J. Aquat. Plant Manage. 34: 60-68

Microorganisms Associated with Hydrilla in Ponds and Lakes in North Florida[1]

YASSER M. SHABANA AND R. CHARUDATTAN[2]

ABSTRACT

The diversity and frequency of microorganisms associated with hydrilla [*Hydrilla verticillata* (L.F.) Royle] were determined by dilution-plating of water, sediment (soil), and hydrilla plant samples from four man-made ponds and two lakes in north Florida. Aqueous dilutions were plated on six selective and nonselective culture media: Sneh & Stack selective medium (SS), Komada's (K), PART selective medium (PART), chitin agar (CA, selective for actinomycetes), nutrient agar (NA), and hydrilla extract plus half-strength potato dextrose agar (HPDA). Four hundred and fifty eight different organisms (211 bacteria, 202 fungi, 44 actinomycetes, and 1 cyanobacterium) were recovered from 48 samples taken from the four ponds using the six media. Two hundred and ten (101 bacteria and 109 fungal isolates) were recovered from 15 samples taken from Rowell Lake. Seventy seven (31 bacteria, 45 fungi, and 1 cyanobacterium) were recovered from 10 samples from Orange Lake. The most effective medium for isolating the greatest variety of fungi was K. Nutrient agar and HPDA were best for isolating the largest diversity of bacteria, and CA and HPDA were best for actinomycetes. Fungi belonging to several plant pathogenic genera including *Botryosporium*, *Cercosporidium*, *Chaetophoma, Diplodia,* and *Pyrenochaeta* were found mainly in hydrilla and soil samples. Very few actinomycetes were recovered, mostly from pond soils, with a few from pond hydrilla. The frequency and diversity of the microorganisms isolated confirmed the occurrence of a rich microbial flora associated with hydrilla and the efficacy of the media and the isolation technique used.

Key words: Submersed aquatic plant, *Hydrilla verticillata*, microbial diversity, aquatic weed, fungi, bacteria, actinomycetes.

INTRODUCTION

To discover effective microbial agents for use in biological or integrated control of hydrilla, it is necessary to understand the microbial ecology of this plant under natural conditions. However, the microflora associated with hydrilla is poorly understood (Charudattan 1990). This investigation was therefore designed to characterize the microbial flora (pathogens and nonpathogens) of hydrilla at selected sites in north Florida by using six selective and nonselective culture media. It has been shown that even organisms that alone have minimal effect on the host plant may contribute to the integrated control of a submerged aquatic weed (Sorsa et al. 1988). In view of this, the microorganisms associated with hydrilla in this study will be screened for their pathogenic or phytotoxic activities against hydrilla. Those that are effective in killing hydrilla will be tested for their host specificity and may then be developed as bioherbicides. It has been suggested that such organisms could be manipulated to improve their biocontrol efficacy by introducing genes for the production of a herbicidal metabolite, a lytic enzyme, or possibly a plant hormone (Pennington 1984). This is one of our long-term objectives; the present study is the first phase.

MATERIALS AND METHODS

Study Sites and Sampling Methodology. Three field sites consisting of four man-made ponds located at the Center for Aquatic Plants, University of Florida, Gainesville and two natural freshwater lakes around Gainesville, Orange and Rowell Lakes, were used for this study. At each of these sites, two stations (sub-sites) were established for sampling. Water, sediment, and hydrilla samples were collected from each station. Water samples were taken by submerging sterile 5-dram glass vials and opening them 10 cm under the water surface. Soil samples were taken from ponds by submerging an inverted, sterile 5-dram vial into the sediment and retrieving the soil. Sampling of soil from lakes was carried out by pushing a 3-m long, 2.25-cm-diam PVC tube into the soil, then closing the upper end of the tube with a rubber stopper, and retrieving the soil. Hydrilla samples were taken by gathering apical shoots of hydrilla (about 20 cm in length) at the water surface and/or 30 cm under the surface. The shoots were transported to the laboratory in sterile 5-dram vials kept on ice and were processed within 3 to 4 h of collection. Plant samples were surface sterilized with 3% H_2O_2 for 3 min and ground with a sterile pestle and mortar prior to making dilutions. Sampling was conducted in November 1994, January 1995, and March 1995 for ponds, Orange Lake, and Rowell Lake, respectively.

Media Used for Isolation. Six selective and nonselective culture media were used to isolate various types of microorganisms: Sneh & Stack selective medium (SS) (Sneh and Stack 1990), Komada's medium (K) (Komada 1975), PART selective medium for *Pythium* and *Phytophthora* (PART)[3], chitin

[1]Published with the approval of the Florida Agricultural Experiment Station as J. Series No. R-05082. This research was supported by funds provided under a cooperative agreement (# 58-6629-4-008) between USDA-ARS and the Center for Aquatic Plants, IFAS, University of Florida.

[2]Assistant Professor, Plant Pathology Dept., Faculty of Agriculture, Mansoura University, Egypt, currently Postdoctoral Research Associate, Plant Pathology Dept., University of Florida; and Professor, Center for Aquatic Plants, Plant Pathology Dept., University of Florida, Gainesville, FL 32611. Received for publication March 26, 1996 and in revised form June 28, 1996.

[3]PART medium consisted of 5 mg pimaricin, 250 mg ampicillin (sodium), 5 mg rifampicin, 200 µg thiamine HCl, 8.5 g DIFCO cornmeal agar, 4.5 g agar, and 500 ml distilled water, and prepared as follows: cornmeal agar was autoclaved for 15 min, and amendments were added and mixed.

agar selective medium for actinomycetes (CA) (Lingappa and Lockwood 1962), nutrient agar (NA) (DIFCO laboratories, Detroit, Michigan), and hydrilla extract plus half-strength potato dextrose agar (HPDA)[4]. Because our results of dilution plating of pond samples showed that growth of the recovered microbes on CA plates was sparse and the bacterial colonies overgrew on the actinomycete and fungal colonies, this medium was supplemented with dextrose (10 g/l), streptomycin sulphate (0.3 g/l), and chloramphenicol (0.1 g/l) when used for isolating from lake samples. Also, HPDA medium when used for isolating from lake samples was supplemented with the above-mentioned antibiotics at the same rate to suppress bacterial overgrowth.

Dilution Plating, Colony Counting, and Culture Storage. Aqueous dilutions of pond water, plant, and soil samples were plated directly on 9-cm agar plates of different media as follows: 1/200 of all sample types for SS, K, and PART media, and for CA, HPDA, and NA media, 1/2000 of water and plant samples and 1/20,000 of soil samples. Aqueous dilutions of lake samples were the same as for the pond samples except for the water samples plated on CA, HPDA, and NA; these were plated at 1/200. Each diluted sample was pipetted at 0.25 and/or 0.5 ml per agar plate and spread with a flame-sterilized glass rod. Two or three replicates were used. Inoculated plates were incubated in the dark at 28 C. Microbes recovered on NA and HPDA plates were counted 10 days after inoculation while those that appeared on the other media were counted 4 weeks post-inoculation. Different organisms recovered on each medium were code-numbered and stored on suitable agar slants [potato dextrose agar (PDA, DIFCO) for fungi and oatmeal agar (OMA, DIFCO) for actinomycetes]; bacteria were stored in sterile water tubes. Working cultures of fungi and actinomycetes were transferred to 6-cm PDA and OMA plates, respectively, and exposed to diurnal light (12 h cycle, 37 $\mu E.m^{-2}.s^{-1}$) from two 40-W cool-white fluorescent lamps suspended 45 cm above the plates to enhance sporulation. Identical-looking colonies of the recovered microbes on different media were considered as the same microbe. Calculations were made to standardize the unit of comparison to be colony forming unit (CFU)/ml of 1/200 or 1/2000 dilution as appropriate.

Identification. Because of the large number of recovered microbes, only the most frequently recovered organisms were identified. Fungal isolates were identified by cultural characteristics and microscopic examination. Bacterial strains were tested for Gram reaction. Organisms that show pathogenic or phytotoxic activity against hydrilla in an *in vitro* assay will be identified to the species level.

Statistical Analyses. The data were analyzed with the Statistical Analysis System (SAS Institute 1988). The number of colonies for each type of microbe was log-transformed to induce homogeneity among treatments, and all multiple comparisons were first subjected to analysis of variance (ANOVA). Significant differences among treatment means were determined with Least Significant Means test. Because the numbers of isolations are cumulative (not means of samples), Chi-square (X^2) procedure was employed for each type of microbe.

RESULTS

Recovery of Microbes from Ponds. Data obtained from direct dilution plating of pond samples are presented in Tables 1 through 3 and Figures 1 through 5. Many of the recovered colonies did not appear until after 3 weeks of incubation except on NA and HPDA; the bacteria were recovered on them within 6 to 8 days after plating.

According to the Chi-square procedure used for testing for microbial diversity, there were significant associations between the medium and the sample type with respect to fungi ($X^2 = 17.67$, $P = 0.02$); however, the medium and the sample type had only a marginally association with respect to bacteria ($X^2 = 16.64$, $P = 0.08$) and no significant association existed with respect to actinomycetes. On total, pond waters yielded 17 fungal colonies/ml (20 different isolates) and 448 bacterial colonies/ml (56 different strains on 4 media only; excluding CA and HPDA which had too numerous bacterial colonies to count). No actinomycetes were recovered on the six media used for pond waters (Table 1, Figure 1). Sixty nine fungal isolates with a total of 41 colonies/ml, 44 bacterial strains (96 colonies/ml on 4 media only, CA and HPDA excluded), and three isolates of actinomycetes (7 colonies/ml) were recovered from pond hydrilla samples. Seventy seven fungal isolates (399 colonies/ml), 108 bacterial strains (3641 colonies/ml on 4 media only, CA and HPDA excluded), and 44 isolates of actinomycetes (933 colonies/ml) were recovered from pond soils (Table 1, Figure 1).

For the microbial frequencies obtained from pond samples, there were significant effects of medium, sample type, and medium x sample type interaction. Hydrilla-potato dextrose agar and K were the most effective media ($P < 0.03$) for recovering fungi when isolating from water (Figure 2a) and hydrilla (Figure 2b) samples; however, there were no differences between these media and SS when isolating from plant, and between CA and HPDA when isolating from soil (Table 1, Figure 2c). The least effective ones in this regard were NA and PART; there were no differences between these media and CA and SS when isolating from water samples (Table 1). Hydrilla-potato dextrose agar and CA followed by NA were the best media for isolating bacteria from water, plant, and soil (Table 1, Figure 2a-c) while SS and PART (for isolating from water and plant) and SS and K (for isolating from soil) were least effective for isolating bacteria. Regardless of the sample type, the best media for isolating microorganisms in terms of frequency were CA, HPDA, and K for fungi ($P \leq 0.02$); CA, HPDA, and then NA for bacteria ($P \leq 0.007$); and CA followed by HPDA for actinomycetes ($P \leq 0.0008$) (data not shown). The actinomycetes were recovered only on CA and HPDA, with the former being better than the latter. The majority of actinomycetes were isolated from the soil samples and very few of them were recovered from hydrilla samples (Table 1, Figure 2a-c). Thus, CA and HPDA were the best media for isolating microbes although the growth of most microorganisms on these media are very weak or sparse. With regard to the microbial diversity, these two media were moderate for fungi but still the best for acti-

[4]HPDA was prepared as follows: 150 g/l of fresh hydrilla sprigs were boiled in tab water for 45 - 60 min, extracted through 4 layers of cheesecloth, and mixed (1:1 v/v) with half-strength DIFCO potato dextrose agar.

Table 1. Comparison of six media and three sample types (water, plant, and soil) for enumeration of microbes recovered from ponds in north Florida.

Medium	Microbial type	Water		Plant		Soil	
		Isol'ns[a]	Colonies[b]	Isol'ns	Colonies	Isol'ns	Colonies
SS	Fungi	1	0.25 b[c]	13	5 ab	25	11.25 c
	Bacteria	0	0 d	2	2.13 d	1	0.38 e
	Actinomycetes	0	0 a	0	0 c	0	0 c
K	Fungi	12	6 a	39	14.17 a	32	21.50 bc
	Bacteria	7	8.92 c	6	41 c	5	4.25 d
	Actinomycetes	0	0 a	0	0 c	0	0 c
PART	Fungi	0	0 b	0	0 c	0	0 d
	Bacteria	2	2 d	0	0 e	3	19.67 c
	Actinomycetes	0	0 a	0	0 c	0	0 c
CA	Fungi	2	1.67 b	5	5 b	12	283.33 a
	Bacteria	6	TM[d] a	5	TM a	4	TM a
	Actinomycetes	0	0 a	2	5 a	31	700 a
HPDA	Fungi	4	7.50 a	10	13.33 a	8	83.33 ab
	Bacteria	23	TM a	18	TM a	45	TM a
	Actinomycetes	0	0 a	1	1.67 b	13	233.33 b
NA	Fungi	1	1.67 b	2	3.33 bc	0	0 d
	Bacteria	18	436.67 b	13	53.33 b	50	3616.67 b
	Actinomycetes	0	0 a	0	0 c	0	0 c

[a]No. of isol'ns = microbial diversity.
[b]No. of colonies = microbial frequency presented as CFU/ml. Values are averages of 4 ponds × 2 subsites × 2-3 replicates.
[c]Values within a column for each type of microbe followed by the same letter(s) are not significantly different according to LS means test ($p \leq 0.03$).
[d]Too many to be counted.

nomycetes. Nutrient agar and HPDA but not CA were the best for bacteria (Figure 3).

The most frequent microorganisms recovered from pond samples and their frequencies in each sample type are presented in Table 2. Only five genera of fungi were frequently recovered on the six media but the recovery varied from one medium to another. Two of these fungi, namely, *Cercosporidium* and *Chaetophoma* are known as plant pathogens (Table 3). Both fungi were recovered from hydrilla and soil. *Cladosporium* and *Penicillium* were recovered from all three types of samples. Whereas the former was isolated only from the ponds, the latter was more ubiquitous, occurring in both the ponds and the lakes. Twelve different bacteria and four actinomycetes were frequently retrieved from pond samples but their recovery also differed from one medium to another. Cyanobacteria (= blue green algae) were predominant on PART medium and the majority was retrieved from soil with a few from water samples (Table 2).

Recovery of Microbes from Orange Lake. According to the Chi-square procedure used for testing for microbial diversity, there was an association between the medium and the sample type with respect to fungi ($X^2 = 17.18$, $P = 0.03$); however, the medium and the sample type had no association with respect to bacteria ($X^2 = 14.74$, $P = 0.14$). Only two isolates of fungi (2 colonies/ml) and 11 strains of bacteria (3 colonies/ml on 5 media; excluding NA which had too numerous bacterial colonies to count) were recovered from the Orange Lake water. Two fungal isolates (2 colonies/ml) and 8 strains of bacteria (4 colonies/ml on 5 media only; excluding NA) were isolated from Orange Lake hydrilla. Soil samples taken from Orange Lake had larger diversity and higher frequency of fungi and bacteria: 26 fungal isolates (130 colonies/ml) and 23 strains of bacteria (48 colonies/ml on 5 media; excluding NA). No actinomycetes were recovered from Orange Lake samples on any of the 6 media (Table 4, Figure 4a).

An ANOVA confirmed the effects of medium, sample type, and medium x sample type interaction on the microbial frequencies resulted from Orange Lake samples. When isolating from water and hydrilla samples, all six media were equivalent with no differences among them in recovering fungi and bacteria except NA which was the most effective in isolating bacteria from both sample types, and CA which was the best for isolating greatest frequencies of fungi from hydrilla samples (Table 4). Chitin agar was also the most effective in isolating fungi from soil samples with no difference with HPDA. Komada's and SS were the second most effective while PART and NA were the least effective in retrieving fungi from the soil. However, NA followed by PART were the best for recovering bacteria from soil samples. Regardless of the sample type, the best media for isolating microbes in terms of frequency were CA, HPDA, and then K for fungi ($P = 0.04$); and NA followed by PART for bacteria ($P \leq 0.03$) (data not shown). Thus, similar order of best media as for ponds occurred also in Orange Lake with regard to the frequency of fungi but they differed slightly in terms of bacterial frequency.

In general, Orange Lake soil had a larger quantity of microflora than hydrilla or water samples (Figure 4a) and this finding was also similar to that obtained from ponds. Only four genera of fungi and five strains of bacteria were frequently recovered on the six media but their recovery differed from one medium to another. One of the fungal genera recovered, *Diplodia*, is known to include plant pathogenic species and was retrieved from the soil. The saprophyte *Penicillium* was predominant on CA and HPDA (Table 5). Cyanobacteria were predominant on PART medium, as with pond samples, and were recovered only from soils.

TABLE 2. ORGANISMS RECOVERED MOST FREQUENTLY FROM PONDS ON DIFFERENT MEDIA.

Medium	Type of organism	Microbe	Frequency of recovery from[a]		
			Water	Plant	Soil
SS	Fungi	*Cercosporidium* sp.	0	0.50	1.06
	Bacteria	B502 (G+)[b]	0	0.63	0
K	Fungi	*Cladosporium* sp.	1.71	1.63	1.08
		Staphylotrichum sp.	0	0.58	2.25
	Bacteria	B1003 (G+)	3.63	7.75	0.29
		B1007 (G+)	0	6.75	0
		B1006 (G+)	0	0	1.08
PART	Fungi	—	—	—	—
	Bacteria	B4002 (cyanobacterium)	0.17	0	7.50
CA	Fungi	*Chaetophoma* sp.	0	0.04	0.25
	Bacteria	B2001 (G-)[c]	TM[d]	TM	TM
		B2002 (G±)[e]	3.00	0.42	0
		B2004 (NT)[f]	7.58	0.63	3.83
	Actinomycetes[g]	A2001	0	0	0.42
		A2006	0	0	0.42
HPDA	Fungi	*Penicillium* sp.	0.17	0	0.04
	Bacteria	B3025 (G-)	TM	0	TM
		B3018 (G+)	6.17	0.04	7.58
	Actinomycetes	A3001	0	0	0.29
		A3014	0	0	0.17
NA	Fungi	—	—	—	—
	Bacteria	B2 (G+)	5.92	0.08	3.46
		B4 (G+)	4.46	0.25	0.21
		B34 (NT)	1.25	0	0.92

[a]Values are averages of the number of CFU/ml recovered from 4 ponds × 2 subsites × 2-3 replicates.
[b]Gram-positive.
[c]Gram-negative.
[d]Too many to be counted.
[e]Gram-variable.
[f]Not tested for Gram reaction because of confluent growth.
[g]Actinomycetes were recovered only on CA and HPDA media.

TABLE 3. GENERA OF FUNGI MOST FREQUENTLY RECOVERED FROM SAMPLES OF PONDS AND LAKES IN NORTH FLORIDA.

Genus	Isolated from					
	Ponds[a]	OL[a]	RL[a]	Water[b]	Hydrilla[b]	Soil[b]
Botryosporium	-	-	+	-	+	-
Cercosporidium	+	-	-	-	+	+
Chaetophoma	+	-	+	-	+	+
Cladosporium	+	-	-	+	+	+
Diplodia	-	+	-	-	-	+
Hansfordia	-	-	+	-	+	-
Penicillium	+	+	+	+	+	+
Pyrenochaeta	-	-	+	-	+	-
Staphylotrichum	+	-	-	-	+	+

[a]Ponds = four man-made ponds planted with hydrilla; OL = Orange Lake; RL = Rowell Lake. The lakes had natural infestations of hydrilla.
[b]Refer to water, hydrilla, or soil samples from pond and lake locations.

Recovery of Microbes from Rowell Lake. According to the Chi-square procedure used for testing for microbial diversity, there were associations between the medium and the sample type with respect to fungi ($X^2 = 25.88$, $P = 0.004$) and bacteria ($X^2 = 19.37$, $P = 0.04$). Twelve fungal isolates (3 colonies/ml) and 17 strains of bacteria (9 colonies/ml on 5 media only; excluding NA which had too numerous bacterial colonies to count) were recovered from Rowell Lake waters (Table 6). Twenty one isolates of fungi (15 colonies/ml) and 13 bacterial strains (7 colonies/ml on 5 media only; excluding NA) were isolated from hydrilla samples. Soil samples yielded 11 fungal isolates (12 colonies/ml) and 8 strains of bacteria (0.1 colony/ml on 5 media only; excluding NA). No actinomycetes were retrieved from Rowell Lake samples on any of the 6 media (Table 6, Figure 4b).

With regard to microbial frequency, the medium, the sample type, and the medium x sample type interaction influenced the quantity of the microflora presented in Rowell

Table 4. Comparison of six media and three sample types (water, plant, and soil) for enumeration of microbes recovered from Orange Lake.

Medium	Microbial type	Water		Plant		Soil	
		Isol'ns[a]	Colonies[b]	Isol'ns	Colonies	Isol'ns	Colonies
SS	Fungi	0	0 a[c]	0	0 b	9	12.67 b
	Bacteria	0	0 b	1	0.67 b	2	1.33 cd
K	Fungi	0	0 a	1	0.67 b	8	28.67 b
	Bacteria	0	0 b	1	1.33 b	5	6.67 c
PART	Fungi	0	0 a	0	0 b	0	0 c
	Bacteria	0	0 b	0	0 b	1	230 b
CA	Fungi	1	6.67 a	1	13.33 a	7	473.33 a
	Bacteria	0	0 b	0	0 b	0	0 d
HPDA	Fungi	1	6.67 a	0	0 b	2	266.67 a
	Bacteria	2	13.33 b	1	20 b	0	0 d
NA	Fungi	0	0 a	0	0 b	0	0 c
	Bacteria	9	TM[d] a	5	TM a	15	TM a

[a]No. of isol'ns = microbial diversity.
[b]No. of colonies = microbial frequency presented as CFU/ml. Values are averages of 2 subsites and 2-3 replicates.
[c]Values within a column for each type of microbe followed by the same letter(s) are not significantly different according to LS means test ($p \leq 0.02$).
[d]Too many to be counted.

Lake samples. Hydrilla-potato dextrose agar and PART were the most effective in isolating fungi from water samples but there were no differences between PART and the rest of the six media (Table 6). Chitin agar, HPDA, and PART were the best for recovering fungi from hydrilla; however, the latter two had significantly the same effect as K, while the least effective medium in that regard was NA. Komada's and PART were better than the rest of the media in isolating fungi from soil samples. Similar to the findings from Orange Lake, NA was the most effective in retrieving the largest quantity of bacteria from water, hydrilla, and soil samples taken from Rowell Lake. Regardless of the sample type, SS, K, PART, CA, and HPDA were equivalent but better than NA ($P \leq 0.03$) in isolating fungi (data not shown).

Predominantly six genera of fungi were recovered from Rowell Lake samples (Table 7). Three of them, namely, *Botryosporium, Chaetophoma,* and *Pyrenochaeta* are known as phytopathogenic genera. The first and the second were isolated from hydrilla samples only and the third was recovered from hydrilla and soil (Table 3). Thirteen strains of bacteria were frequently retrieved from lake samples, 11 of them were recovered on NA (Table 7). Unlike ponds and Orange Lake, cyanobacteria were not found in Rowell Lake samples.

Comparison of Ponds and Lakes. The four ponds and the two lakes used in this study seemed to have, in general, similar diversity of fungi and bacteria. However, actinomycetes were found only in ponds, with ponds 1 and 2 yielding greater number of them than ponds 3 and 4 (Figure 5a). With

Table 5. Organisms recovered most frequently from Orange Lake on different media.

Medium	Type of organism	Microbe	Frequency of recovery from[a]		
			Water	Plant	Soil
SS	Fungi	F802[b]	0	0	1.50
	Bacteria	-	—	—	—
K	Fungi	F1502	0	0	3.83
		Diplodia sp.	0	0	1.33
	Bacteria	-	—	—	—
PART	Fungi	-	—	—	—
	Bacteria	B4501 (cyanobacterium)	0	0	56.5
CA	Fungi	*Penicillium* sp.	0.17	0.33	0
	Bacteria	-	—	—	—
HPDA	Fungi	*Penicillium* sp.	0	0	0.50
	Bacteria	-	—	—	—
NA	Fungi	-	—	—	—
	Bacteria	B301 (G+)[c]	TM[d]	TM	TM
		B303 (G±)[e]	0.83	TM	0.83
		B309 (G+)	0	0	TM
		B316 (G±)	0	0	TM

[a]Values are averages of the number of CFU/ml recovered from 2 subsites × 2-3 replicates.
[b]Fungus collection accession numbers; unidentified isolate.
[c]Gram-positive.
[d]Too many to be counted.
[e]Gram-variable.

Table 6. Comparison of six media and three sample types (water, plant, soil) for enumeration of microbes recovered from Rowell Lake.

Medium	Microbial type	Water		Plant		Soil	
		Isol'ns[a]	Colonies[b]	Isol'ns	Colonies	Isol'ns	Colonies
SS	Fungi	2	2 b[c]	4	2.67 cd	3	3 b
	Bacteria	2	2 c	0	0 c	0	0 b
K	Fungi	0	0 b	6	4.67 bc	3	34.67 a
	Bacteria	1	2 c	1	0.67 c	1	0.67 b
PART	Fungi	2	3 ab	5	25 ab	5	33 a
	Bacteria	0	0 c	3	35 b	0	0 b
CA	Fungi	2	2 b	4	40 a	0	0 b
	Bacteria	3	2 c	0	0 c	0	0 b
HPDA	Fungi	6	13 a	2	20 ab	0	0 b
	Bacteria	4	41 b	0	0 c	0	0 b
NA	Fungi	0	0 b	0	0 d	0	0 b
	Bacteria	7	TM[d] a	9	TM a	7	TM a

[a]No. of isol'ns = microbial diversity.
[b]No. of colonies = microbial frequency presented as CFU/ml. Values are averages of 2 subsites and 2-4 replicates.
[c]Values within a column for each type of microbe followed by the same letter(s) are not significantly different according to LS means test ($p \leq 0.05$).
[d]Too many to be counted.

regard to the frequencies of microbes recovered, all sample sites were more or less equivalent with respect to fungi except for Orange Lake which yielded the largest number of fungal colonies, albeit with least diversity of fungi (Figure 5a & b). Larger variation was apparent among sample sites with respect to frequencies of bacteria. Ponds 1 and 2 showed higher frequencies of bacteria and actinomycetes than those obtained from Ponds 3 and 4. In general, lakes had fewer bacteria than ponds; Rowell Lake had the least population of bacteria (Figure 5b) although it showed a fair amount of bacterial diversity (Figure 5a).

In general, genera of fungi regarded as plant pathogenic were recovered predominantly from hydrilla and soil samples, whereas water samples yielded only saprophytic or weakly pathogenic genera (i.e., *Penicillium* and *Cladosporium*).

DISCUSSION

Despite the well-known limitations of plate-dilution technique, namely its proclivity to favor isolation of fast-growing, heavily sporulating saprophytes over slower growing and sparsely sporulating plant parasites and its total inability to isolate fastidious microbes and obligate parasites, it is useful as a general-purpose method for enumerating and isolating a wide range of microorganisms especially when selective media and media containing ingredients suppressive to the

Table 7. Organisms recovered most frequently from Rowell Lake on different media.

Medium	Type of organism	Microbe	Frequency of recovery from[a]		
			Water	Plant	Soil
SS	Fungi	*Pyrenochaeta* sp.	0	1.33	0
	Bacteria	—	—	—	—
K	Fungi	F1757[b]	0	0.67	33.33
	Bacteria	—	—	—	—
PART	Fungi	F4758	0	20	29
	Bacteria	B4751 (G-)[c]	0	33	0
CA	Fungi	*Hansfordia* sp.	0	25	0
	Bacteria	—	—	—	—
HPDA	Fungi	*Botryosporium* sp., *Hansfordia* sp.	0	10	0
		Penicillium sp.	5	0	0
	Bacteria	B3752 (NT)[d]	24	0	0
NA	Fungi	—	—	—	—
	Bacteria	B405 (G-)	0	TM[e]	166.6
		B401 (G-), B403 (G-), B412 (G-), B414 (G+)[f]	TM	0	0
		B404 (NT), B415 (G-), B416 (G+), B418 (G-)	0	TM	0
		B407 (NT), B410 (G-)	0	0	TM

[a]Values are averages of the number of CFU/ml recovered from 2 subsites × 2-4 replicates.
[b]Fungus collection accession number; unidentified isolate.
[c]Gram-negative.
[d]Not tested for Gram reaction because of confluent growth.
[e]Too many to be counted.
[f]Gram-positive.

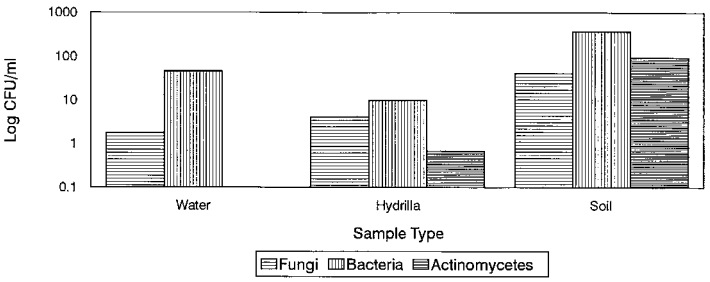

Figure 1. Microbial frequencies in water, hydrilla, and soil samples from ponds. Bars represent the log-transformed means of 4 ponds × 2 subsites × 2-3 replicates. Columns of bacteria represent the transformed number of colonies recovered on 4 media only; CA and HPDA were excluded since they had too numerous colonies to count.

fast-growing saprophytes are employed. For our purpose of characterizing the most frequent hydrilla-associated microorganisms, the technique has proved to be quite useful. Considerable microbial diversity and frequency were evident, with certain types of microbes predominating in each milieu (water, soil, and hydrilla).

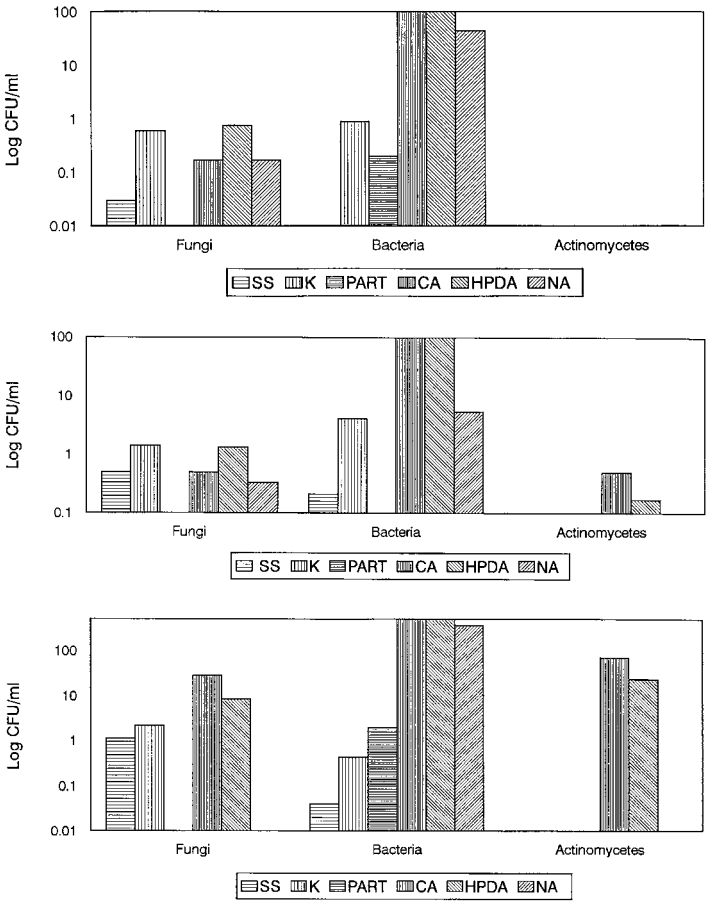

Figure 2. Microbial frequencies in pond-water (upper), -hydrilla (middle), and -soil (lower) samples on 6 media. Bars represent the log-transformed means of 4 ponds × 2 subsites × 2-3 replicates. Number of colonies recovered on PART medium was an average of 2 ponds × 2 replicates. Bacterial colonies recovered on CA and HPDA were too numerous to count but were represented in the graph with the probable largest values of frequency.

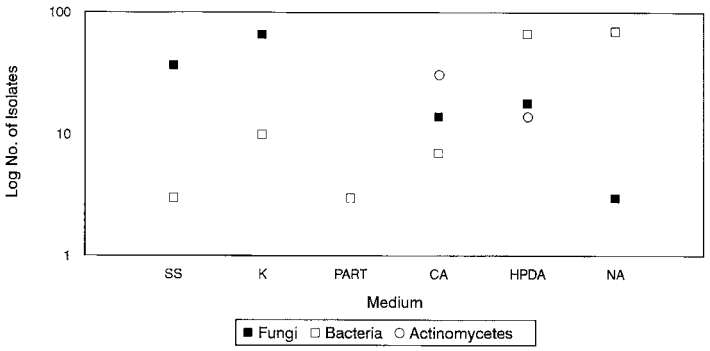

Figure 3. Microbial diversity obtained from pond samples on 6 media. Points represent the total number of different isolates recovered from water, hydrilla, and soil samples from four ponds each was sampled from two stations.

We have focused on the basic problem of selecting proper media for dilution plating to enumerate microorganisms from water, plant, and soil samples. In general, there was variation in the types of organisms isolated from the same sample depending on the medium employed. Samples from a particular site yielded different microbes when plated on different media; hence the employment of six selective and nonselective media in the present investigation is justified. Another reason for utilizing different media was that for each type of microbe one or two media are appropriate. Media containing low concentrations of organic substances (low-nutrient media) give higher colony counts than those containing high concentrations of organic substances (high-nutrient media) (Chand et al. 1992). This is in agreement with our results since CA and HPDA (low-nutrient media) provided the highest colony counts for fungi, bacteria, and

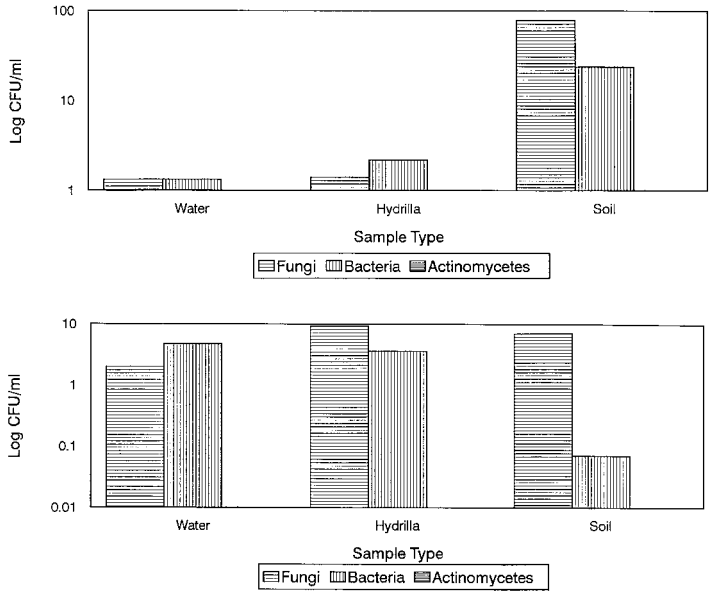

Figure 4. Microbial frequencies resulting from water, hydrilla, and soil samples from Orange Lake (upper) and Rowell Lake (lower). Bars represent the log-transformed means of 2 subsites × 2-4 replicates. Columns of bacteria represent the transformed number of colonies recovered on 5 media only; NA was excluded since it had too numerous colonies to count.

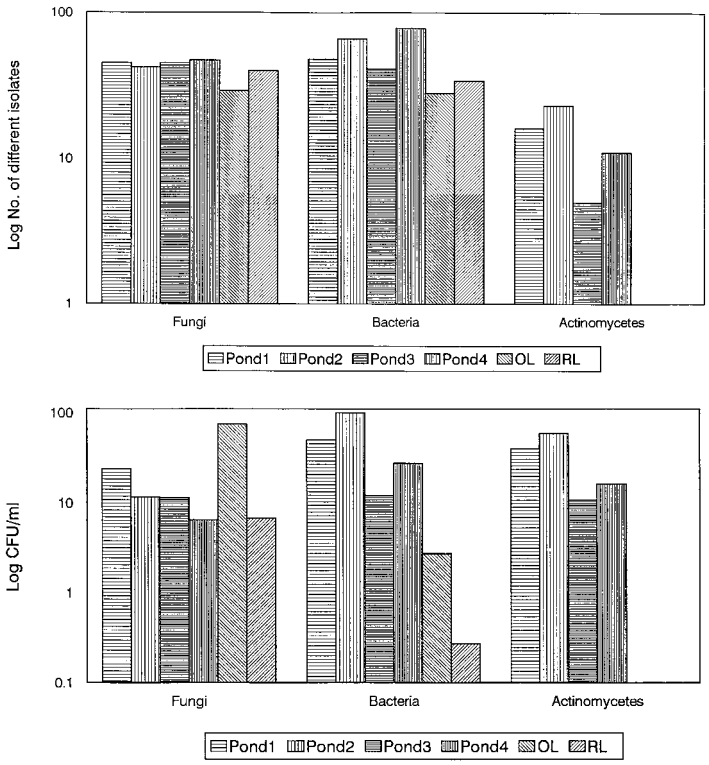

Figure 5: (upper) Microbial diversity in ponds and lakes. Bars represent the total number (log-transformed) of different isolates recovered on the 6 media pooled together, (lower) Microbial frequencies in ponds and lakes. Bars represent the log-transformed means of 2 subsites × 2-4 replicates. Columns of bacteria represent the transformed number of colonies recovered on the 6 media except CA and HPDA (for ponds) and NA (for lakes) since they had too numerous colonies to count. Data were divided by 10 and then log-transformed to magnify the small numbers. OL = Orange Lake; RL = Rowell Lake.

actinomycetes. Although CA and HPDA were best in that regard, there was a problem of overgrowth with *Penicillium* and/or bacteria on these media. The bacterial overgrowth was overcome by supplementing the medium with the antibiotics streptomycin sulphate and chloramphenicol, but the antibiotics depressed microbial growth on these media. Also, colonies too numerous to count or confluent growth occurred more often on CA, HPDA, and NA. On the contrary, K and the selective cultural media SS and PART gave distinctive colonies and fewer microbial counts. This again may be due to the presence of different fungicides and antibiotics in these media that suppressed the microbial growth. It can be further explained by the observation that microbial colonies did not appear on these media until 3 weeks after plating. Ramsay (1976) selected NA for studies on freshwater planktons, because it gave as great or greater diversity of bacteria than other media. In our study, NA was among the best for isolating a large variety and high counts of bacteria; it was comparable to CA and HPDA. However, Chand et al. (1992) found lower bacterial counts in high-nutrient medium such as NA presumably due to the rapid overgrowth of fast-growing bacteria (Hattori 1976). Our results agree in part with their results since we found lower bacterial count on NA (high-nutrient) than on CA and HPDA (low-nutrient) only when plating pond samples; the situation was reversed when lake samples were plated, possibly due to the addition of antibiotics to CA and HPDA used for plating the lake samples.

On the basis of colony characteristics and morphological properties, organisms were classified into different isolates. Chand et al. (1992) found that the bacterial cell shape and biochemical properties (Gram-stain reaction, bacterial spore-stain reaction, oxidative/fermentative use of glucose, motility, and oxidase test) confirmed that colonies with identical physical properties (color, size, and morphology) belonged to the same bacterial group. Similar observations were also recorded by Ramsay (1977). During purification and subculturing of microbial cultures, we observed that the colony characteristics of each isolate remained stable if medium and incubation conditions were not changed. Analogous results were obtained by Chand et al. (1992) with bacteria.

It should be noted that plant pathogenic genera of fungi were recovered only from hydrilla and soil but not from water samples. These fungi were recovered from asymptomatic plants; they are therefore weak or opportunistic parasites or occur at concentrations below the threshold necessary for disease initiation. The absence of hydrilla-associated actinomycetes is not surprising since actinomycetes generally are not plant pathogenic. However, they are strong phytotoxin producers and normally are preponderant in soil.

This is the first detailed investigation of microorganisms associated with hydrilla. Representative organisms isolated in the study are being evaluated in a bioassay to determine their pathogenic and phytotoxic capabilities on hydrilla.

ACKNOWLEDGMENTS

This research was supported with funds provided under a cooperative agreement No. 58-6629-4-008 between USDA-ARS and the Center for Aquatic Plants, University of Florida, Gainesville. We thank Jay Harrison for assistance with statistical analyses. Special thanks are due to James DeValerio for his generous technical assistance.

LITERATURE CITED

Chand, T., R. F. Harris and J. H. Andrews. 1992. Enumeration and characterization of bacterial colonies of a submersed aquatic plant, Eurasian watermilfoil (*Myriophyllum spicatum* L.). Appl. Environ. Microbiol. 58: 3374-3379.

Charudattan, R. 1990. Biological control of aquatic weeds by means of fungi. Pages 186-201 *in* A. H. Pieterse and K. J. Murphy eds. Aquatic Weeds: The Ecology and Management of Nuisance Aquatic Vegetation. Oxford University Press, New York, NY. 593 pp.

Hattori, T. 1976. Plate count of bacteria in soil on a diluted nutrient broth culture medium. Rep. Inst. Agric. Res. Tohoku Univ. 27: 23-30.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Protect. Res. 8: 114-125.

Lingappa, Y, and J. L. Lockwood. 1962. Chitin media for selective isolation and culture of actinomycetes. Phytopathol. 52: 317-323.

Pennington, J. C. 1984. Feasibility of applying genetic engineering technology to aquatic plant control. U.S. Army Engineers, Aquatic Plant Control Research Program Information Bulletin, A-84-2. U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Ramsay, A. J. 1976. Heterotrophic bacteria and their relationship with plankton in a New Zealand freshwater lake. N. Z. J. Marine Freshwater Res. 10: 77-90.

Ramsay, A. J. 1977. Aerobic heterotrophic bacteria isolated from water, mud, and macrophytes of Lake Grasmere, New Zealand. N. Z. J. Marine Freshwater Res. 11: 541-557.

SAS Institute. 1988. SAS Statistical Software Release 6.03, Cary, NC.

Sneh, B., and J. Stack. 1990. Selective medium for isolation of *Mycoleptodiscus terrestris* from soil sediments of aquatic environments. Appl. Environ. Microbiol. 56: 3273-3277.

Sorsa, K. K., E. V. Nordheim, and J. H. Andrews. 1988. Integrated control of Eurasian watermilfoil, *Myriophyllum spicatum*, by a fungal pathogen and a herbicide. J. Aquat. Plant Manage. 26: 12-17.