* Ki135, *Pichia kluyveri* Pe114, *S. cerevisiae* Gr112	[52]
Spontaneous wine fermentation, Romania	31	Tolerance of ethanol (≤15%), SO_2 (≤29 mg/L); β-glucosidase, esterase, lipase, protease, pectinase;	Four *S. cerevisiae* strains	[53]
Wine-related isolates, Culture Collection of Wine Yeasts, Slovakia	29	Tolerance of 18 and 37°C, pH (3.0), ethanol (15%), glucose (30%), SO_2 (≤60 mg/L); β-glucosidase, pectinase, esterase, protease; H_2S; acetic acid; volatile production	*S. cerevisiae* PDA W 10, *Lachancea thermotolerans* 5 –1 –1, *Metschnikowia pulcherrima* 125/14	[15]
Spontaneous fermentations of desert-grown grapes, Mexico	24	Tolerance of glucose (35%) plus SO_2 (15 mg/L); fermentation kinetics; volatile production	*Candida thaimueangensis*, *C. apicola* and *Hanseniaspora* sp. strains	[12]
Soil, grapes, ferments, Yalu River Valley, China	12	Tolerance of glucose (≤35%), ethanol (≤18%), SO_2 (≤300 mg/L), low pH (2.0–4.0)	*S. cerevisiae* BZ1 and EZ2	[54]
Various origins, CBS-Westerdijk Fungal Biodiversity Institute, Netherlands	10	Fermentation kinetics; volatile and metabolite production	Various non-*Saccharomyces*	[55]
Baiju yeast, China	4	Growth and viability of co-cultures in fermentations of Cabernet Sauvignon grapes (22.8% sugar, pH 3.38, 260 mg/L yeast assimilable nitrogen, 25°C); Volatile production; Sensory	*Zygosaccharomyces bailii* and *Pichia kudriavzevii* strains	[24]
Specific traits				
Spontaneous fermentations, Greece	190	Tolerance of SO_2 (500 mg/L); killer status; β-glucosidase; acetic acid; H_2S; fermentation kinetics; sensory	Various species	[••16]
Grapes, University of Basilicata Culture Collection, Italy	122	Amino acid decarboxylase activity	Isolates of *S. cerevisiae*, *Zygosaccharomyces bailii*, *Hanseniaspora* spp.	[27]
Wine-related, AWRI Wine Microorganism Culture Collection (WMCC), Australia	103	Late-fermentation flocculation	*S. cerevisiae* AWRI1688 and AWRI1759	[25]
Wineries and other niches, IATA-CSIC collection, Spain	81	Production of tryptophan-derived indolic compounds	Various strains	[26]
Collections, commercial strains or spontaneous fermentations, Italy	33	Inhibition of other yeast and bacteria	Various strains	[56]
Collections, commercial strains or spontaneous fermentations, Germany	14	Glycosides and ester formation in *Saccharomyces* and non-*Saccharomyces* co-fermentations	*Torulaspora delbrueckii* strains	[57]
Collections at Università degli Studi di Firenze and University of California-Davis	9	Increased esters; increased polysaccharides (galactomannoprotein) leading to reduced protein instability in *Schizosaccharomyces pombe* and *S. japonicus*	*Schizosaccharomyces japonicus* UCD2489	[48,49]
Grapes, Maule Region, Chile	2	Bio-protection (reduced use of SO_2)	*Candida oleophila* and *C. boidinii*	[58,••59]

[a] *Hanseniaspora uvarum* strains only.

environment, the grapes ripen at higher temperatures (> 40°C) to achieve high sugar content (> 26°Brix) and pH musts. Consequently, the authors speculated that the associated microflora would be inherently better suited to such harsh fermentation conditions. Isolates recovered included *Aureobasidium*, *Sporobolomyces*, *Candida* and *Hanseniaspora* species, which all grew with 350 g/L glucose and 100 mg/L SO_2, while acetic acid yield remained modest (0.2–1.0 g/L). Importantly, several of the predominant species found produced high amounts of higher alcohols, while *Candida thaimueangensis* produced high amounts of esters, thereby also introducing possibilities for flavour enhancement with these yeasts. If it is shown that such strains do in fact struggle less under harsh conditions, they will likely lead to greater sustainability in the winemaking process since shorter fermentations generally require less energy and additives (see below).

In instances where isolates have been collected from the field and their identity is not known, they are typically identified by way of sequencing of the ITS regions or by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry biotyping. Recent examples of the applications of these methods are readily found in the literature [13–••16]. Such identifications might be supported or expanded upon through a bank of broad typing procedures that include microscopic appearance, CO_2 production and growth under various conditions, sporulation, tolerance of several stressors and expression of enzymatic activities. These methods and examples of results obtained for 26 strains isolated from wine-related samples in Slovakia are conveniently summarised in a compendium by Sidari and coworkers [15] and will no doubt benefit other research groups working in the field.

Revisiting the past

The pursuit of new isolates from the field is not necessarily the only way forward. To quote Boundy-Mills and co-workers, "The optimization of specific biotechnology applications rarely starts by the sampling and isolation of new yeast strains" [17]. Culture collections already house an enormous number and diversity of yeast species and strains thereof. The opportunity therefore exists to (re-)visit these collections in search of strains with specific properties. Such searches can either be restricted to a single species, closely related strains or else can be quite broad. As an example of the former, we surveyed 172 isolates of a single species, *Lachancea thermotolerans*, sourced from large culture collections and individual research laboratories [18]. Side-by-side comparisons of these identified isolates with desirable oenological properties [19], one of which was eventually commercialised due to its ability to bioacidify wine [20].

It is also worth recognising that the identities of strains in culture collections are not necessarily inherently accurate, especially for isolates collected many decades ago and characterised via more traditional methods. For this reason, it is important to acknowledge the value of efforts to confirm the identities of historic isolates through contemporary methods [••21]. Such work ensures that strains appear under the correct species name and are not missed from single-species comparisons, such as that mentioned above, purely because of misidentification.

Repurposing

A final opportunity for winemaking arises from the use of yeast strains intended as starters in other beverage or food fermentations or else isolated from these (excluding spoilage agents). By implication, such strains are likely to broadly meet biosafety requirements and be well suited to fermentation processes. As a case in point, Ravasio and coworkers screened 60 yeasts, mainly of food or drink origin, to identify those that might offer sensory benefits to lager beer fermentations [22]. Several strains were found to produce more aroma compounds than the lager reference strain, and co-fermentation of *Wickerhamomyces anomalus* with the reference resulted in an increased fruity-flavour profile. Postigo et al. [23] had similar success in their study of 141 strains of wine-related origin for their suitability in brewing. Efforts specific to wine fermentation include the evaluation of non-*Saccharomyces* yeast from the distilled beverage Baiju (Table 1), which involves a fermentation strikingly different to winemaking. Cooked sorghum most typically undergoes a solid-state fermentation involving a simultaneous saccharification of starches to sugars that are fermented by yeast, including *Saccharomyces*, *Zygosaccharomyces* and *Pichia* [24]. The authors reasoned that since Baiju yeasts are exposed to high fermentation temperatures, they would be more reliable even in wine fermentations. In keeping with this, when sequentially inoculated into fermentations of Cabernet Sauvignon grapes, the non-*Saccharomyces* showed little loss of viable cell numbers towards the end of fermentation despite the attainment of ~9% ethanol content.

Screening

Ideally, screening of candidate yeast would be performed under the precise winemaking conditions (grape variety, juice composition, fermentation tank dimensions, intended wine style and so on) in which the yeast is to be applied. Of course, such a tailored approach is not financially or practically feasible for most research groups, especially for large numbers of candidate strains. Instead, evaluation conditions are necessarily a compromise in many regards. Trials are typically conducted on a small scale (microlitres to litres), with no or limited control of oxygen-ingress, using a limited number of juices or defined media, and usually involve a stepwise approach to progressively narrow down the field of candidates. Short-listing of candidates through these processes might be based on broad criteria such as rapid and complete fermentation with an absence of undesirable

fault compounds (e.g. acetic acid, ethyl acetate, hydrogen sulfide, volatile phenols) or it may be quite narrow, seeking a specific attribute [25], metabolite(s) [26] or enzymatic activity [27]. In terms of the former, a recent evaluation of 190 isolates from Greek wine regions also included a novel Hierarchical Cluster Analysis of results from five simple phenotypic tests (resistance to killer toxin and SO_2, low H_2S and acetic acid production and β-glucosidase activity) that was applied together with sensory impacts of the strains to help identify the most promising candidates [••16].

Other examples of the efforts to screen large numbers of candidate strains at a small scale are numerous. At one instrumentational extreme, Gutiérrez et al. [28] plated strains onto solidified grape juice and sniffed them after incubation to detect the intensity and general 'pleasantness' of each. By comparison, evaluations have been performed in multi-well plates, a format previously shown to be comparable to shake flasks with fermentation locks [29], and then analysed by precision instruments such as GC-MS [30]. Increasingly, high-throughput methods utilising automation and robotics are helping researchers assess large numbers of candidate strains [31]. Screening, at least at an early stage and for specific attributes, might not even require extensive culture of the candidate yeasts. Several phenotypes have a monogenic basis encoding specific enzymatic capabilities, such as the release of thiols (*IRC7* encoding cysteine-S-β-lyase [32]) and production of phenolic off flavour (*PAD1* encoding phenylacrylic acid decarboxylase [33]). As such, rather than having to utilise growth assays and potentially expensive volatile analyses, it may be easier to screen for or against strains possessing these genes by molecular methods, as is commonly done for other beverage strains [34].

Whatever the attribute and however the data are collected, at some point, evaluations under more wine-like conditions are required. However, with individual groups using different conditions and media, the risk that findings between labs cannot be compared is elevated. As such, efforts to develop a standardised strain evaluation protocol and to confirm the reproducibility of findings between labs are commendable. A consortium of 17 research groups that constitute the Italian Group of Microbiology of Vine and Wine (GMVV) have successfully validated a method for characterisation of *Saccharomyces cerevisiae* strains [••35], showing good agreement between labs for assessment of fermentation performance and production of key metabolites in both a synthetic medium and grape musts. Such a standardised approach will help evaluate future new strains by labs across the many wine regions of the world.

What of sustainability in winemaking?

Wine can be argued to be a luxury. Its production takes up agricultural land, demands resource inputs and creates GHG emissions, while its irresponsible consumption can have serious adverse health effects. However, wine is also a critical element of many societies, cultures and traditions. Grapegrowing, winemaking and allied industries and tourism provide employment, especially in regional locations and have helped hone agricultural and biotechnological processes. Whatever the perception, it is essential that the industry strives for greater sustainability, both economic and environmental.

While estimates vary, a recent review suggests that viticulture and winemaking release between 0.15 and 0.45 kg of CO_2 per 750 ml bottle of wine produced [36]. The largest contributor (40%) to the carbon footprint of winemaking is the electricity used (especially for refrigeration), followed by fugitive emissions of CO_2 (23%), diesel combustion (16%) and chemicals used in winemaking (10%) [37]. Therefore, if wine *must* be made, it is imperative that it is done as efficiently as possible to yield the least environmental impact and the highest quality product that provides the greatest economic return to the producer. Several of the yeasts referred to in Table 1 offer reliable fermentation with desirable sensory contributions. As such, they will afford one or more of the following benefits: increased winery throughput, reduced demand for electricity, nutrient or chemical additions, less wine quality erosion or waste and thereby increased profitability. But beyond mere process efficiency and sensory complexity, yeasts that offer specific sustainability gains are also being sought. Fining for protein stability is just one example of this.

Hazes due to unstable pathogenesis-related (PR) proteins in white wines are a constant threat. The additions of bentonite fining agents that are commonly made to avoid such hazes result in wine quality degradation and volume losses (3–10%) estimated at USD 1 billion per year, with spent bentonite representing a disposal issue [38]. Moreover, protein instability may be getting worse due to climate change. Higher growing temperatures, reduced rainfall and increased attack of vines by fungal pathogens raise the level of PR proteins in grapes [39], mainly the thaumatin-like proteins and chitinases (CHIs) [40]. Consequently, yeasts have been sought to tackle protein instability in various ways, ideally also reducing the need for bentonite and wine losses. Developments span the aims of i) avoiding the accumulation of PR proteins in the grapes, ii) preventing their aggregation and precipitation or iii) removing them from the juice/wine.

As reviewed by Sipiczki [41], several species of *Metschnikowia* inhibit the growth of several microbes including the grapevine pathogen, *Botrytis cinerea*. The mechanisms appear to include iron immobilisation by pulcherrimin production, depletion of nutrients or competition for space on the plant surface for fungal colonisation.

The application of yeast to suppress fungal attack in turn reduces the PR protein content of grapes [42] and thereby the subsequent haze threat and bentonite demand. Whilst such examples are promising, more work is required to match the successes seen for yeast-based bioprotectants in other fruits, especially in a post-harvest/storage context [43,44].

In terms of preventing existing PR proteins from becoming a haze problem that then requires intervention and inputs, several yeasts offer promise. Potentially unstable wine proteins interact with wine components to aggregate and precipitate. Yeast-derived polysaccharides such as mannoproteins compete for these wine components, thereby blocking haze development [•45]. While it has been known for some time that mannoproteins released by *Saccharomyces cerevisiae* can suppress haze formation [46], the amounts released and their effectiveness seem insufficient [47], thereby turning research attention to other species. A screen of *Schizosaccharomyces* spp. strains (Table 1) revealed examples with ~7-fold higher polysaccharide release than the *S. cerevisiae* reference [48,49].

In a final strategy, selected yeasts are being put forward as a means of removing PR proteins, namely CHIs, rather than merely blocking their aggregation. Since CHIs bind chitin, strains whose cell wall is rich in chitin provide a means of binding these PR proteins and removing them from the wine with settling of the yeast. Studies with various *S. cerevisiae*, *S. paradoxus* and hybrid strains rich in cell wall chitin demonstrate their potential for reducing haze formation [50].

Conclusion

Far from being a thing of the past, bioprospecting for superior strains of yeast for winemaking continues to deliver results. It is helped by efforts to seek new strains from outside the heavily surveyed niches and regions of the world. As a complement to this is the fact that existing culture collections are many and significant in size with often readily searchable databases and a growing likelihood that strain identities and phenotypic information have been reviewed and updated. Strains nominally isolated from or used in other fermentation processes should not be overlooked either as they represent a pool of strains likely to already be adapted a fermentation processes. While improved fermentation efficiency and enhancement of wine quality will likely deliver wine production with fewer carbon emissions, reduced chemical use and greater value, other sustainability targets are also being sought. For example, various strategies for preventing or reducing protein haze formation in white wines will reduce the cost, losses and environmental impact associated with managing this risk with bentonite. Whatever the application, efficient and specific screening methods will greatly assist in producing a shortlist of promising candidates, which will in turn need to be evaluated under conditions as close to the intended application context as possible. Further exploitation of promising strains will of course need to be undertaken with due consideration of the Nagoya Protocol (www.cbd.int/abs), but a key advantage of bioprospected strains, unless modified, will be that they are not subject to restrictions imposed on genetically modified strains.

Author contribution

This review is entirely the work of the author.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The author declares no conflict of interest.

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