Identification of a Marine Green Alga *Percursaria percursa* from Hypersaline Springs in the Middle of the North American Continent

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The chlorophycean alga *Percursaria percursa* (Ulvaceae, Ulvales, Chlorophyceae), typical of marine inter-tidal zones, is reported for the first time from hypersaline springs located along the north-western shore of Lake Winnipegosis in Manitoba. Although not usually found inland, *P. percursa* is the dominant member of microbial mat communities that thrive in shallow pools at the outlets of hypersaline springs.

Key Words: *Percursaria percursa*, chlorophyta, hypersaline, springs, marine algae, Manitoba.

This study was undertaken to characterise the members of microbial mat communities growing in the pools and outflow channels of hypersaline springs in North-Western Manitoba. Studies on the microbial ecology of hypersaline waters has previously been limited to marshes and standing bodies of water such as salt lakes and salt production ponds (Oren 2002), so very little is known about the microbes inhabiting flowing water sources such as springs. Stable hypersaline springs are uncommon, and well-defined springs amenable to scientific study, particularly at low temperatures, are relatively rare. However, these springs can provide important information about the survival of microbes under extreme conditions. They also provide a modern analogue for the extensive microbial mats that characterised life on Earth during the Precambrian.

The highly saline pools, marshes, and saltpans on the northern margins of Lake Winnipegosis, Manitoba, have created a unique ecosystem dominated by organisms characteristic of marine habitats (Patterson et al. 1997). The flora and fauna have been described previously, with plant species composition similar to prairie saltpans (Burchill and Kenkel 1991), and animals that included marine representatives (Chordata, Protozoa, Rotifera, and Arthropoda) (McKillop et al. 1992). The distribution of the vegetation was linked to the salt tolerance of the species (Burchill and Kenkel 1991). It has been suggested (Patterson et al. 1997) that these marine species were transported to the springs area in Manitoba by birds (including Canada Geese) from coastal areas such as Hudson Bay, 700 km northeast of this site, and the Gulf of Mexico. Colonization of the saline springs could be no sooner than the retreat of the continental ice sheet, which covered the region up until about 10 000 years ago. Patterson et al. (1997) show that a saline ecosystem has been in place since at least 5500 years ago. Colonization by marine species occurred soon thereafter (Patterson et al. 1997).

Geochemical and stable-isotope analyses indicate that spring waters originate as meteoric water (Grasby et al. 2000). There was a large influx of glacial meltwater into the underlying bedrock during the Pleistocene, which dissolved the underlying beds of halite (NaCl), sylvite (KCl) and similar salts deposited by evaporation of Devonian seawater. The retreat of ice sheets has released the waters, discharging now as saline springs (Grasby et al. 2000; Grasby and Chen 2005). There are numerous saline springs along the western shore of Lake Winnipegosis (Grasby 2000). Microbial mats have only been observed at springs in the Dawson Bay area (on the north-western shore of the lake), further west along the Red Deer River (Manitoba), as well as springs near Pelican Bay (east of Dawson Bay). These hypersaline springs flow over a saltpan of barren, iron stained surficial material (McKillop et al. 1992). The high salt concentrations severely limit plant growth in the area, and life within the springs themselves is restricted to microorganisms.

Study Site

The hypersaline springs are located along the western shore of Lake Winnipegosis in North-Western Manitoba. As shown in Figure 1, the springs are in Manitoba, just south and west of Dawson Bay in the northern extent of Lake Winnipegosis. The locations and chemistry of the spring outlets are summarised in Table 1. Water samples were collected and preserved in the field. The water was passed through a 0.45 μm filter. Samples for Fe analysis were acidified to pH <2 with ultrapure NHO_3. Samples for anion analysis were untreated. Anions were measured by ion liquid chromatography, and Fe by ICP-MS. The salinity of springs studied here, the feature that defines the springs
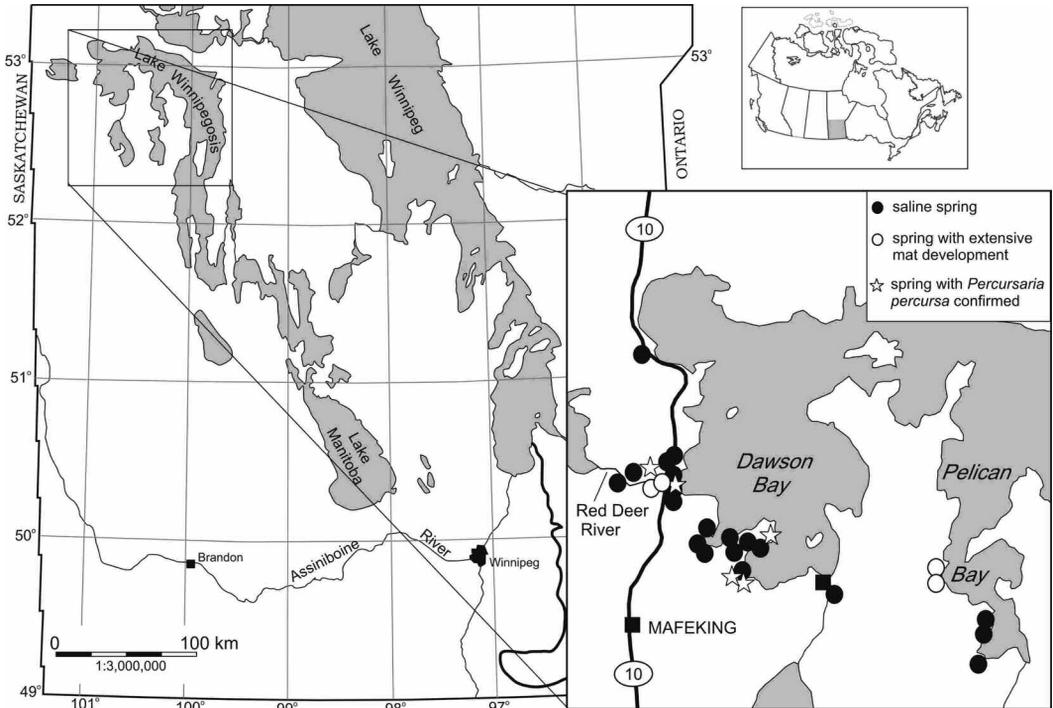
FIGURE 1. Regional map showing locations of saline springs, indicating which ones have been observed to have microbial mats of *P. percursa*.

and limits growth in these areas, ranges from below seawater to twice seawater concentrations. The salinity is mostly from NaCl (from halite), with minor differences in composition compared to typical seawater. The salt from these springs has been used historically by early settlers (Petch 1987). Chloride and sulfate concentrations in springs containing microbial mats are typically 24 to 36 g/l and 2.1 to 3.5 g/l, respectively (Table 1). Gas bubbles rising from the spring sediments were observed frequently, often becoming trapped by the overlying mat in springs with confluent growth, leading to the appearance of medium to large swellings of the mat fabric. The gas composition is dominantly N_2 (95%) with traces of CO_2 (1.8%), He (1.5%), O_2 (1.0%), Ar (0.8%), and CH_4 (0.02%) (McKillop et al. 1992), with the latter two potentially supporting microbial growth.

Each site consisted of multiple spring outlets, most of which formed pools around the origin, the oldest of which have formed raised cauldrons. Microbial mats grew on the surface and around the margins of the pools (Figure 2). There was considerable red goethite (FeO•OH) staining in areas surrounding the springs, but not in the spring sources themselves, which are dominated by calcite and quartz. Areas surrounding the springs also sometimes exhibited a characteristic "brainy" texture due to the decomposition of the algal mats beneath the evaporitic gypsum crust. The springs

flow year-round and the salinity keeps some of the larger ones from freezing, even in winter (McKillops et al. 1992). Microbial mat growth was observed in freezing temperatures in the fall and spring. Samples of the microbial mats were collected in October 2001, May 2002, and August 2003. Samples were stored on ice or in a freezer (-20°C) until they were analysed by microscopy at the University of Manitoba using a Leitz Diaplan (Germany) microscope.

Results and Discussion
Organism
Percursaria percursa (C. Agardh) Bory De St. Vincent is a green alga of the order Ulvales (Ulvophyceae, Chlorophyta) that is phylogenetically related to *Ulva intestinalis* (O'Kelly et al. 2004). It forms distinct biseriate simple threads of varying breadth (Bliding 1963). The thalli are typically unbranched, up to 3 cm long, flexuous and contorted. The double row of cells is usually in exact symmetry (Figure 3), with each cell containing a single parietal band-like chloroplast similar to those seen in species of the genus *Ulothrix*. Under intense light, the chloroplast forms a narrow parietal girdle in the middle of the cell, whereas under low light levels the chloroplast becomes well developed and less band-like (Bliding 1963). The number of pyrenoids in the chloroplasts of *P. percursa* varies between one and three with most specimens typically

TABLE 1. Locations and physicochemistry of spring waters west of Lake Winnipegosis.

sample number	Latitude	Longitude	TDS [c]	Cl	SO$_4$	Fe	
	NAD 87		mg/l	mg/l	mg/l	ug/l	pH
M993013 [a]	52°48.105'	100°48.750'	64510	35700	3460	1700	6.87
M003005	52°47.977'	100°49.430'	63488	35300	3220	1900	6.70
M993031	52°40.893'	100°4.798'	61637	34300	3200	1200	6.75
M993016 [a]	52°45.086'	100°52.926'	61327	33700	3170	1100	6.50
M993029 [b]	52°38.235'	100°22.120'	61015	35400	1130	1700	6.29
M993028 [b]	52°41.561'	100°21.176'	60300	33000	3170	1900	6.35
M993027 [b]	52°41.259'	100°20.988'	59404	32800	3180	1100	6.70
M993019 [b]	52°51.752'	101°08.166'	58646	32500	2970	2500	6.60
M993014 [b]	52°47.703'	100°51.817'	58197	32100	2990	2300	6.64
M003003 [a]	52°52.103'	101°04.169'	56545	31500	2780	2000	6.78
M993020 [b]	52°51.289'	101°08.159'	55861	31300	2840	2700	6.73
M003006 [b]	52°47.919'	100°53.118'	55855	31200	2770	2100	6.56
M993026 [b]	52°51.783'	101°05.392'	55626	31000	2820	<150	6.34
M983075 [a]	52°52.031'	101°03.271'	54825	31000	2800	2100	6.97
M993011 [b]	52°52.603'	101°02.886'	54543	30200	2790	<150	7.40
M993025	52°45.806'	100°52.845'	49068	27400	2600	<150	6.99
M003002 [a]	52°52.191'	101°05.563'	48522	27200	2400	1900	6.86
M993037	51°37.429'	99°58.472'	43680	22300	4590	<150	8.35
M983076 [a]	52°52.526'	101°02.359'	42405	23700	2100	3200	7.05
M003004 [b]	52°45.266'	100°53.429'	38566	21500	2090	1000	6.91
M993038	51°49.099'	99°43.493'	37208	19900	3000	<150	8.38
M993035	51°37.424'	99°56.777'	37189	18900	3690	240	7.99
M993034	51°43.390'	99°57.692'	35856	18900	3010	<150	7.96
M983077	52°54.092'	101°01.125'	32786	18500	1610	1000	6.98
M983079	52°47.205'	100°58.041'	32356	18100	1620	110	7.88
M983073	51°55.587'	100°09.384'	28857	15500	2300	2400	7.39
M993036	51°36.570'	99°51.542'	28765	14400	3360	<150	7.95
M993017	52°46.802'	100°57.852'	23733	12700	1080	340	6.48
M983074	52°00.408'	100°08.480'	22319	11700	1400	2300	7.40
M993041	52°02.191'	100°09.754'	21648	10800	2250	<150	6.94
M003007	52°57.587'	101°01.299'	21100	12100	1011	<150	6.87
M993032	51°53.731'	100°09.068'	20096	10300	1500	<150	5.98
M993021	52°43.719'	100°40.582'	18236	9970	899	<150	7.62
M993033	51°50.039'	100°07.305'	17453	8320	1580	<150	7.84
M993022	52°42.632'	100°41.958'	16946	9730	808	<150	7.10
M993039	51°48.000'	99°43.792'	14371	1400	2460	<150	7.99
M003001	51°34.591'	99°41.305'	11914	5530	1790	390	6.93
M993024	52°44.569'	100°44.862'	7189	3720	321	<150	8.28
M993015	52°46.525'	100°53.561'	5892	2850	151	<150	6.62
M993012	52°53.878'	101°02.030'	5027	2490	180	<150	7.75

[a] Hypersaline springs with *P. percursa*
[b] Hypersaline springs with algal mats, possibly *P. percursa*
[c] Total dissolved solids (TDS)

having two (Celan 1979). Although the thalli are usually biseriate, it is common to find some thalli that are uniseriate in part. Cells in the material collected from the saline springs bordering Lake Winnipegosis are a typical size (Abbott and Hollenberg 1976), between 10 – 18 μm wide and 24 – 28 μm long. The thalli are light green because of the chloroplasts, but the cells from this site are often covered by amorphous rust-coloured clusters that can impart a brown, to rust, to orange, to red colour to the mats as a whole. This has led to the illusion that these mats are dominated by red and green algae (Bezys et al. 1997; Wadien 1984), when in fact only green algae (Chlorophyta) are present.

This species is usually found with other algae, frequently forming floating entangled masses (often called green tide or sea lettuce) in upper tidepools and salt marshes. In North America, this taxon is found along the Pacific coast (Abbott and Hollenberg 1976; Setchell and Gardner 1920, Garbary et al. 1985), the Atlantic coast (South 1984; Wynne 1986), and in the Arctic (Setchell and Gardner 1920). Typically a marine organism, there is one previous report that *P. percursa* may also occur inland in environments where salt concentrations are elevated (Kornmann 1956). Although reported in many locations, this filamentous alga usually does not occur in large quantities, and is generally a minor contributor to blooms dominated by closely-related species of *Cladophora* and *Enteromorpha* (Hayden and Waaland 2002; Setchell and Gardner 1920; Taylor et al. 2001). In the hypersaline springs
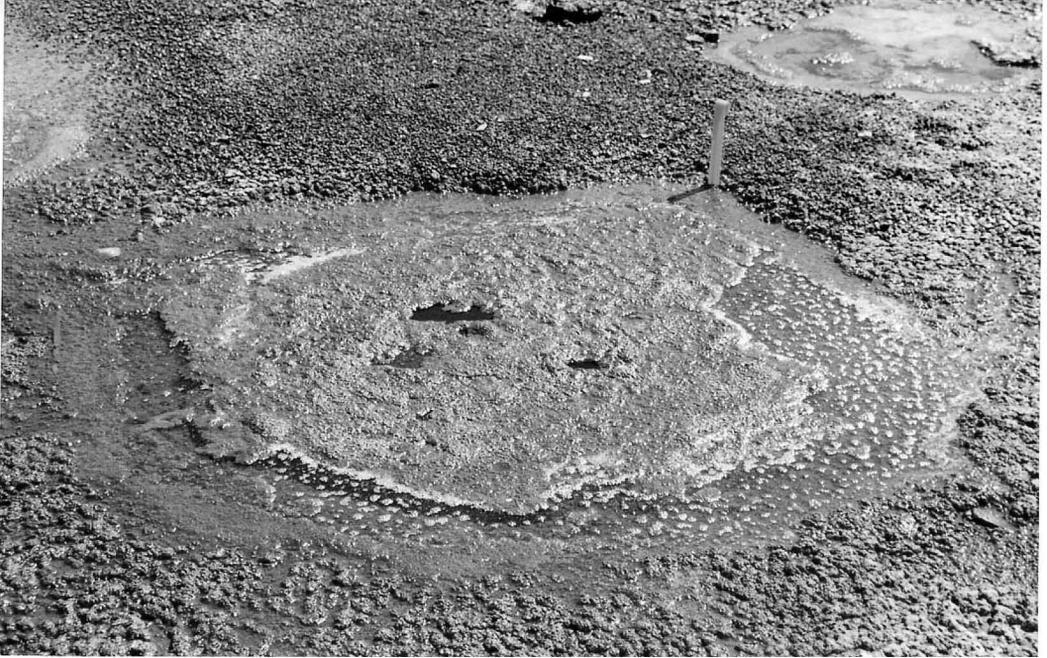
FIGURE 2. Photograph of a hypersaline spring containing extensive mats of *P. percursa*. A 15 cm tent peg inserted beside the spring is included for scale.

near Lake Winnipegosis, it was by far the dominant organism, associated with various pennate diatoms and cyanobacteria (including members of the Oscillatoriaceae) (unpublished data) but not other filamentous green algae.

Habitat and distribution

There are numerous saline springs along the western shore of Lake Winnipegosis (Grasby 2000; Bezys et al. 1997; McKillop et al. 1992) as well as in north-eastern Alberta (Grasby and Chen 2005), however, prolific growth of *P. percursa* has only been confirmed at the Dawson Bay salt springs located on the north-western shore of the lake. Similar algal growths have been observed at other sites west of Pelican Bay and south of Red Deer River, but detailed analyses have not been done to confirm *P. percursa.* Salinity at these sites is typically greater than that of the springs located further south in Manitoba, and this may explain the dominance of *P. percursa.* The salinity of the springs is thought to be increasing over time (Patterson et al. 1997), and although the full range salinity tolerance is unknown, it does not seem to be limiting growth of this organism, but rather promoting it. *P. percursa* can grow in diluted seawater over a range of 0-34 PSU (practical salinity units), with an optimum of 24 PSU (Taylor et al. 2001), but tolerance above seawater has not been tested. *P. percursa* has a broad tolerance to environmental conditions of irradiance (9-175 μmol m^{-2} s^{-1}) and temperature (at least 10-30°C), as well as
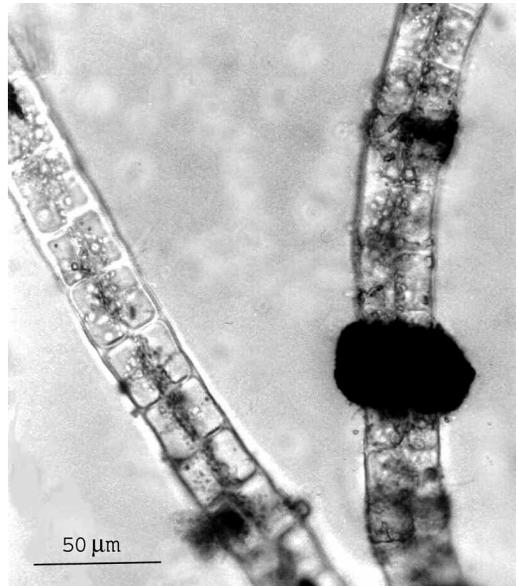

FIGURE 3. Photomicrograph (1000×) of filaments of *Percursaria percursa* from a hypersaline spring west of Lake Winnipegosis. The biseriate cells are paired in two longitudinal rows in each filament. Note the large accumulation of material on the right strand that imparts a rust-red color to the mats as a whole, although the filaments themselves are green. The scale bar represents 50 μm.

N and P concentrations [1-200 μM PO_4^{3-}, 10-1000 μM NO_3^-, 1-100 μM NH_4^+] (Taylor et al. 2001). Growth of "green tide" algae like *P. percursa* is generally enhanced by eutrophic conditions, but the springs west of Lake Winnipegosis are pristine discharges, with the only potential sources of significant amounts of N and P from sporadic incidences of bird defecation.

The distribution of *P. percursa* was compared to water chemistry parameters by principle component analysis (PCA) using The Unscrambler® 7.6, a multivariate statistical software package. The first two axes of the PCA explained 86% of the total variance and revealed differences in the water chemistry between hypersaline springs with and without mat communities (Figure 4). PCA axis 1 explained 65% of the total variation between the springs and clearly separated hypersaline springs with mat communities from those without. Total dissolved solids (TDS = the sum of major cations and anions) and chloride explained 95.2 and 94.6% of the variance in the scores of the hypersaline springs on axis 1, respectively. All hypersaline springs with mat communities were situated to the right of axis 1 and positively correlated to TDS and chloride, indicating that high salinities are required for the growth of *P. percursa*. Iron and sulphate concentrations also appeared to be correlated to the presence of mats and explained 49.0 and 56.5% of the variance on axis 1, respectively. Additionally, PCA axis 2 explained 21% of the variation between the hypersaline springs. On this axis pH was the most important factor, explaining 55.3% of the variance on axis 2, and indicated that the growth of *P. percursa* occurred in springs with lower pH.

In addition to the above parameters, other factors must also determine the distribution, as not all springs at each site contain mats of *P. percursa*. The temperature of the waters varied seasonally, ranging from 5°C (temperature of the source water) up to 30°C in the summer, but densities did not correlate with temperature. Growth of the organism is restricted to springs with high flow rates that form open pools, and it is not observed in low-flowing seeps in the area even if the water chemistry is compatible. In addition, distinct pools or cauldrons just a few meters apart, or even touching, had extensive growth of mats in one pool and nothing in the other. Considering the motility of the species, and its widespread distribution in the area, lack of colonization would not explain the patchy distribution. There could be a subtle difference in the chemistry of the water at each outlet, although chemical measurements to date indicate that all the water from these sites originates from the same source. There could be a difference in predation to explain the disparity of mat development in different springs. Studies
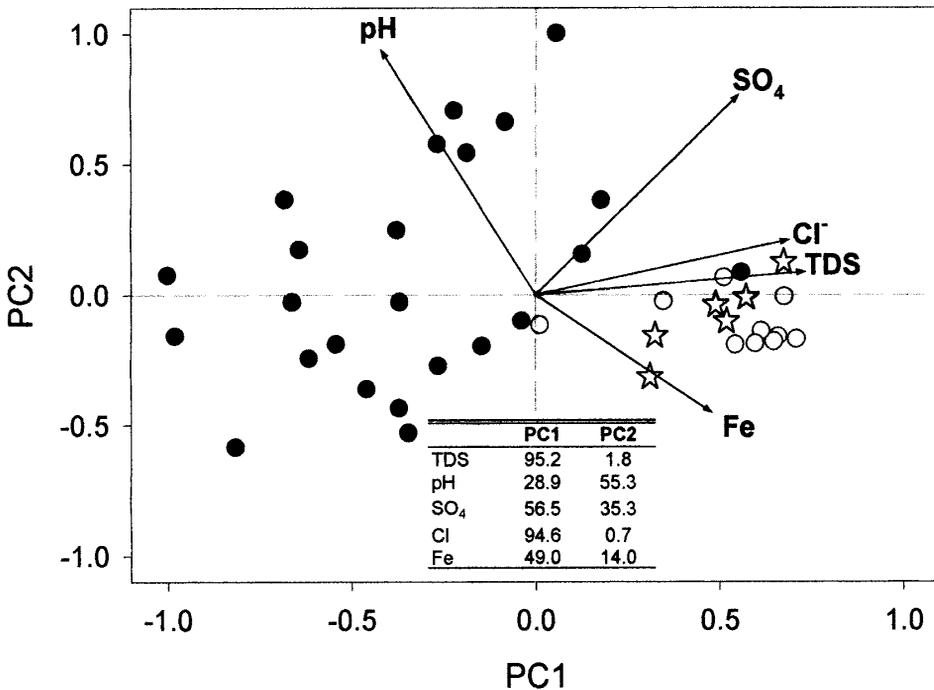

FIGURE 4. Principle component analysis (PCA) ordination biplot displaying grouping of hypersaline springs with respect to water chemistry. The symbols represent springs without *P. percursa* (closed circles), with mat communities not yet confirmed as *P. percursa* (open circles), and with confirmed mats of *P. percursa* (open stars). The inset table lists the relative contributions (in %) of each chemical parameters to the variance on each of the two axes.

of rotifer populations in areas containing *P. percursa* found that this predator is limited by high salt concentrations as well as higher temperatures (Saunders-Davies 1998). The dynamics of growth and predation require further study at this site.

Further research

The ability of *P. percursa* to grow and thrive in this extreme environment certainly merits further scientific investigation. The extent to which this dominant primary producer supports an entire ecosystem of microbes in the mat community is the subject of ongoing research. This organism supports a dynamic community of anaerobes including sulphate-reducing bacteria and methanogens. The potential for methanogenic fermentation of *P. percursa* biomass at lower salinities has been demonstrated previously, and proposed as a future mechanism for the production of a renewable source of biogas (Schramm and Lehnberg 1984). The extent to which this organism affects the precipitation and mineralization of carbonates (calcium carbonate) and iron (hematitic- to limonitic) at these sites (Bezys et al. 1997), causing the distinctive reddish brown sinter or tufa mounds of springs on the salt pans, also requires further investigation. These mats also likely support a unique community of invertebrates and epiphytic algae, which to date have not been investigated to our knowledge.

Acknowledgments

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