In vitro selection of duckweed geographical isolates for potential use in swine lagoon effluent renovation

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Abstract

Plant-based systems for nutrient sequestration into valuable biomass have the potential to help avoid the environmental problems associated with the disposal of large volumes of animal waste. The objective of this study was to select superior duckweed (Lemnaceae) genotypes for the utilization of nutrients in animal wastes. A two-step protocol was used to select promising duckweed geographic isolates to be grown on swine lagoon effluent. Forty-one geographic isolates from the worldwide germplasm collection were used in an in vitro screening test, because they were noted to be fast-growing genotypes during routine collection maintenance. In vitro screening was accomplished by growing geographic isolates on a synthetic medium that approximated swine lagoon effluent in terms of nutrient profile, total ionic strength, pH, and buffering capacity. Large differences among geographic isolates were observed for wet weight gain during the 11-day growing period, percent dry weight, and percent protein in dry biomass. Total protein production per culture jar differed 28-fold between the most disparate of the 41 geographic isolates and was the variable used for selection of superior geographic isolates. The challenge of eight of the 41 geographic isolates with full-strength swine lagoon effluent in the greenhouse led to the selection of three that are promising as genotypes to be grown on lagoon effluent. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Duckweed; Growth; Lemnaceae; Lemna gibba; Lemna minor; Protein production; Spirodela punctata; Swine waste; Wolfla; Wolflella

1. Introduction

Enormous quantities of nutrients are present in animal wastes produced at modern, large-scale animal production facilities, a situation that has created global environmental concern (Williams, 1995). Technologies are needed for effective utilization of waste stream nutrients so that they do not contribute to surface and ground water contamination. Alternative plant-based systems for sequestering nutrients into biomass potentially may avoid the environmental problems associated with disposal of animal wastes.

Land application of animal waste is common and relatively successful in the midwestern US where deep, permeable soils and integration of feed crop and animal production allow nutrient recovery from the manure stream via land application systems with feed crops such as corn. However, in the southeastern US, swine farms are not associated with large tracts of land, corn and soybean yields are lower than in the Midwest, and the utilized crops are selected for nutrient uptake rather than usefulness (such as feed grains). These factors result in the transformation of animal waste nutrients from one problematic form to another, i.e. large amounts of low value biomass that is stockpiled in nutrient rich areas.

Build-up of some waste constituents in the soils to which animal waste has been applied over an extended period of time has been documented. This is particularly true in the case of poultry litter, which can lead to soil accumulation of excess phosphorus (Heathman et al., 1995; Chapman, 1996) and copper and zinc (Mitchell et al., 1992; van der Watt et al., 1994). Elevated nitrogen loading rates can increase the NO3-nitrogen levels in forage biomass to a level that raises toxicity concerns (Harvey et al., 1996) because excess nitrate is reduced to nitrite in the digestive tract of grazing ruminants, and the resulting excess nitrite limits blood oxygen transport (Ball et al., 1996).

Treatment technologies that are effective only during a given crop’s active growing season require storage of...
large volumes of waste at year-round animal production facilities at a significant cost to the producer. In general, duckweeds grow over a longer period compared to crops with a determinate growth habit (e.g. maize, soybeans). The length of the effective wastewater renovation period for a duckweed-based system might be extended by selecting geographic isolates that perform well in the early spring and late autumn. These would compliment the geographic isolates shown to be most efficient for biomass/protein production during the heat of the summer.

It is logical to focus on duckweed species (Lemnaceae) for use in aquatic systems designed for recovery of nutrients in animal wastes. Dry matter production is high compared with other crops, e.g. 20.4 t/ha/yr reported for Spirodeles polyrrhiza (Edwards et al., 1992) and 54.8 t/ha/yr for Lemna gibba (Oron et al., 1988; Oron, 1990). Effective removal of nutrients from wastewater through duckweed production has been demonstrated (Harvey and Fox, 1973; Sutton and Orones, 1975; Buddhavarapu and Hancock, 1991). When applied to nutrient utilization from animal waste, the tendency of duckweed to assimilate NH$_4^+$ efficiently (Monselise et al., 1987) and in preference to NO$_3^-$ (Monselise and Kost, 1993) is a valuable trait. An obvious end-use for dried duckweed is as an animal feed supplement, as it typically contains between 24% and 45% protein (Edwards et al., 1992; Leng et al., 1995; Oron, 1990; Oron et al., 1988) and has been shown to be an adequate substitute for high protein supplements such as soymeal in feed for poultry (Truax et al., 1972; Haustein et al., 1990, 1991, 1994), swine (Leng et al., 1995; Rodriguez and Preston, 1996; Hang et al., 1997; Van et al., 1997), dairy cattle (Culley and Epps, 1973; Rusoff et al., 1977; Rusoff et al., 1978; Rusoff et al., 1980; Leng, 1990), and fish (Shireman, 1978; Jauncey and Matty, 1979; Gaigher et al., 1984; Mbagwu and Adeniji, 1988; Hassan and Edwards, 1992; Ray and Das, 1994).

Other features that make duckweed an attractive target for nutrient utilization systems include its great genetic diversity and the fact that it clones itself. The wide geographic distribution of populations within the 36 species within Lemnaceae suggests that significant variation will be detected among genotypes for traits such as the ability to grow well on different types and concentrations of wastewater, the ability to proliferate at high or low temperatures, protein production, and suitability of the biomass produced for different end uses. This creates the opportunity to select superior geographic isolates and to match suitable geographic isolates to specific environments and/or waste streams. Once such a match is made, the genetic make-up of the population used for nutrient sequestration should stay relatively constant as the vast majority of progeny are produced through asexual reproduction from mother fronds.

When considered as a new crop, a logical starting point for research with duckweed is improvement with respect to desired traits. Generally, the first step in a crop improvement effort is to acquire a diverse genetic base upon which to build the program. This step has been accomplished by the transfer of the worldwide duckweed germplasm collection from Dr. Elias Landolt (ETH, Zurich, Switzerland) to the Environmental Biotechnology Laboratory at North Carolina State University. The collection consists of nearly 1000 geographic isolates belonging to 36 species within the four genera in Lemnaceae: Lemna, Spirodeles, Wolffia, Wolffia. The next step is to select genotypes that exhibit desirable traits, particularly important in cases such as duckweed for which asexual propagation will be used. The objective of the research presented was to identify one or more duckweed geographic isolates especially suited to growth on swine lagoon effluent.

2. Methods

2.1. In vitro screening

An in vitro protocol was used for the selection of promising geographic isolates to be grown on swine lagoon effluent in the greenhouse. This was done because in vitro conditions permitted the testing of a large number of genotypes in a relatively small area. Also, the growth chamber provided the uniformity needed to evaluate genotypes without the confounding influence of fluctuating environmental conditions. Forty-one geographic isolates were used, chosen from the collection based on the observation that they exhibited rapid growth during routine culture maintenance. The 41 geographic isolates represented 12 species, at least one species from each of the four genera within Lemnaceae (Table 1). Eight of the geographic isolates were native to the United States.

The culture medium was designated swine artificial medium (SAM) as it was formulated to closely resemble the nutrient profile, total ionic strength, pH, and buffering capacity of swine lagoon effluent commonly found in North Carolina (Table 2). The carbon source in SAM was 3.0% sucrose, 1.15 g/l citric acid was used as a buffering agent, and the pH after autoclaving was 7.0. Medium was autoclaved at 121°C for 18 min.

Glass baby food jars (6.0 cm outer diameter x 7.0 cm tall) with polypropylene caps (Magenta Corp., Chicago IL) served as culture vessels. Each jar was inoculated with fronds from one of the 41 geographic isolates such that 35% of the medium surface area was covered. This was accomplished by completely covering the surface of a water-filled 10 ml beaker with fronds before placing them into the larger culture vessels and was done to reduce bias by differing frond sizes among geographic
isolates. Previous experiments indicated biomass production would likely be biased in favor of geographic isolates with large fronds when inoculating with a fixed number of fronds and in favor of geographic isolates with small fronds when inoculating with a fixed initial fresh weight. The variable of most importance when considering the practical implementation of a system is the quantity of biomass/protein produced when a given surface area is covered at the outset of a given culture period.

Prior to culture initiation, the duckweed geographic isolates had been precultured for 2 weeks on Schenk and Hildebrandt (1972) medium (SH) supplemented with 3.0% sucrose and adjusted to pH 5.8. Jars were placed into a 23°C growth chamber with a 16-hour photoperiod and a photosynthetic photon flux density of 40 lmol/m²/s provided by wide spectrum fluorescent tubes. The culture period was 11 days.

The experiment was conducted as a completely randomized design. Each geographic isolate was represented by three jars, and the experiment was repeated once. Thus, there was a total of six jars per geographic isolate and a total of 246 jars. Replicates were placed on separate shelves in the growth chamber. Frond fresh weight was recorded for each jar at the start and end of the culture period. These two values were used to calculate the fresh weight gain during the culture period. Fronds were blotted dry with paper towels and placed into an incubator maintained at 37°C for 72 h prior to recording frond dry weight for each jar. Frond protein content was determined using the Lowry protein determination procedure (Stoscheck, 1990). Total protein production for each jar was calculated by multiplying frond fresh weight gain by frond percent dry weight and multiplying the product by percent protein content on a dry weight basis.

**2.2. Statistical analysis**

Statistical analyses were accomplished using SAS (SAS Institute, Cary, NC). Analysis of variance were
done using a data set with only the six species (33 geographic isolates) which were each represented by more than two geographic isolates in order to examine the influence of species on the observed variables. The independent variables were placed in the following order in the statistical model: replicate, species, and geographic isolate within species followed by the one-way interactions of replicate with species and with geographic isolate within species. Once it was determined from the general linear models procedure that each of the observed variables varied significantly among species, Duncan's critical range tests were used to separate species means for dependent variables.

After the strong influence of geographic isolate on the dependent variables was revealed, a second set of analyses was done using the entire data set to focus on geographic isolates. The independent variables in the analyses of variance were placed in the following order: replicate, jar within replicate, geographic isolate, and replicate by geographic isolate interaction. Duncan's critical range tests were used to separate geographic isolate means for dependent variables after the general linear models procedure showed that each of the observed variables varied significantly among geographic isolates. Pearson correlation coefficients were calculated between pairs of independent variables using the 41 geographic isolate means for each variable: fresh weight gain, percent dry weight, percent protein on a dry weight basis, and total protein production.

### 2.3. Growth of selected geographic isolates on swine lagoon effluent in the greenhouse

The six duckweed geographic isolates (i.e. 15% of the original 41) that were most highly ranked on the basis of absolute protein production from the in vitro screening experiment were used to observe growth and performance on non-diluted swine lagoon effluent in the greenhouse. Two additional geographic isolates were included based on the fact that efficient genetic engineering protocols have been developed for them (Stomp and Rajbhandari, unpublished data): *Lemna minor* geographic isolates 8627 and 8744 which were ranked 14 and 30, respectively. The eight geographic isolates were grown in the growth chamber in large flasks to produce enough fronds to inoculate large tubs in the greenhouse. The medium was SH supplemented with 3.0% sucrose and adjusted to pH 5.8, and growth chamber conditions were the same as described previously.

Plastic tubs were placed into insulated boxes with bottom heat in the greenhouse. Day-time air temperatures fluctuated between 19°C and 34°C, but water temperature was maintained at 25 ± 2°C. The test was conducted during late spring and early summer without supplemental lighting or shading. The 24 l non-diluted swine lagoon effluent (taken from the NCSU Field Laboratory) placed into each tub created a 36×80 cm² surface area. Non-diluted swine lagoon effluent was used to provide the greatest challenge to the eight geographic isolates. Water lost to evaporation was replaced with tap water twice a week.

One tub per geographic isolate was used in each of two sequential replicates. A sufficient number of fronds was placed in each tub to completely cover the water surface at the start of each replicate. Qualitative observations of frond health were the only recorded data during the 4-week growing period.

### 3. Results

#### 3.1. In vitro screening

Forty-one duckweed geographic isolates were grown in vitro on buffered SAM for 11 days. Data from the six species that were each represented by more than two geographic isolates showed that species influenced all the observed variables (p < 0.01): initial fresh weight, fresh weight gain, percent dry weight, percent protein on a dry weight basis, and total protein production. The variation among geographic isolates within species (p < 0.01) was found to be as great or greater than that of species for all variables. Influence of replicate and one-way interactions with replicate were not significant (p > 0.05) for any variable.

Data from all 41 geographic isolates were used in analyses to focus on geographic isolate as a variable because of its clear influence on all variables. As expected, the initial frond fresh weights varied (p < 0.01) among geographic isolates when an equal surface area was used to inoculate each vessel. Fresh weight gain during the culture period differed (p < 0.01) among geographic isolates with an 11-fold difference between the geographic isolates exhibiting the most and least growth (Table 4). Percent dry weight for fronds differed (p < 0.01) among geographic isolates with a 3-fold difference between the highest and lowest geographic isolates (Table 4). Protein content based on dry weight differed (p < 0.01) among geographic isolates with a 9-fold difference exhibited between the geographic isolates with the highest and lowest concentrations (Table 4). The significant influence of jar within replicate (p = 0.03) in the case of percent dry weight was considered an artifact. In no other case was the influence of replicate, jar within replicate, or replicate by geographic isolate interaction significant.

Geographic isolate rankings differed markedly among fresh weight gain, percent dry weight, and protein content. For example, *Lemna aequinoctialis* 8230 was ranked first for fresh weight gain, sixth for percent dry weight, and 35th for protein content. *Lemna gibba* G3 was ranked 27th and 37th for fresh weight gain and
percent dry weight, respectively, but was first in protein content. For this reason, the variable “total protein production” was calculated to yield a single variable by which the most productive geographic isolates were selected. Geographic isolates were highly variable (p < 0.01) in total protein production, ranging from 1.04 mg/jar for *Wolffia oblonga* 8817 to 28.87 mg/jar for *Spirodela punctata* 7776. Total protein production was not influenced by replicate, jar within replicate, or replicate by geographic isolate interaction.

The six most highly ranked geographic isolates according to total protein production are shown in Table 5. These six geographic isolates tend to be relatively highly ranked based on fresh weight gain, but there was no consistent trend with regard to ranking according to percent dry weight or protein content (Table 5).

### Table 5

<table>
<thead>
<tr>
<th>Species – geographic isolate</th>
<th>Total protein production (g)</th>
<th>Fresh weight gain (g/jar)</th>
<th>Dry weight (%)</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Spirodela punctata</em> – 7776</td>
<td>1 0.24</td>
<td>7 9.3</td>
<td>9 22.2</td>
<td>17 11.6</td>
</tr>
<tr>
<td><em>Lemma gibba</em> – 8678</td>
<td>2 0.22</td>
<td>21 7.1</td>
<td>29 16.4</td>
<td>2 18.7</td>
</tr>
<tr>
<td><em>Lemma minor</em> – 7501</td>
<td>3 0.21</td>
<td>14 7.8</td>
<td>21 19.3</td>
<td>14 13.8</td>
</tr>
<tr>
<td><em>Spirodela punctata</em> – 7488</td>
<td>4 0.21</td>
<td>9 9.2</td>
<td>5 23.8</td>
<td>24 9.5</td>
</tr>
<tr>
<td><em>Lemma obscura</em> – 7720</td>
<td>5 0.20</td>
<td>10.3 5</td>
<td>38 12.8</td>
<td>6 15.4</td>
</tr>
<tr>
<td><em>Lemma aquinoncials</em> – 8715</td>
<td>6 0.18</td>
<td>2 13.1</td>
<td>16 20.4</td>
<td>30 6.6</td>
</tr>
<tr>
<td>Critical range</td>
<td>0.04</td>
<td>1.6</td>
<td>2.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Table 3

Performance of duckweed species (represented by three or more geographic isolates each) grown in vitro on buffered swine artificial medium for 11 days

<table>
<thead>
<tr>
<th>Species</th>
<th>Fresh weight gain (g)</th>
<th>Percent dry weight</th>
<th>Protein content (%)</th>
<th>Total protein production (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lemna aequinocialis</em></td>
<td>8.6 ab</td>
<td>27.0 a</td>
<td>6.3 d</td>
<td>0.17 c</td>
</tr>
<tr>
<td><em>Lemna gibba</em></td>
<td>6.6 c</td>
<td>15.2 c</td>
<td>16.9 a</td>
<td>0.23 a</td>
</tr>
<tr>
<td><em>Lemna minor</em></td>
<td>6.7 c</td>
<td>18.7 c</td>
<td>13.4 b</td>
<td>0.20 b</td>
</tr>
<tr>
<td><em>Lemna minuta</em></td>
<td>8.9 a</td>
<td>15.7 e</td>
<td>9.1 c</td>
<td>0.17 c</td>
</tr>
<tr>
<td><em>Spirodela polyrhiza</em></td>
<td>8.1 b</td>
<td>20.2 b</td>
<td>6.5 d</td>
<td>0.16 c</td>
</tr>
<tr>
<td><em>Wolffia oblonga</em></td>
<td>1.8 d</td>
<td>17.4 d</td>
<td>8.9 c</td>
<td>0.02 d</td>
</tr>
</tbody>
</table>

### Table 4

Fresh weight gain, percent dry weight, and protein content on a dry weight basis for the most disparate of 41 duckweed geographic isolates screened over an 11-day period growing in vitro on buffered swine artificial medium

<table>
<thead>
<tr>
<th>Geographic Isolate</th>
<th>Fresh weight gain (g/jar)</th>
<th>Dry weight (%)</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest ranked</td>
<td>1.4</td>
<td>12.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Critical range</td>
<td>1.6</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Highest ranked</td>
<td>15.2</td>
<td>37.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Mean of all 41</td>
<td>7.0</td>
<td>19.5</td>
<td>18.9</td>
</tr>
</tbody>
</table>

### Table 2

Performance of duckweed species (represented by three or more geographic isolates each) grown in vitro on buffered swine artificial medium for 11 days

The three species of *Lemna* and the two species of *Spirodela* were highly variable in total protein production. For this reason, the variable “total protein production” was calculated to yield a single variable by which the most productive geographic isolates were selected. Geographic isolates were highly variable (p < 0.01) in total protein production, ranging from 1.04 mg/jar for *Wolffia oblonga* 8817 to 28.87 mg/jar for *Spirodela punctata* 7776. Total protein production was not influenced by replicate, jar within replicate, or replicate by geographic isolate interaction.

Three of the six correlations between pairs of dependent variables, calculated using geographic isolate means, were significant. High percent dry weight was associated with relatively low protein content (r = −0.49, p < 0.01), and strains with high protein content tended to have high total protein production (r = −0.47, p < 0.01). The closest association (r = −0.64, p < 0.01) was found between fresh weight gain and total protein production (Fig. 1).

### 3.2. Growth of selected geographic isolates on swine lagoon effluent in the greenhouse

Characteristics of the swine lagoon effluent used for the greenhouse experiment are shown in Table 6. None of the geographic isolates grew rapidly, but their
Fig. 1. Fresh weight gain and total protein production by 41 duckweed geographic isolates during an 11-day growing period in vitro on buffered swine artificial medium. Pearson correlation coefficient for the two variables based on geographic isolate means (n = 6); r = 0.64, p = 0.0001. Geographic isolate symbols: + tested in vitro only, □ selected for in vitro performance followed by poor greenhouse growth, ● selected for in vitro performance followed by good greenhouse growth, ○ not selected in vitro and exhibited poor greenhouse growth, ● not selected in vitro and exhibited good greenhouse growth.

Table 6

<table>
<thead>
<tr>
<th>Characteristics of unmodified swine lagoon effluent used to grow eight duckweed geographic isolates in the greenhouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>18.71 mM</td>
</tr>
<tr>
<td>6.92 mM</td>
</tr>
</tbody>
</table>

responses differed markedly as judged by non-quantified, visual estimates. *Lemna gibba* 8678 appeared to tolerate the lagoon effluent better than the other geographic isolates and maintained healthy fronds that multiplied slowly, and *Spirodea punctata* 7776 remained relatively healthy without noteworthy multiplication. These geographic isolates were ranked second and first, respectively, in the in vitro screening experiment. The remaining geographic isolates of the six selected in the first experiment did not tolerate non-diluted swine lagoon effluent, responding with slow senescence of the majority of fronds.

The two geographic isolates included because they can be efficiently genetically engineered differed in response to non-diluted swine lagoon effluent. The rapid senescence of *Lemna minor* 8744 fronds was not surprising given its ranking of thirteenth in the first experiment. However, fronds of *Lemna minor* 8627 exhibited about 50% survival (without noteworthy multiplication) despite its ranking of fourteenth in the in vitro screening experiment. Qualitative observations were uniform between replicates and led to the following ranking of the eight geographic isolates according to health during 4 weeks on non-diluted swine lagoon effluent in the greenhouse: 1 – *L. gibba* 8678, 2 – *S. punctata* 7776, 3 – *L. minor* 8627, 4 – *L. minor* 7501 and *S. punctata* 7488, 5 – *L. obscura* 7720 and *L. aequinoctialis* 8715, 6 – *L. minor* 8744.

### 4. Discussion

The promise for selecting outstanding duckweed genotypes for use in waste nutrient utilization systems was demonstrated. Clear differences were found among species and geographic isolates for traits that are important for production of valuable biomass on swine lagoon effluent: fresh weight gain, percentage dry weight, protein content on a dry weight basis, and total protein production over a defined growing period.

The large influence of geographic isolate on the observed traits suggested that it would be practical to focus attention on the geographic isolate rather than the species level. This approach is supported by observations of geographic isolates that respond relatively well or poorly for a given variable compared to the species to which it belongs. For example, while *Lemna gibba* produced the most total protein as a species during the in vitro trial, its geographic isolates ranged in rank from 2nd to 18th among the 41 tested. The spread for the most disparate *Lemna minor* geographic isolates according to total protein production was from 3rd to 30th.

Response differences among geographic isolates were expected given that 41 were used from 12 species within the four *Lemnaceae* genera. Similar performance differences were reported when 11 duckweed geographic isolates were grown on partially treated domestic sewage (House et al., 1999). The fact that the three promising geographic isolates in the present work are in two genera and that each is within a different species points to the usefulness of selecting directly at the geographic isolate level rather than starting at the genus or even species level. These three geographic isolates originate from widely divergent points, i.e. India, Australia, and Denmark, which indicates that selection from a genetically diverse duckweed collection for a given trait could afford significant improvement compared to the duckweeds that happen to grow locally. If a selected duckweed is intended for use in a non-confined system, it would be wise to screen a larger number of duckweed isolates endemic to a relatively limited geographic area given the concern for the potential invasiveness of exotic isolates.

The single trait total protein production resulted from the combination of three other traits and was used for selection in this work. However, care must be taken in choosing selection criteria since selection can be
accomplished via several methods. For example, one could focus on the set of geographic isolates that emerges when only those are selected that are in the top 50% of all desired traits. Use of such a method would result in the selection of four geographic isolates, two of which were among the six selected using the single combined trait. Likewise, selection of traits in sequence could be used to define a select group of geographic isolates. This was done with the 41 geographic isolates by selecting the top 21 with regard to fresh weight gain (the trait most closely associated with total protein production) and then selecting the top 11 of those 21 with regard to percent protein (important in choosing geographic isolates of potentially greater value). Finally, the top six from among those 11 geographic isolates were selected according to percent dry weight. Among the six selected via this method were the top four selected via this method were the top four selected using the single trait total protein production, including the two that performed well on non-diluted swine lagoon effluent in the greenhouse. One of the two that were changed from the total protein production selection method was Lemna minor 8627, ranked 14th in that method but shown to be promising for growth in the greenhouse on swine lagoon effluent.

Caution must be used in assuming that the types and magnitudes of variation in a controlled in vitro system would be seen in a field system. Light level, daylength, temperature, and temperature fluctuations are some of the variables that would significantly influence duckweed biomass and protein production and that differ greatly between the growth chamber and in situ systems. Batches of swine lagoon effluent will vary in nutrient levels, another factor that will influence duckweed performance. The ranking of these 41 geographic isolates would likely change if all were tested in the greenhouse or field compared to rankings in vitro. Greenhouse and field tests utilizing a large number of geographic isolates would be cumbersome and costly compared to the in vitro screening method used here. Therefore, greenhouse and field trials will continue with L. gibba 8678, S. punctata 7776, and L. minor 8627 because they grew on non-diluted swine lagoon effluent in the greenhouse after having been selected for total protein production in vitro on medium that closely resembled typical swine lagoon effluent. The goal of the continued research will be to define a continuous-flow swine lagoon effluent treatment system that simultaneously produces biomass of value.

References


