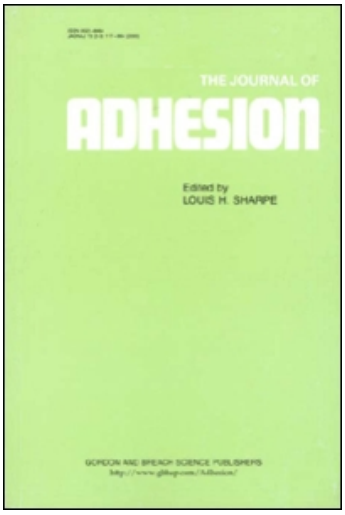

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Screening Microalgal Cultures in Search of Microbial Exopolysaccharides with Potential as Adhesives

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Nearly 800 cultures from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Collection of Living Microalgae (CCLM) were screened for exopolysaccharide (EPS) production by examining the viscosity of conditioned media supernatants. We first established dose-response relationships for the viscosity of reference polysaccharides dissolved in microalgal growth media. Then, using the 40 most viscous CCLM cultures, we confirmed that the viscosity of culture supernatants correlated well with their total sugar (and thus EPS) content. The conditioned medium with the highest viscosity (6.55 cP, equivalent to 1.16 g/L xanthan gum) was produced by a non-axenic isolate of the cyanobacterium Microcystis aeruginosa *f.* flos-aquae*. Two types of bacteria, designated CSIRO501 (Gram-positive) and CSIRO505 (Gram-negative), were subcultured from it. At 20 dry wt% sugar, an exopolymer preparation from CSIRO505 contained substantially more EPS than its counterparts from CSIRO501 or from axenic* Microcystis *cultures, and it resembled a well-known bacterial EPS (xanthan gum) in being much more effective at bonding wood than PVC. It bonded wooden lap joints with a dry shear strength of 1.5 MPa, four times the value obtained in equivalent tests of a commercial polyvinyl acetate (PVA) glue.*

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INTRODUCTION

Microalgae and bacteria living in aquatic ecosystems commonly secrete extracellular polymeric substances. These can be formed as capsular material that closely surrounds the producing microbial cell, or material that is released more widely into the surrounding environment as a dispersed slime [1–3]. The production of this extracellular material requires a significant metabolic investment, but the ecological advantage afforded by the process offsets the energy cost to the organism [4]. A large proportion (40–95%) of this polymeric material is exopolysaccharide (EPS), but it may also include proteins, nucleic acids, and lipids [5].

In dilute solutions, such as occur in natural marine systems, surfaces absorb and concentrate dissolved organics [6], including EPS. EPS plays an important role in cellular attachment and adhesion to surfaces, increasing survival compared with growth in an unattached state [7,8]. It forms a highly hydrated matrix [9], which provides a layer of protection to cells against toxic compounds [10,11] or against digestion by other organisms [12]. EPS may also prevent cellular desiccation [13–15] or damaging ice-crystal formation [16]. Thus, EPS forms the architectural network of biofilms and aggregates, protecting cells and facilitating intercellular interactions [1,17].

In laboratory cultures, microalgae and bacteria generally produce the largest quantity of EPS during stationary phase when a nutrient such as nitrogen is limiting growth [1,2,18]. Released EPS usually increases the viscosity of the growth media [19–21], and can be recovered from the culture broth with relative ease compared with the isolation of attached capsular EPS [22,23].

There has been a growing interest in the isolation and identification of new microbial polysaccharides with biotechnological potential. Polysaccharides produced by microorganisms have a range of uses as adhesive, gelling, flocculant, and thickening agents in the glue, oil, paper, paint, textile, cosmetic, food, and beverage industries. A “green” (*i.e.*, environmentally friendly) adhesive based on a microbial polysaccharide that has a high shear strength on aluminium, epoxy glass, and wood has been developed by Montana Biotech (Rock Hill, SC, USA) [24]. High-value applications for EPS exist in the biomedical field [25–28], where their uses include pharmaceutical formulation, cancer therapy, drug delivery, and bone healing. Topical applications of EPS include wound dressings for patients with burns, chronic ulcers, or extensive tissue loss.

Microalgae are unicellular photosynthetic organisms. The Commonwealth Scienctific and Industrial Research Organisation (CSIRO) Collection of Living Microalgae (CCLM, http://www.cmar.csiro.au/microalgae/), is housed in the Hobart, Australia, Laboratories of CSIRO Marine and Atmospheric Research and is the largest collection of its kind in Australasia [29]. It consists of a living bank of more than 800 unialgal strains, and represents most phyla/classes of marine microalgae and some freshwater species. While most microalgal species in the CCLM are eukaryotes, the collection includes the non-eukaryotic cyanobacteria (photosynthetic members of the domain Bacteria informally known as "blue-green algae") in its scope. In addition, non-axenic microalgal strains contain resident populations of bacteria which are usually not photosynthetic. The majority of the CCLM strains have been isolated from Australian waters, while other strains have been sourced from the Antarctic and Southern Ocean Environment, European, South Pacific, North Pacific, Atlantic, and Asian waters. The CCLM is, therefore, a vast resource of biodiversity and a potential source of new and interesting EPS, some of which may be suitable for commercial exploitation.

This communication presents the results of a survey in which CCLM cultures were screened for EPS production by measuring the viscosity of conditioned growth media. Rheometry has been used to assess the potential presence of microbial EPS in laboratory cultures [20,30,31] as well as in industrial-scale fermentation processes [32,33]. In addition, several studies have described viscosity-based screens for selection of EPS-producing isolates [34–36]. To our knowledge, rheometry has not previously been used as a tool to rapidly assess EPS production in large microalgal strain libraries, so some validation was required. First, the rheological behaviour of reference polysaccharides in culture growth media was investigated. Second, since the phenol/sulphuric acid assay for sugars [37] can be used to quantify the EPS content of liquid microbial cultures [38–40], we compared the results of this assay with viscosity measurements for the most viscous CCLM cultures. We also assayed protein content and compared it with viscosity. The culture CS 566/01, identified as the alga *Microcystis aeruginosa* f. *flos-aquae* (Wittrock) Kirchner 1898, had the highest viscosity in the survey. This non-axenic algal culture had a resident population of bacteria, and two such strains were isolated from it. Since (amongst other things) we are seeking EPS that might serve as new commodity adhesives, crude preparations of the EPS from these isolates were tested for dry shear strength on relevant substrata. The adhesion results were compared with those from reference adhesives and related to the EPS and protein content of the preparations.

MATERIALS AND METHODS

Sampling of the CCLM

CCLM cultures were maintained on a variety of liquid media; most commonly, MLA based on Milli-Q^{TM} water [41] (Millipore Australia Pty., Ltd., North Ryde NSW, Australia) or f/2 media based on sea water [42]. Additional media types were also used for growth of microalgal strains. They included GSe [43,44], fE/2, and f [42] in modified form [45]. Both fE/2 and f are natural seawater-based media; GSe contains seawater and Milli-Q water in a 4:1 ratio by volume.

Two rounds of sampling CCLM were undertaken for this study. In the first round, at the time of routine inoculation of CCLM cultures into fresh media, 1.5 mL aliquots of cultures were aseptically removed from 767 parent cultures into sterile 1.5 mL plastic tubes (Eppendorf South Pacific Pty. Ltd., North Ryde, NSW, Australia). At this point, the cultures were mostly in the stationary phase, with culture age (*i.e.*, time since last inoculation) typically as follows: cyanobacteria, 12–14 weeks; thraustochytrids, 1–2 weeks; eukaryotic unicellular algae, 6–7 weeks. Aliquots were maintained under normal growth conditions until the time of analysis, which was performed within 1 week of sampling.

Several months later, the CCLM was revisited for a second round of sampling which focussed on the 40 isolates found in the first round to produce the most viscous EPS. From this set of 40 cultures, which were descended from those sampled in the first survey, 10 mL aliquots were removed for viscosity, total sugar, and protein analyses. The ages of the 40 cultures (*i.e.*, time elapsed since last inoculation) for this subsampling were as follows: cyanobacteria, 12–14 weeks; eukaryotic unicellular algae, 10–13 weeks. The latter cultures were, therefore, ~4 weeks older in the second sampling round than earlier generations of the corresponding cultures had been in the first round. Aliquots were maintained as described previously until the time of analysis.

For analysis, samples from both rounds of the survey were centrifuged for 10 min, 450 g, at 20°C (Sorvall RT6000, DuPont, Wilmington, DE, USA) and subjected to viscosity measurements and biochemical analyses as described below.

Rheometry

Three commercially available polysaccharides were used to prepare standard curves for viscosity measurements: dextran sulfate sodium salt (500,000 Da, cat. no. D8906, Sigma-Aldrich St. Louis, MO, USA),

dextran sulfate sodium salt (5000 Da, cat. no. 31404, Fluka, Buchs, Switzerland), and xanthan gum (ZNARO6003, Kelzan AR, Merck & Co. Inc., Chicago, IL, USA). Polysaccharides (10 mg mL) were dissolved in ultra-clean water (Milli-Q, Millipore Australia Pty.), MLA, or f/2 media. An aliquot was removed for measurement of pH. Solutions were diluted with Milli-Q water to obtain test solutions for standard curves, with polysaccharide concentrations ranging 0–10 g/L for dextran sulfate and 0–2 g/L for xanthan gum.

To measure viscosity, a 1 mL aliquot of sample (reference polysaccharide solution or CCLM culture supernatant) was placed in the 1 mL sample cup of a Brookfield cone and plate microviscometer (cone spindle CPE-42, gap 0.0005 inch, Model LVT, Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) connected to a recycling water bath which maintained the samples at 25°C during measurement. Samples were allowed to equilibrate for 2 min; the viscosity was then measured three times at 30 rpm, once after each of 3 sets of 10 revolutions, and mean values calculated. The cup and cone were rinsed with 70% (v/v) ethanol in water solution between samples and wiped clean/dry.

Biochemical Composition

After viscosity measurement, culture supernatants (approximately 10 mL) were supplemented with NaN_3 (final concentration 0.4 mg/mL, mL, Ajax Chemical Company, Sydney, NSW, Australia) to prevent bacterial spoilage, and stored at 4°C for up to 1 week. Samples were analysed for total sugars [37,46] in triplicate. For each reaction, 600 μL of concentrated sulfuric acid (Pronalys, May & Baker, Melbourne, VIC, Australia) was added to 200 μL of sample in a borosilicate glass test tube, followed rapidly by 120 μL of 5% (w/v) phenol (Sigma-Aldrich Chemical Company, Milwaukee, WI, USA) solution in water. Samples were mixed thoroughly and left to cool at room temperature for 30 min, then transferred to a 1 mL disposable plastic cuvette. Absorbance at 490 nm was measured using a Bio-Rad SmartSpec™ 3000 spectrophotometer, with Milli-Q water as blank. Unconditioned (*i.e.*, fresh sterile) culture media were also tested as negative controls, since none of the media contained added sugars. The total sugar content of each sample was interpolated from a standard curve for D-glucose (BDH Chemicals Ltd., Poole, UK) in Milli-Q water, in the range 0–225 μg/mL.

Samples of supernatants from microalgal cultures prepared as above were stored at 4°C for up to 2 weeks after viscosity measurement and analysed for protein using a Pierce Coomassie Plus Assay

Kit (Rockford, IL, USA). Again, Milli-Q water was used as a blank and unconditioned culture media were tested as negative controls. The protein content of each sample was interpolated from a standard curve for bovine serum albumin (Sigma-Aldrich Chemical Company, St. Louis, MO, USA) in Milli-Q water, in the range 0–25 μg/mL.

Isolation of Bacteria from Non-Axenic CCLM Strain CS-564/01

Simple Medium was prepared in Milli-Q water and contained 3 g/L peptone (Acumedia Manufacturers, Inc., Baltimore, MD, USA), 1 g/L yeast extract (Oxoid Ltd., Hampshire, UK), and 15 g/L agar (Difco Bacto Laboratories, Pty., Ltd., Liverpool, NSW, Australia). Simple Medium with added glucose (SM+Glc) included 30 g/L glucose (BDH Chemicals Australia Pty., Ltd., Kilsyth, VIC, Australia), which was autoclaved separately as a 30% (w/v) stock solution and added prior to pouring plates.

To isolate bacteria resident in CS-564/01, a 100 μL aliquot of this culture was spread on a plate of SM or SM+Glc. Plates were incubated at 20°C for one week. Individual colonies were repeatedly subcultured by streaking until only one colony morphology was present on each plate. Gram staining (ProSciTech, Kirwan, QLD, Australia) was used for initial characterization of bacterial isolates. Bacteria were examined by phase-contrast at 1000 × magnification using a Zeiss Axioplan microscope (Carl Zeiss Pty. Ltd., North Ryde, NSW, Australia).

Production and Enrichment of Bacterial EPS

The two bacterial species isolated from CS-564/01 were subcultured onto SM+Glc and incubated at 20°C for 3 weeks. For each culture, mucoid growth was scraped from the agar plate with a sterile metal spatula. This material (comprising bacterial cells and their extracellular secretions) was placed in a sterile plastic 15 mL centrifuge tube. A 1 mL aliquot of aqueous NaN_3 (50 mg/mL) was added and the mixture incubated at 25°C for 2 hr to kill the bacteria. An additional 10 mL Milli-Q water was added and the tubes were centrifuged (2000 g, model 5810, Eppendorf, Hamburg, Germany) for 10 min at 20°C to pellet cells and other particulate matter. The supernatant was then centrifuged at 4000 g for 1 hr at 20°C in a 15 mL Amicon Ultrafiltration device (30,000 Da molecular weight cut-off PES; Millipore, North Ryde, NSW, Australia). The retentate (a concentrate of secreted bacterial components of high molecular mass) was freeze-dried and weighed.

Production and Enrichment of Microalgal EPS

Axenic cyanobacterial strains CS-566/01-A01 and CS-566/01-A15 (both *Microcystis* f *pseudofilamentosa*), and CS-591/04-A12 and CS-588/02-A11 (both *Nodularia spumigena*), were each used as inocula for 2×20 mL of MLA medium. After 2 weeks incubation at 20°C and light intensity of 530 lux, these cultures were used to inoculate 2×250 mL of MLA medium. After a further 2 weeks under the same growth conditions, each 250 mL culture was used to inoculate 1800 mL of MLA medium in a 2 L Schott bottle. The bottles were fitted with screw cap lids through which air was bubbled into the medium *via* sterile 0.2 μm air filters (Midisart 2000, Sartorius Stedmin Australia Pty. Ltd., East Oakleigh, VIC, Australia). A second air filter was fitted to the exit line to prevent culture contamination.

After 6 weeks, the growth of the axenic cyanobacterial strains was stopped by the addition of 0.4 g/L NaN_3. EPS was purified according to the methods of Richert *et al.* [47]. The cultures were centrifuged at 7000 g for 1.5 hr at 20°C to remove cells and particulate material (Sorvall, K.I. Scientific Pty. Ltd., North Ryde, NSW, Australia). The viscous supernatants were filtered under air pressure (stainless steel pressure filtration unit, cat. no. 16249, Sartorius Stedmin Australia Pty. Ltd., East Oakleigh, VIC, Australia) first through a glass fiber filter (GF/C, Whatman International Ltd., Maidstone, UK) to remove any remaining cellular material. Filtration was repeated through a 0.45 μm cellulose nitrate filter (Sartorius Australia, Pty. Ltd.). The 2 L filtrates were then subjected to diafiltration against MilliQ water (30,000 Da molecular weight cut-off, PES, Vivaflow 200, Sartorius Australia, Pty. Ltd.) to remove media components and small molecular weight contaminants and then ultrafiltration to concentrate the polysaccharide containing solutions. The retentates (each a concentrate of secreted cyanobacterial components of high molecular mass) were freeze-dried and weighed.

Gel Permeation Chromatography

Selected exopolymer preparations were analysed by gel permeation chromatography (GPC) using a TSK-Gel 5000PW column (Tosohas, Montgomeryville, PA, USA) in a LC20 liquid chromatography system (Shimadzu, Kyoto, Japan) with a flow rate of 0.5 mL/min (void peak at 8.5 min, retained peaks commencing at 10 min). Samples were dissolved and run in 50 mM ammonium bicarbonate (99% purity, w/w). Where necessary, samples were heated to 50°C and/or sonicated before loading to maximise the amount of dissolved material. Visibly

undissolved material was removed by centrifugation, and all samples were passed through a 0.45 μm nylon filter before injection onto the column. In-line monitoring of column eluate was by absorbance at 280 nm (aromatic amino acid residues) and 190 nm (most chemical groups), or by evaporative light scattering detection. The column was calibrated using pullulan standards of weight-averaged molecular mass (M_w) 788,000, 404,000, 212,000, 112,000, 47,300, 22,800, 11,800, and 5,900 Da (Shodex P-82). For these the polydispersity index (M_w/M_n) was 1.1 ± 0.03, except for the largest standard, for which it was 1.23.

Adhesive Testing

Milli-Q water was added to the freeze-dried preparations of bacterial and cyanobacterial high molecular mass material to yield a final concentration of 20% (w/w) solids. Each mixture was then homogenised with a plastic pestle (Eppendorf, Hamburg, Germany) to form a thick opaque paste.

Reference materials were also prepared for adhesion tests. Xanthan gum (Fluka, Buchs, Switzerland) was prepared as a 20% (w/v) solution in Milli-Q water. A 1 mL aliquot of a domestic polyvinyl acetate adhesive, PVA Craft Glue™ (Selleys, Melbourne, Australia), was freeze-dried and weighed to determine its solids content. A 500 μL aliquot of the Selleys Craft Glue was then diluted to 20% solids (w/v) in Milli-Q water. Approximately 50 μL of each 20% (w/v) preparation was freeze-dried and used to prepare a 1 mg/mL solution in Milli-Q water for total sugars and protein analysis, as described above.

Lap-joints of wood and plastic were subjected to destructive testing in order to establish dry shear strength. ASTM specifications were followed as far as possible. However, American oak veneer proved unsuitable as a wooden substratum due to buckling of the test pieces, so medical tongue depressors made of maple wood were used instead. Lap-joints were created as described previously for "coating tests" [48]. To define the area for overlap (2.66 cm^2), a circle 1.8 cm in diameter was marked in pencil on one end of a tongue depressor (thickness 1 mm, Livingstone Int. Pty., Ltd., Rosebery, Australia) using the rounded end of a second tongue depressor. ASTM Designation D3163-01 was followed for plastic lap joints. Strips (2.54 cm wide × 10.16 cm long) were cut from polyvinyl chloride (PVC) report covers (thickness: 200 μm, Cat. no BCP20CLR200, Ibico, Australia) that had been cleaned with a 70% ethanol in water solution (v/v). A line was drawn 1.27 cm from the end of the plastic strips with a permanent-ink marker to define the area of overlap (3.23 cm^2).

Paired adherends were laid side-by-side on a flat aluminium tray. Since the high viscosity of most of the samples precluded delivery by pipette, a 50 μL drop of 20% (w/v) adhesive preparation was applied to the center of the marked end using a 1 mL syringe (Terumo Corp., Binan, Philippines) and spread over the area to be bonded. The complementary test piece was then positioned so that it overlapped the marked area and formed a lap joint. A stainless steel bar was laid on top of the joint to apply pressure of 0.2 g/mm^2 for 2 hr. When the weight was removed, the joints were cured for $\geq$1 week in a sealed plastic box at 23% relative humidity, achieved by including an open beaker containing 250 mL of saturated potassium acetate solution (Chem Supply, Gillam, SA, Australia). Shear strength testing was performed using a Hounsfield tensile tester (Model H50KM, Hounsfield Test Equipment Ltd., Surrey, UK), fitted with an 800 N load cell, at a cross-head speed of 10 mm/min. At least three tests were carried out for each adhesive on each test material, except where the joints were too weak to test (see Results).

RESULTS

Viscosity of Reference Polysaccharide Solutions

The relationship between viscosity and concentration for dextran sulfate and xanthan gum dissolved in water and microalgal growth media is shown in Table 1. The smaller dextran sulfate polysaccharide (5,000 Da) had almost no effect on viscosity, whereas viscosity increased in direct proportion to the concentration of the larger polysaccharide (500,000 Da) and of xanthan gum. The highest viscosities were obtained in Milli-Q water and the lowest in f/2 medium, with intermediate values seen in MLA medium.

Screening the CCLM

A total of 767 unialgal strains, representing more than 220 species, was surveyed. This included more than 176 species in over 113 genera, representing 19 different taxonomic classes (Figs. 1 and 2). The supernatants of about 110 strains ($\sim$14% of the total) had a viscosity significantly greater than culture medium alone (Fig. 2). Of the 40 strains with the highest viscosities (Fig. 2 and Table 2), over half (23 strains) belonged to the Cyanobacteria (blue-green algae), the only non-eukaryotic class in the CCLM. Five belonged to the Dinophyceae (dinoflagellates) and three to the haptophyte class, Prymnesiophyceae. There were four strains belonging to the Bacillariophyceae, two centric and two pennate diatoms. The green algal classes Chlorophyceae

TABLE 1 Viscosity Calibration Data

	Viscosity[a] (cP) at 25°C		
Conc. (mg/mL)	MilliQ water	f/2 medium	MLA medium
Dextran sulfate 5000 Da			
0	0.86	0.84	1.09
2500	0.88	0.95	0.87
5000	0.96	1.02	1.08
10000	0.92	1.09	1.04
Dextran sulfate 500,000 Da			
0	0.86	0.84	1.09
2500	4.12	1.05	1.97
5000	5.35	1.19	3.12
10000	7.76	1.54	4.41
Xanthan gum			
0	0.91	1.08	1.09
50	1.59	1.51	0.98
200	2.94	1.71	1.53
400	4.32	2.42	2.37
500	4.86	2.97	2.99
600	5.31	3.46	3.59
800	6.64	4.85	4.48

[a] $n = 3$–6, standard error $\leq 3.6\%$ of mean.

and Prasinophyceae each had two representatives in the 40 strains with the highest viscosities measured in this survey (Table 2).

In the first round of sampling, viscosities in this set of 40 culture supernatants ranged from 1.55 to 6.55 cP (Table 2). Standard curves generated from the data in Table 1 were used to convert culture viscosities to xanthan gum concentrations in the relevant growth medium (f/2, MLA), with the f/2 calibration used as an approximation for GSe cultures as both media are based on seawater. These concentrations ranged from 1155 mg/L xanthan equivalents for CS-564/01 (whose algal component is known to be *Microcystis aeruginosa* f. *flos-aquae*) to 100 mg/L xanthan equivalents for CS-866 (an unidentified prymnesiophyte). Table 2 lists the xanthan equivalents calculated for the 40 most viscous culture supernatants.

Approximately 14% of the microalgal strains analysed (106 of 767 cultures) were designated as axenic (*i.e.*, containing no bacteria culturable on SM agar plates). Of the cultures producing the 40 most viscous supernatants, 6 (15%) were designated as axenic, 28 (70%) were designated as non-axenic (*i.e.*, bacteria present in the culture), and 6 (15%) were of unknown status (Table 2).

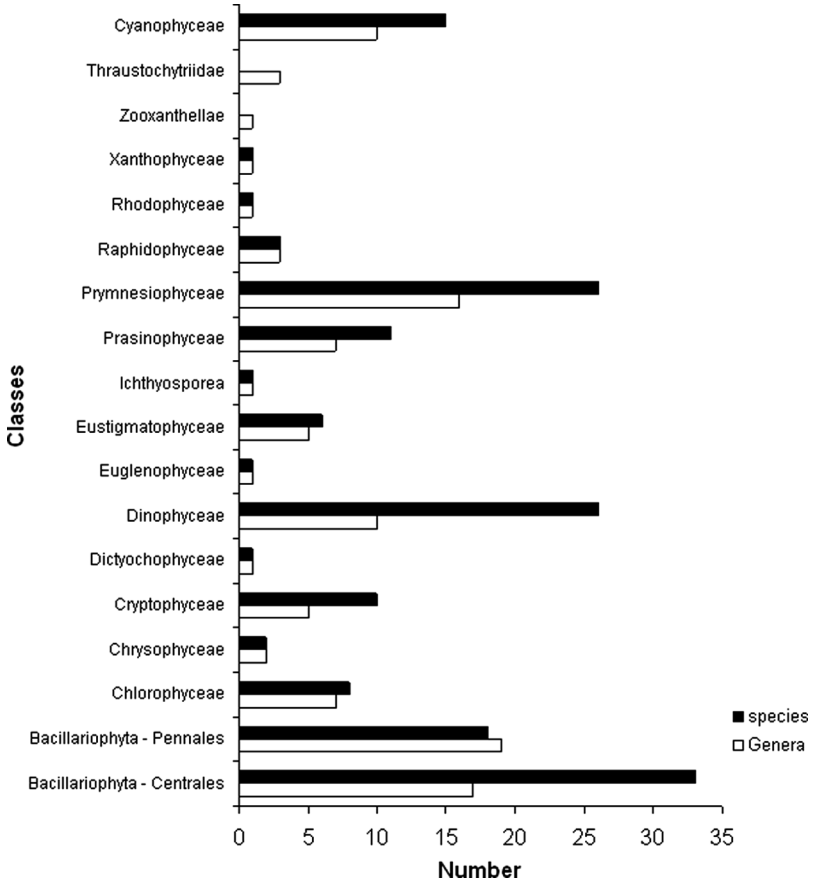

FIGURE 1 Taxonomic breakdown of microalgal strains surveyed for viscosity of parent culture broth after centrifugation. Abundances of genera and species in each class are represented by white or black bars, respectively. All groups belong to the Eukaryota domain except the Cyanophyceae, which are cyanobacteria from the domain Bacteria.

A subsequent generation of the 40 strains determined to have the highest viscosities in the original survey were subsampled, and the culture supernatants were measured for viscosity and total sugar content. The former ranged up to ~4 cP while the latter ranged from 0 to 300 μg/mL glucose equivalents. The viscosity of each supernatant was plotted against its sugar content (Fig. 3).

The protein content of the second-round supernatants ranged from 0 to 30 μg/mL (data not shown). Protein content was generally an order

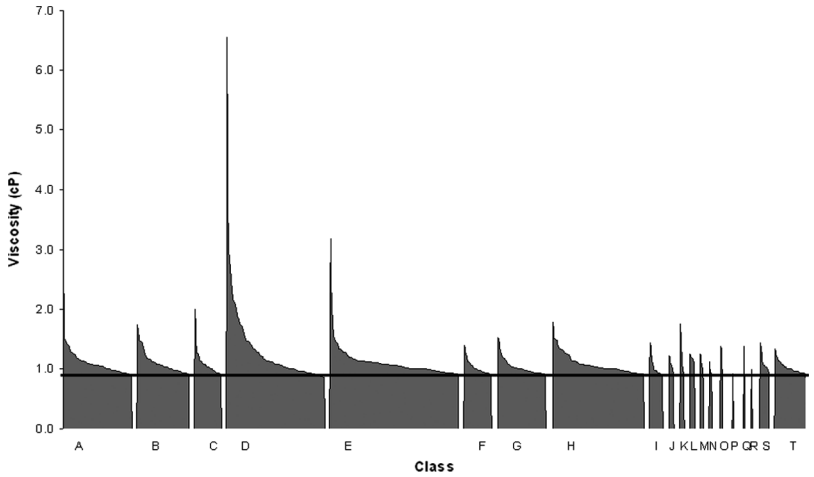

FIGURE 2 Summary of viscosity data according to classes analysed in a survey of parent cultures from CSIRO Collection of Living Microalgae. Viscosity (at 25°C) of parent culture broth was measured after centrifugation at 450 g for 10 min at 20°C. Horizontal black line represents the background viscosity of the culture media. Letters represent classes as follows: A: Bacillariophyta–Centrales, B: Bacillariophyta – Pennales, C: Chlorophyceae, D: Cyanophyceae, E: Dinophyceae, F: Eustigmatophyceae, G: Prasinophyceae, H: Prymnesiophyceae, I: Cryptophyceae, J: Raphidophyceae, K: Rhodophyceae, L: Thraustochytriidae, M: Zooxanthellae, N: Chrysophyceae, O: Dictyochophyceae, P: Euglenophyceae, Q: Ichthyosporea, R: Xanthophyceae, S: Mixed algae, and T: Unidentified. All groups belong to the Eukaryota domain except the Cyanophyceae, which are cyanobacteria from the domain Bacteria.

of magnitude lower than the corresponding sugar content, and plots of viscosity against protein content revealed a poor correlation between the two data-sets ($R^2 = 0.243$; data not shown).

Bacterial and Cyanobacterial EPS Preparations

Efforts to isolate bacteria from cyanobacterial strain CS-564/01, whose algal component was known to be *Microcystis aeruginosa* f. *flos-aquae*, yielded two strains; other strains may also have been present, but were not pursued. Both of the bacterial isolates showed enhanced mucoid morphology when grown on solid media with added glucose (SM + Glc). One isolate, which grew as orange colonies containing Gram-positive rods of approximately $1.0 \times 0.4\,\mu m$, was designated CSIRO501; the other, which grew as off-white colonies containing Gram-negative rods of approximately $1.4 \times 0.4\,\mu m$, was

TABLE 2 Details for Cultures from the CSIRO Collection of Living Microalgae Ranked in Order of Decreasing Viscosity of Parent Culture from First Round of Sampling

Strain designation	Species	Phylum/ class	Axenic	Source environment	Growth media	Age at first round of sampling (wk)	Viscosity of supernatant (first round) (cP)	Equivalent concentration of Xanthan gum (mg/L)	Age at second round of sampling (wk)	Viscosity of supernatant (second round) (cP)
CS-564/01	*Microcystis aeruginosa* f. *flos-aquae*	Cyanophyceae	No	Sewage treatment plant, Tas, Aus	MLA	12	6.55	1155	14	3.99
CS-877	*Gymnodinium dorsalisulcum*	Dinophyceae	No	Broome, WA, Aus	f/2	7	3.18	536	13	1.08
CS-538/01	*Anabaena circinalis*	Cyanophyceae	No	Murrumbidgee River, NSW, Aus	MLA	14	3.17	528	14	2.43
CS-31	*Stephanopyxis turris*	Bacillariophyta – Centrales	No	La Jolla, CA, USA	GSe	6	3.01	501	12	0.96
CS-567/02	*Microcystis aeruginosa* f. *sphaerodictoides*	Cyanophyceae	No	Gippsland Lakes, Vic, Aus	MLA	12	2.94	490	14	2.99
CS-566/ 01-A15	*Microcystis* f. *pseudofilamentosa*	Cyanophyceae	Yes	Sewage treatment plant, Vic, Aus	MLA	12	2.80	466	14	3.14
CS-566/ 01-A01	*Microcystis* f. *pseudofilamentosa*	Cyanophyceae	Yes	Sewage treatment plant, Vic, Aus	MLA	12	2.73	455	14	2.84
CS-591/ 04-A12	*Nodularia spumigena*	Cyanophyceae	Yes	Orielton Lagoon, Tas, Aus	MLA	12	2.41	400	14	1.24
CS-338	*Microcystis aeruginosa*	Cyanophyceae	No	Burrunjuck Dam, NSW, Aus	MLA	6	2.29	381	12	0.99
CS-292/01	*Prorocentrum mexicanum*	Dinophyceae	No	Wilson Inlet, WA, Aus	GSe	6	2.28	346	12	2.06
CS-554/01	*Microcystis aeruginosa* f. *aeruginosa*	Cyanophyceae	No	Irrigation Canal, Griffith, NSW, Aus	MLA	12	2.21	368	14	2.84
CS-537/02	*Anabaena circinalis*	Cyanophyceae	No	Mt Bold Reservoir, SA, Aus	MLA	14	2.15	358	14	2.14
CS-537/01	*Anabaena circinalis*	Cyanophyceae	No	Mt Bold Reservoir,	MLA	14	2.10	350	14	1.00

(*Continued*)

TABLE 2 Continued

Strain designation	Species	Phylum/ class	Axenic	Source environment	Growth media	Age at first round of sampling (wk)	Viscosity of supernatant (first round) (cP)	Equivalent concentration of Xanthan gum (mg/L)	Age at second round of sampling (wk)	Viscosity of supernatant (second round) (cP)
				SA, Aus						
CS-588/ 02-A11	*Nodularia spumigena*	Cyanophyceae	Yes	Gippsland Lakes, Vic, Aus	MLA	12	2.08	347	14	1.25
Mar.Nod. (ex151)	*Nodularia sp.*	Cyanophyte	No	Unknown	f	6	2.03	300	13	1.00
33,WALDe CDun	Dunaliella-like	Chlorophyceae	No	Saline Lake, WA, Aus	f	6	2.01	300	13	1.14
CS-788/13	*Protoceratium reticulatum*	Dinophyceae	No	CSIRO wharf, Derwent Estuary, Tas, Aus	GSe	6	1.99	262	10	0.97
CS-545/17	*Anabaena circinalis*	Cyanophyceae	No	Craigbourne Dam, Tas, Aus	MLA	14	1.95	260	14	1.45
CS-576	*Microcystis viridis*	Cyanophyceae	No	Lake Kasumigaura, Ibaraki, Japan	MLA	12	1.85	247	14	1.93
CS-531/01	*Anabaenopsis cf. tanganyikae*	Cyanophyceae	No	Blackwood River, WA, Aus	MLA	14	1.85	247	14	1.13
CS-537/03	*Anabaena circinalis*	Cyanophyceae	No	Mt Bold Reservoir, SA, Aus	MLA	14	1.79	238	14	1.52
CS-457	*Prymnesiophyte* sp.	Prymnesiophy- ceae	No	Exmouth Gulf, WA, Aus	f/2	6	1.79	211	12	0.99
CS-25	*Porphyridium purpureum*	Rhodophyceae	Yes	Halifax, Nova Scotia, Canada	f/2	6	1.76	207	12	1.29
CS-542/02	*Anabaena* sp.	Cyanophyceae	No	Orielton Lagoon, Tas, Aus	MLA	14	1.75	233	14	1.67
GRA 2 Amphora	Amphora-like	Bacillariophyte	No	Geordy River, Tas, Aus	f	6	1.75	205	13	1.13

CS-337/02	*Anabaena circinalis*	Cyanophyceae	No	Burrinjuck Dam, NSW, Aus	MLA	6	1.73	230	14	1.65
CS-323/01	*Alexandrium minutum*	Dinophyceae	No	Port River, SA, Aus	GSe	6	1.72	202	10	1.24
CS-512/04	*Microcystis* sp.	Cyanophyceae	No	Swan River, WA, Aus	MLA	12	1.71	228	14	2.69
CS-633	*Dunaliella* sp.	Chlorophyceae	No	Burton Lake, Antarctica	fE/2	6	1.69	200	14	0.97
CS-537/05	*Anabaena circinalis*	Cyanophyceae	No	Mt Bold Reservoir, SA, Aus	MLA	14	1.69	225	14	1.52
CS-337/01	*Anabaena circinalis*	Cyanophyceae	No	Burrinjuck Dam, NSW, Aus	MLA	6	1.65	220	14	1.10
CS-780	*Navicula* cf *jeffreyi type*	Bacillariophyta - Pennales	No	St Helens, Tas, Aus	f/2	4	1.57	146	5	1.22
CS-554/ 01-A01	*Microcystis aeruginosa* f. *aeruginosa*	Cyanophyceae	Yes	Irrigation Canal, Griffith, NSW, Aus	MLA	12	1.56	208	14	0.92
40, WALT Oscill	Oscillatoria-like	Cyanophyte	No	Saline Lake, WA, Aus	f	6	1.53	102	13	1.24
CS-347	*Skeletonema name pending*	Bacillariophyta - Centrales	No	Gulf of Carpentaria, Aus	f/2	6	1.53	102	10	0.96
CS-324/13	*Alexandrium minutum*	Dinophyceae	No	Port River, SA, Aus	GSe	6	1.53	102	10	1.04
CS-317	*Tetraselmis* sp.	Prasinophyceae	No	Moreton Bay Prawn Farm, Cleveland, Qld, Aus	f/2	6	1.52	101	12	1.00
CS-749	Nephroselmis-like	Prasinophyceae	No	Derwent River, Tas, Aus	GSe	4	1.51	101	5	0.95
CS-438/5	*Prymnesium* sp.	Prymnesiophy-ceae	No	Serpentine River, WA, Aus	GSe	6	1.51	101	12	0.97
CS-866	Prymnesiophyte unknown	Prymnesiophy-ceae	No	Dunalley, Tas, Aus	GSe	12	1.50	100	12	1.20

designated CSIRO505. For each species, biomass was harvested from culture plates and the exudates were processed to yield preparations of high molecular mass components.

The cyanobacterium of CS-564/01 was not isolated to purity for axenic culture. Instead, since two of the axenic cyanobacterial strains listed in Table 2 (CS-566/01-A01 and CS-566/01-A15) were *Microcystis* species closely related or identical to the cyanobacterial component of CS-564/01 (see Discussion), these were grown separately in liquid cultures. Purification of high molecular mass components from 4 L of conditioned media for each strain yielded 134 and 233 mg of solids, respectively.

To investigate the heterogeneity and size range of macromolecules present in our EPS preparations, we performed Gel Permeation Chromatography (GPC) on selected samples. Since axenic cyanobacterial strains contained only one microbial species, we expected them to contain the simplest and best defined EPS populations. Accordingly, high molecular mass preparations from the four top-ranking axenic microalgal strains in Table 2 (CS-566/01-A01, CS-566/01-A15, CS-591/04-A12, and CS-588/02-A11) were subjected to GPC (Figs. 4a–d). The resulting chromatograms were relatively complex, with proteins (*i.e.*, coincident A_{280} and A_{190} peaks) eluting mainly between 17 and 21 min, and non-protein macromolecules such as EPS (*i.e.*, A_{190} peaks with low or no A_{280} signals) conspicuous in the void and early included volume (9–12 min) of some chromatograms. The

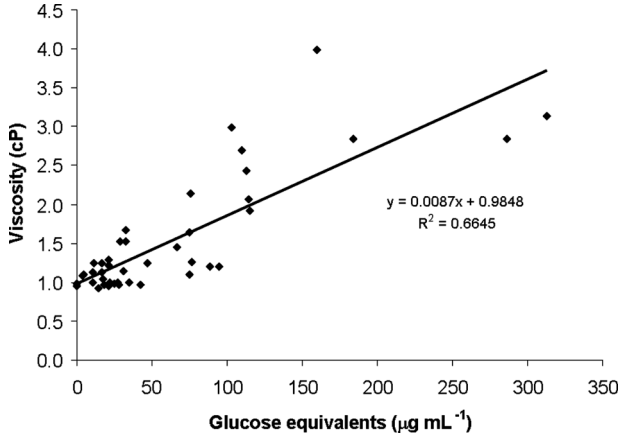

FIGURE 3 Viscosity (at 25°C) *versus* total sugars (expressed as glucose equivalents) for 40 culture supernatants from the CSIRO Collection of Living Microalgae.

monodisperse A_{280}-only peak at ~21 min in most of the chromatograms probably reflects solutes of low molecular mass present in the inclusion volume. The chromatograms for the CS-566/01 preparations, which both come from *M. pseudofilamentosa* strains, were similar in profile and in peak heights (Figs. 4a,b). The void and early included volumes of both contained substantial EPS peaks, with asymmetry (leading edge shoulders) suggestive of incomplete solubilization despite the samples having been sonicated. Calibration of the column with size standards (Fig. 4c, inset) indicated that linear polysaccharides excluded from this column would be >800,000 Da; branched ones would, of course, be larger. The two other preparations, both from

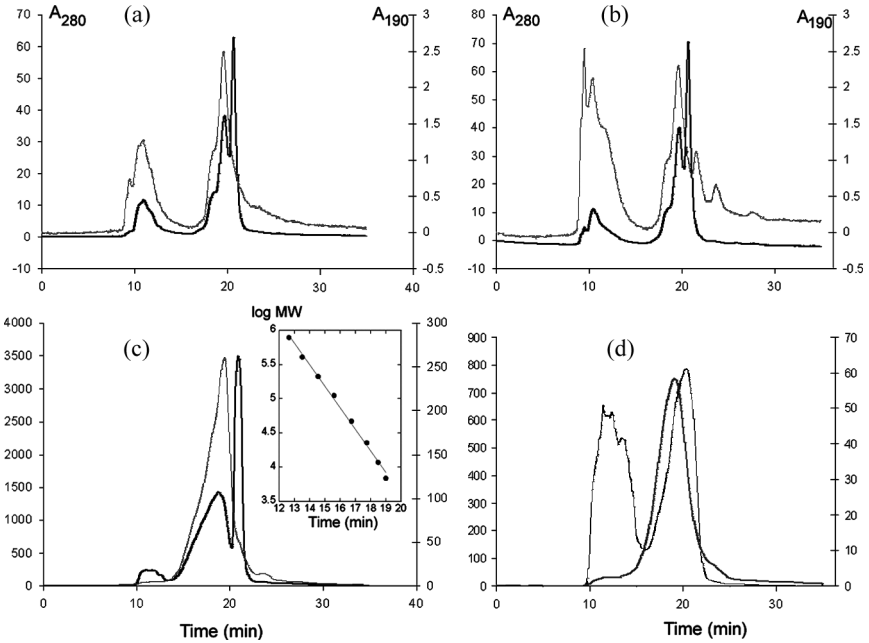

FIGURE 4 GPC chromatograms of exopolymer preparations from axenic cyanobacterial strains (a) CS-556/01-A01, 5–10 μL (b) CS-556/01-A15, 5–10 μL (c) CS-591/04-A12, 100 μL, and (d) CS-588/02-A11, 100 μL. The different loadings were used to offset the low solubility and high viscosity of some samples. Absorbance at 280 nm is indicated (in milliAU) on the left-hand vertical axis of each panel (black chromatogram), while that at 190 nm is indicated similarly on the right-hand axis (grey chromatogram). Where sample concentrations permitted the use of detection by light scattering (not shown), the signal typically mirrored the A_{280} response. The inset in panel (c) shows the calibration curve obtained with pullulan size standards (R = 0.998).

Nodularia spumigena strains, contained amounts of UV-absorbing species comparable with the CS-566/01 samples, but lacked EPS peaks in the void and early included volume (Figs. 4c,d). The large asymmetric and mainly-A_{280} peak beginning in the void volume of Fig. 4d suggests that this sample contained many protein aggregates. Overall, the GPC separations revealed that the cyanobacterial exopolymer preparations were far from homogeneous. Although all of our high molecular mass preparations undoubtedly represent enriched rather than pure EPS, for the sake of convenience we will refer to them as "EPS preparations".

Adhesive Testing and Biochemical Composition

Results of dry shear strength tests for the microbial EPS preparations and reference adhesives bonding wood (maple) and plastic (PVC) adherends are shown in Fig. 5. The highest observed shear strength was 1.50 MPa, and was measured for CSIRO505 bacterial EPS bonding wooden adherends. Visual examination of broken joints revealed mainly cohesive failure with tendril formation. The same adhesive was ineffective at bonding plastic, yielding an observed shear strength of 0.04 MPa. Bacterial EPS from CSIRO501 was ineffective on both

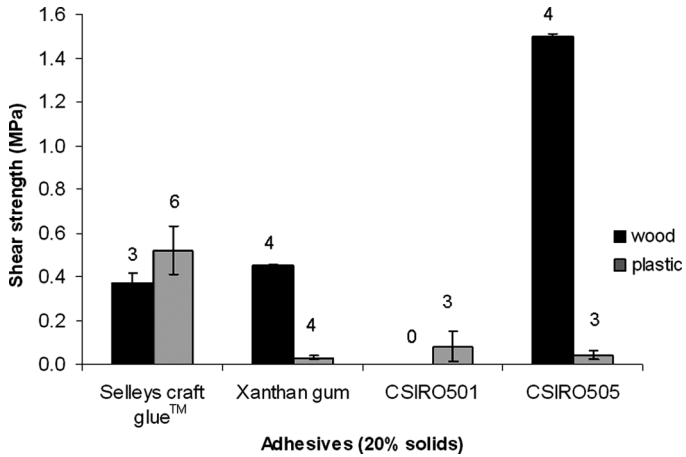

FIGURE 5 Dry shear strengths from lap-joints of wood (maple) and plastic (PVC). Adhesives were applied at 20% solids (w/w) and the joints cured at room temperature and low (23%) relative humidity for $\geq$1 week. Numbers over bars indicate *n*, the number of samples tested. Error bars show the standard error of the mean.

substrata; it bonded plastic adherends with a low shear strength of 0.08 MPa, and its wooden joints were so weak that they did not survive to the testing stage. Qualitative examination of broken joints indicated that the adhesive had a gummy texture with very low cut strength and poor attachment to adherends. Joints prepared using cyanobacterial EPS from CS-566/01-A01 and CS566/01-A15 did not survive until testing, and are consequently not shown in Fig. 5. Inspection of failed joints revealed that the material was very brittle and did not appear to have wet wooden surfaces well. A domestic PVA adhesive (Selleys Craft Glue) gave dry shear strengths of 0.37 and 0.52 MPa for wooden and plastic joints, respectively. Xanthan gum displayed significant adhesive strength (0.45 MPa) when used on wood, but was ineffective on plastic (0.03 MPa).

The percentage of total sugars and protein in each of the preparations tested for adhesive strength is reported in Fig. 6. Total sugars accounted for 95 dry wt% of xanthan gum, while CSIRO501 and CSIRO505 contained 8 and 20 dry wt% total sugars, respectively, and 25 and 19 dry wt% protein, respectively. Selleys Craft Glue had little (<5 dry wt%) of either component. The purified cyanobacterial EPS from CS-566/01-A01 and CS-566/01-A15 consisted of 11 and 12 dry wt% total sugars, and 13 and 10 dry wt% protein, respectively.

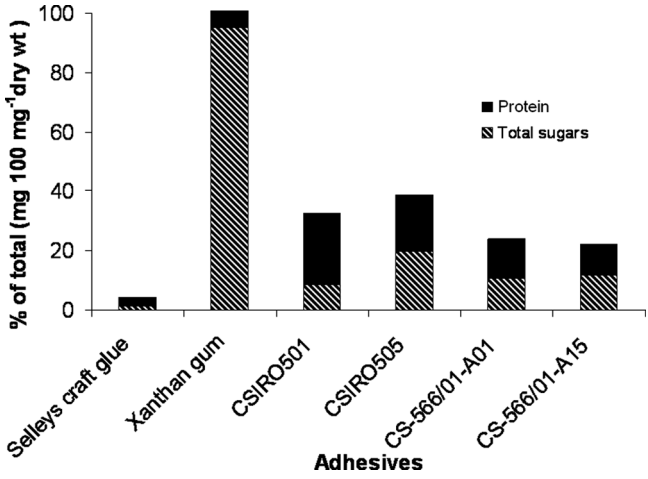

FIGURE 6 Carbohydrate and protein content of adhesives, reported as dry wt%. Composition data come from colorimetric assays which yield masses in terms of reference species, namely bovine serum albumin (for protein) and glucose (for sugar). Here, these values are expressed as a percentage of the mass measured for samples dried under vacuum.

Preliminary analyses indicated that the CS-566/01 EPS contained mainly neutral sugars, with <5% (w/w) uronic acids (C.M. Nichols, unpublished data).

DISCUSSION

The supernatants of microbial cultures in stationary phase are complex solutions in which EPS (if present) is just one of many components. However, the polymeric nature of EPS—which encompasses the possibility of branched and/or highly charged species—means that the EPS content (by weight) is likely to be a major determinant of culture viscosity. Although the relationship is far from straightforward, with molecular mass, structure, charge, composition, the presence of non-sugar residues, the potential for non-covalent aggregation, and the presence of mono- or divalent ions all contributing to the observed viscosity, rheometry has previously been used as an expedient way to monitor EPS production in microbial fermentations [20,30]. Although popular in some fermentation-based industries, viscosity measurements have not previously been used as a discovery tool for screening a national collection of microorganisms.

The CCLM is an extensive library of microalgal and bacterial biodiversity sampled from varied environments across a wide biogeographical range, and therefore, constitutes a promising source of novel polysaccharides, which may have commercial potential. Measuring the viscosity of cultures, sampled at the time of routine subculturing, was chosen as a relatively rapid way to assess the concentration of EPS in CCLM cultures. About 750 of the ≥800 cultures were assessed in this way.

Three commercially available polysaccharides were used as analogues to microbial polysaccharides in a range of molecular masses. Dextran sulphate is a polyanionic branched-chain polymer of glucose. Xanthan gum, a major commercial polysaccharide formed by the bacterial plant pathogen *Xanthamonas campestris* [49], is also a polyanionic branched-chain polymer, but it contains several different types of sugar residue and has a very high weight-average molecular mass (approximately 1,400,000 Da) [49]. The ionic strengths of the media most commonly used for growing CCLM microalgae rank Milli-Q water < MLA media < f/2, so the dose-response data of Table 1 support and extend previous studies demonstrating that the presence of dissolved salts lowers the viscosity of dilute solutions of polysaccharides [50]. For all but the smallest molecule, viscosity was directly proportional to the polysaccharide concentration expressed as mass per unit volume. At similar concentrations, the viscosities of

the three polysaccharides ranked in the order dextran sulfate (5,000 Da) < dextran sulfate (500,000 Da) < xanthan gum, highlighting the dependence of viscosity on the molecular mass of the polymer [51].

The 40 highest viscosity values in the first round of CCLM sampling (Table 2) correspond to xanthan gum equivalents of 100 to 1155 mg/L, and represent high yields in cultures not yet optimised for EPS production [52]. Over half of this subset of 40 species are non-eukaryotes belonging to the Cyanobacteria, a group well known to include EPS producers [53,54]. The remaining isolates are classed as eukaryotes, although non-axenic cultures do contain bacteria as well as algae (discussed below).

The total sugar content of a culture supernatant is a direct indication of its polysaccharide content (by weight) and, therefore, of its EPS content. Our results (Fig. 3) show that this parameter does indeed correlate positively with viscosity ($R^2 = 0.6645$). Our findings suggest that rheometry should afford a simple and fast way to compare EPS production levels within large libraries of microbial cultures. An added advantage is that it is much less hazardous to make viscosity measurements than to perform the sugar assay, which involves handling phenol and concentrated sulphuric acid.

Many microorganisms secrete proteins [5] and, accordingly, polypeptides may be released by the organisms in microalgal cultures [55]. In this study, the protein content of culture supernatants was generally an order of magnitude lower than the corresponding sugar content, and it showed a poor correlation with viscosity. This suggests that culture viscosity is more strongly and consistently influenced by the presence of EPS than by protein.

A high proportion of the CCLM microalgal strains surveyed were non-axenic and, therefore, also contained bacterial species that were associated with the microalgae when the culture was first established. Since healthy microalgal strains commonly have a resident population of non-photosynthetic bacteria that may be free-living, attached to microalgal cells, or even intracellular [56], the bacteria in non-axenic CCLM cultures should not be considered merely as contaminants. Studies have shown that symbiotic relationships may occur between bacteria and microalgae; for example, bacteria can adhere to the site of nitrogen fixation on cyanobacterial heterocysts [17] *via* the heterocyst-produced EPS [57]. These micro-zones around cells facilitate the transfer of nutrients from one species to the other [17]. Similarly, laboratory cultures of *Gymnodium catenatum* include a bacterial community that is essential for successful germination and growth [58]. However, since the surveyed population of CCLM cultures and the subset producing the 40 most viscous supernatants

contained a similar proportion of axenic cultures (14–15%), we conclude that there is no gross correlation between axenic status and EPS production levels.

The most viscous culture in both sampling rounds, CS-564/01, was a non-axenic cyanobacterial strain whose algal component is known to be *Microcystis aeruginosa* f. *flos-aquae*. The EPS of non-axenic microalgal strains may be produced by any or all of the constituent organisms, and these mixed populations must be resolved into separate species to allow the study of individual types of EPS. No effort was made to resolve the cyanobacterium of culture CS-564/01 because closely related or identical strains were already present as axenic cultures which had given high viscosities in the survey (see below). Efforts to resolve bacterial species from CS-564/01 resulted in the isolation of two non-photosynthetic bacterial strains, designated CSIRO501 and CSIRO505. Both strains showed enhanced mucoid morphologies when grown on glucose-containing agar plates; such a response is often the result of increased EPS production [59,60]. The high molecular mass components of these exudates were purified to allow compositional and functional analysis.

Table 2 shows that the two most viscous axenic microalgal strains in the first sampling round were CS-566/01-A15 and CS-566/01-A01, ranking in sixth and seventh place overall. They again displayed high viscosities in the second round, now ranking within the top four. On this basis alone, the EPS from these isolates deserved further investigation. In addition, both of these axenic cultures were *Microcystis* strains that were closely related or identical to the cyanobacterium of non-axenic culture CS-564/01. Recent taxonomic findings indicate that the cyanobacterium of CS-564/01, namely *M. aeruginosa* f. *flos-aquae*, is from the same species as *M. pseudofilamentosa*, the cyanobacteria present in both CS-566/01-A01 and CS-566/01-A15 [61]. For all of these reasons, CS-566/01-A01 and CS-566/01-A15 were grown separately as axenic liquid cultures on a scale that permitted purification of their high molecular mass secretions for compositional and functional analysis.

As we have seen, cultures with high viscosity in the first sampling round usually gave rise to descendants that displayed high viscosities in the second round. However, this was not always the case. In those instances, EPS output may genuinely have declined in the generations between the two rounds of sampling, but other plausible explanations exist. In both rounds of sampling, cultures were sampled in stationary phase of growth, when EPS production was likely to be highest [62]. For technical reasons, however, cultures sampled in the second round were in some cases up to 4 weeks older (*i.e.*, had a post-inoculation

period up to 4 weeks greater) than those sampled in the first round. EPS production in microalgae has been shown to depend on environmental conditions including nutrient availability [63–65], so the observed differences in viscosity between sampling rounds may have been due to variations in nutrient content or cell density. Moreover, after an extended stationary phase, cell death and lysis may have released hydrolytic enzymes that would accelerate the degradation of EPS.

Secreted components of high molecular mass (nominally $\geq$30,000 Da) were purified from monospecific cultures of the microorganisms of greatest interest, namely bacteria CSIRO501 and CSIRO505, and cyanobacteria CS-566/01-A01 and CS-566/01-A15. At best, the combined estimates of protein and carbohydrate content for the bacterial and cyanobacterial exopolymer preparations could account for only 39% and 25% of sample dry weight, respectively. While the nature of the other material remains uncertain, the situation is not without precedent: in other studies, the carbohydrate plus protein content of various vertebrate and invertebrate adhesive secretions could account for only 38–63% of sample dry weight [66]. In the present study, the high molecular mass preparation from CSIRO501 exudate was shown to contain about three times as much protein as polysaccharide, whereas its counterpart from CSIRO505 contained approximately equal amounts of both. The exopolymer preparations from the two cyanobacterial cultures also contained similar amounts of protein and polysaccharide, with most of the latter appearing to have a molecular mass >800,000 Da in GPC. Although all of the secreted polymer samples clearly contain much non-polysaccharide material, for convenience we refer to them as "EPS preparations".

Dry shear strength tests were conducted both on maple wood, a hydrophilic natural substratum, and on PVC plastic, a hydrophobic synthetic substratum. The EPS preparation from CSIRO501 was ineffective on both types of adherend (<0.08 MPa). In contrast, the EPS preparation from CSIRO505 could bond wooden lap-joints with a dry shear strength of 1.5 MPa, four times as great as that afforded by a domestic PVA adhesive (Selleys Craft Glue) tested at the same concentration of solids. Inspections of broken CSIRO505 joints were encouraging, in that bond failure was mostly cohesive and tendril formation in the adhesive residue indicated good energy-absorbing mechanisms. Interestingly, the CSIRO505 preparation's efficacy did not extend to plastic adherends (0.04 MPa). This preparation contained 2.5 times the wt% sugar and, hence, EPS, of the CSIRO501 preparation. Moreover, xanthan gum—another bacterial EPS—displayed the same strong bias, bonding wooden adherends with a much higher dry shear

strength (0.45 MPa) than plastic ones (0.03 MPa). These findings are consistent with the idea that the adhesive strength of bacterial exudates on natural substrata such as wood depends largely upon their EPS content.

Lap joints prepared using EPS preparations from the axenic cyanobacterial strains CS-566/01-A01 and CS-566/01-A15 were too weak to allow measurement of their dry shear strength. Thus, while the results obtained with CSIRO505 vindicate the use of viscosity screening as a platform for discovering adhesive EPS, the CS-566/01 results serve as a reminder that high culture viscosities need not necessarily translate into useful dry bond strengths, even if GPC indicates that the EPS is of high molecular mass (>800,000 Da). Since the CSIRO505 preparation contained about twice the wt% EPS of the CS-566/01 preparations, the higher EPS content of the former may once again have contributed towards its better performance as an adhesive.

Labare *et al*. [67] tested the adhesive strength of polysaccharide adhesive viscous exopolymer (PAVE) produced by periphytic marine bacteria on various surfaces, including metal, wood, and acrylic plastic. Dry shear strengths for PAVE on acrylic plastic ranged from 0.05 to 0.17 MPa, while those on wood ranged from 0.06 to 0.58 MPa. Although procedural differences between the PAVE study and our own preclude direct comparisons, we note that the bond strength of PAVE with wooden and plastic adherends spans ranges similar to those determined for the marine bacterial exudates from our study. In both cases, greater bond strengths could be obtained on wood than on plastic.

Two bacterial EPS adhesives, one from Montana Biotech (MB, Rock Hill, SC, USA) and the other from Specialty Biopolymers (SB, Bozeman, MT, USA), have been described by Haag *et al*. [68,69]; they are assigned molecular masses of 40,000–1,000,000 and 500,000 Da, respectively [70]. Generally, these commodity adhesives displayed high bond strengths on wood: maple lap-joints of MB or SB cured at 53% relative humidity had shear strengths of ~15 MPa. The SB adhesive displayed a particularly impressive shear strength of 25 MPa when cured at 23% relative humidity for one week—curing conditions similar to those used in our study. Both adhesives are purified EPS preparations, with MB containing >95 dry wt% carbohydrate [70]. In contrast, the exopolymer samples from CSIRO501 (8 dry wt% carbohydrate; 25 dry wt% protein), CSIRO505 (20 dry wt% carbohydrate; 19 dry wt% protein), and CS-566/01 (11–12% dry wt carbohydrate; 10–13% dry wt protein) were relatively crude preparations with significantly lower carbohydrate contents. Moreover, the MB and SB adhesives were tested at ~31% (w/v) solids, whereas all of our EPS

preparations were tested at 20% (w/v) solids. The higher strength of the MB and SB preparations probably reflects *inter alia* their higher solids content and much greater EPS purity. Microbial exopolymer preparations can only sometimes cure into useful adhesives but, for those that do, the dry bond strengths on natural substrata seem to reflect the EPS content of the preparations.

There is a genuine and growing need for natural biopolymers as an alternative to those derived from petrochemical feedstocks. Microbial bioadhesives could represent an attractive alternative to synthetic adhesives for some applications, although production economics and performance issues currently pose significant challenges to commercial success. To move forward the discovery phase of our project, further purification of the CSIRO505 EPS, along with assessment of its molecular mass and structure, is in progress. Other promising EPS candidates found in this survey, or identified by applying the viscosity screening technique to other microbial repositories, will also be studied. A better understanding of the composition and structure of these diverse EPS samples should shed light on the mechanisms underpinning their adhesive function. In parallel, empirical optimisation of formulation and curing conditions should lead to improved bond strength, which may ultimately allow the development of new environmentally friendly commodity adhesives.

CONCLUSIONS

Dose-response curves for reference polysaccharides dissolved in various microalgal growth media showed that viscosity increased with the concentration and molecular mass of the macromolecule, but that it decreased with increasing ionic strength. We then screened most of the $\geq$800 cultures from the CSIRO Collection of Living Microalgae (CCLM) for EPS production by examining the viscosity of conditioned media supernatants. Using the 40 most viscous CCLM cultures, we confirmed that the viscosity of culture supernatants correlated well with their total sugar (and thus EPS) content, but not with their protein content. The conditioned medium with the highest viscosity (6.55 cP, equivalent to 1.16 g/L xanthan gum) was produced by CCLM culture CS-564/01, a non-axenic isolate of the cyanobacterium *Microcystis aeruginosa* f. *flos-aquae*. Two types of bacteria, designated CSIRO501 (orange, Gram-positive rods) and CSIRO505 (off-white, Gram-negative rods), were subcultured from CS-564/01. Aqueous preparations of EPS from these bacteria and from axenic *Microcystis* cultures were applied at 20% (w/v) solids to maple wood and PVC plastic adherends and—after curing—were tested for adhesive strength. The

CSIRO505 preparation bonded wooden lap joints with a dry shear strength of 1.5 MPa, four times as strong as bonds obtained in equivalent tests of a commercial polyvinyl acetate (PVA) glue. The CSIRO505 preparation contained 1.7–2.5 times as much EPS as the CSIRO501 or cyanobacterial preparations, and resembled a well-known EPS (xanthan gum) in being many times more effective at bonding wood than plastic. Overall, these findings suggest that culture supernatant viscosity is a good indicator of EPS content, which, in turn, may make a substantial contribution to dry bond strength on wood. Our viscosity screening identified a microbial EPS which, even in a crude preparation (20 dry wt% EPS), outperformed a synthetic commodity adhesive on this commercially important substratum.

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