An initial examination of the contents of commercial aquatic microbial algae suppressing formulations

Paul H. Lord

INTRODUCTION

At least five commercial products are currently marketed with the claim that they inhibit algae growth in fresh water ponds by introducing bacteria. These products are:
1. Pond Saver® by Plant Health Care, Incorporated® (1997);
2. Cygnet Bio Blend® by Aquatic Nuisance Plant Control and Cygnet Enterprises, Incorporated® (1998);
3. Bacta-Pur Klear Bacteria® by Aquatic Eco-systems, Incorporated® (undated);
4. Pond Clarifier® by Organica, Incorporated® (1999); and

These products appear to be based on research initiated in the 1970s (Shilo, 1970; Granhall and Berg, 1972; Burnham, 1973; 1975; 1977; Daft, et al., 1973; Reim et al., 1974; Martin, 1976; Burnham et al., 1976; Darveau and Lynch, 1977; Delucca and McCracken, 1977; Mayfield and Inniss, 1978; Berger, 1979) after bacterial associations with algae cultures were noted in the 1960s (Safferman and Morris, 1962; Mayer et al., 1964; Blasco, 1965; Shilo, 1967). Some of the 1970s research documented the competition for usable nitrogen compounds between aquatic (photosynthetic) algae and aquatic (nonphotosynthetic) bacteria. Follow-up research in the early 1980s continued (Yamakana and Glazer, 1980; Gromov and Mamkaeva, 1980; Burnham et al., 1981, 1984; Burnham, 1981a; 1981b; 1984; Cole, 1982; Halemejko, 1984). However, research in the later 1980s and 1990s, into this potential algae control mechanism, is sparse with only a few known related papers published (Coder and Goff, 1986; Peterson et al., 1993). By way of contrast, there are a larger number of papers published in the last two decades discussing the use of bacteria as inhibitors of various aquatic macrophytes (e.g., Gunner, 1982; 1983; 1984; Galbraith, 1984a; 1984b; Pennington, 1984; 1985; Underwood, 1991; Shearer, 1997). Recent texts devoted to the algae (Vymazal, 1995; Stevenson, et al., 1996) devote less than a half page each to adverse bacterial interactions with them. A review of the literature identified 17 bacteria taxa likely to be used in such formulations. These are identified in Appendix A.

Despite manufacturers’ marketing claims, local experience has shown that only rarely do bacterial formulations make a noticeable improvement in water quality (Harman, 1998). During 1998 the use of Pond Saver in application on a small private pond was supervised by Albright (1999). The bacteria provided no noticeable difference

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whereas the subsequent application of alum significantly improved water clarity (Albright, 2000).

This study examined the contents of commercial microbial products to determine their consistency with earlier research and present-day advertising, and then to investigate their effectiveness. Commercial preparations were cultured, microorganisms isolated and identifications were attempted using standard methods (Rodina, 1972; Bukovsan, 1986; Austin, 1988; Leboffe, 1996; Prescott, 1996). Additionally, algae were cultured in flasks and the formulations were tested, according to manufacturer directions and in excess, to determine algaecidal character. Finally, product content was examined for sand, total phosphorous, and nitrates-nitrites.

MATERIALS AND METHODS

A one liter bottle of Bacta-Pur Klear®, a one pound (.455 kg) box of Organica Pond Clarifier® and a three pound (1.36 kg) box of Pond Saver® were obtained from the previously identified commercial sources. Taking note of Pond Saver’s advertised claim of four billion colony-forming units (CFUs) per gram, dilutions of Pond Saver were initiated per Appendix B (Table 1) to verify the CFUs and to identify colony types. Appendix B, Table 2 was derived from Organica, Incorporated claims of 400 billion bacteria per pound. Assuming Bacta-Pur Klear would provide similar CFUs per dosage, dilutions of that product were initiated per Appendix B, Tables 3, 4, and 5. Liquid Difco® brain heart infusion (BHI) nutrient agar was mixed with 0.1 ml of dilutions 2, 3, and 4 for each product using the pour plate methodology (Bukovsan, 1986) and incubated at 20° (+/- 2°) C. Additionally, a 0.1 ml sample of pure Bacta-Pur Klear was mixed with the BHI nutrient agar and incubated identically. Observations were made at 24 and 48 hours and noted.

After initial observations, a revision of Appendix B, Table 5, was designed (Appendix B, Table 6) to provide satisfactory colony count ranges for Bacta-Pur Klear.

To avoid widely varying CFUs in plates cultured from dilutions of the dry products (i.e., Pond Saver and Pond Clarifier), products were hydrated prior to use. Aquarium bubble stones (AquaFizzle® Art. #A-762) and clear plastic aquarium tubing (Blue Ribbon Pet Products Inc.) plugged with fiber plugs (Brentwood Salon Care® Prod. No. 504200) were autoclaved at 121°C and 15 psi for 20 minutes. Air was then pumped through the tubing, plugs, and bubble stones into the Dilution 0 bottles for 24 hours before it was used to form subsequent dilutions. Buffered water was prepared per APHA, AWWA, WPCF (1992) in order to ensure pH requirements of manufacturers. Each dilution was stirred for a minimum of one minute with a sterilized Teflon® covered magnetic stirrer and then shaken for 30 seconds prior to further dilution and inoculation.

To avoid “cooking” any heat sensitive bacteria, spread plates (as opposed to pour plates) were used. This was accomplished using an ethyl alcohol washed and flamed “L”
shaped bent stirring rod (Drygalski spatula) which was used to push the 0.1ml sample over the entire surface of the petri plate (Bukovsan, 1998; Austin, 1988). The ethyl alcohol was cooled in an ice bath and two separate spatulas were alternately used to prevent overheating with continued use and flaming.

Dilutions of all products were streaked on Difco Plate Count Agar (PCA), Difco Brain Heart Infusion Agar, and NWRI agar prepared per APHA, AWWA, WPCF (1992). All plates were incubated at 20° (+/- 2 °C).

Additionally, an attempt, patterned after Burnham (1973), was made to grow the bacteria from Bacta-Pur Klear and Pond Saver on media prepared from fresh algae (tentatively identified as *Ankistrodesmus falcatus* (Palmer, 1962)). Water from a BFS aquarium that had maintained a monospecific bloom of green algae for several months was filtered using a Whatman® 4.7cm glass micro fibre filter (cat no. 1820 047) and a vacuum pump. When the filter became clogged, the algae and filter were placed in a screw-top 250-ml Erlenmeyer flask and filled to the 125-ml mark with a solution of agar made from 25 g of agar and 500 ml of water heated to boiling. These flasks were then autoclaved at 121°C and 15 psi for 20 minutes. The media was then used in spread plates.

*Anabaena* sp. (tentatively identified as *A. constricta* [cf. Palmer, 1962]) and *Chlorella pyrenoidosa* (identified by Ward’s Natural Science Establishment Incorporated®) were obtained from Ward’s Natural Science Establishment Inc. Attempts were made to culture these algae using a variety of media, light regimens, and mixing techniques. After initial testing, the *Chlorella pyrenoidosa* was cultured using a modified Bristol’s solution (Ward’s Natural Science Establishment, Inc., undated, after Bold 1949). This media was Bristol’s solution (80%) with minor alterations to micronutrients and 20% soil-water medium (Ward’s Natural Science Establishment, Inc., undated). Additionally, *C. pyrenoidiosa* was grown on Ward’s supplied Proteose Agar slants that were made from this media supplemented with proteose peptone and agar (Ward’s Natural Science Establishment, Inc., undated). The *Anabaena* sp. was cultured using a proteose peptone Bristol’s solution medium (Seeber, 1982). Both algae were cultured with a light regimen which maintained a lighted cool-white fluorescent light 18 inches (45.5 cm) from the bottom of the flasks for 16 hours out of every 24 (5:00 AM to 9:00 PM; by use of a Paragon TI+MITE® timer) to simulate summer epilimnion conditions. No shaking was used. *Anabaena* sp. flasks were swirled once every four or five days. *C. pyrenoidiosa* flasks were not mixed or swirled. Lighting procedures were established based on Zeikus (1998) and on recommendations from Ward’s Natural Science Establishment, Inc. technical representatives.

Bacteria cultured were preliminarily compared to the documented characteristics of the bacteria likely to be encountered (Appendix C).
Living cultures of *A. constricta* and *C. pyrenoidosa* were exposed to various dilutions of the bacterial products. These products were tested in extreme concentrations (>2000x recommended first dose). Later they were tested in their recommended first dosage (as calculated in Appendix D). Absorbency at 664nm, as determined with the use of the BFS Milton Roy® Spectrometer, was used as an indicator of algae culture concentration as that wavelength has the maximum absorbency for Chlorophyll$_a$, per APHA, AWWA, WPCF (1992).

The three products were analyzed by Albright (1999) for total phosphorous and nitrate+nitrites per APHA, AWWA, WPCF (1992) using the ascorbic acid method following persulfate digestion and the cadmium reduction method, respectively.

**RESULTS AND DISCUSSION**

Most of the Pond Saver and Pond Clarifier did not dissolve when mixed with water. This was the case whether the material was soaked for minutes or days, bubbled or not. Gritty particulate matter, which looked like common sand, resulted from soaking Pond Saver and Pond Clarifier. This particulate matter made creating dilutions difficult and made counting resulting colonies tedious. Bacta-Pur Klear, on the other hand, had no particulate component.

Product CFU raw data are contained in Appendix E. The best CFU count obtained for each product is as follows:

- **Pond Saver**: 10,000,000/g (as opposed to 4 billion claimed).
- **Pond Clarifier**: 300,000/g (as opposed to 882,000 claimed).
- **Bacta-Pur Klear**: 9,000/ml.

Twelve bacteria and one fungus (most likely a contaminant; see “Discussion and Conclusions”) were cultured from the products. They are described in Appendix F.

More colonies grew using the Drygalski spread plate technique than did using the pour plate technique.

The cooked algae agar media produced only a small proportion of the colonies produced with the other media. PCA media produced the largest colony counts with Pond Saver while BHI Nutrient Agar produced the largest number of colonies with the other products.

Products had immediate visually noticeable clarifying impacts on *C. pyrenoidosa* when added in extreme dosages. The impacts on *Anabaena* sp. were not so pronounced. Tables 1–4 compare spectrophotometric readings (absorbancy at 664 nm) of the algae cultures immediately before and 72 hours after the addition of the algicidal products at extreme (Tables 1 and 2) and recommended (Tables 3 and 4) dosages. That wavelength
was used because chlorophyll $a$ has a maximum absorbancy in that range. The intent was that absorbancy readings would provide relative changes in chlorophyll $a$ concentrations (a proxy for algae densities) following product addition. However, it became evident that, due to the nature of the product, suspended particulate material associated with the products caused turbidity. This undoubtedly affected absorbancy readings and confuses the contribution by algae and the products themselves.

An additional problem was encountered when using *Anabaena* sp. It tended to grow in a filamentous form. Because it could not be homogenized throughout the sample, spectrophotometric readings did not accurately reflect its densities.

Total phosphorous and nitrites-nitrites found in the products are identified in Table 5.

**Table 1.** Anabaena culture absorbency at 664 nm with extreme product dosage.

<table>
<thead>
<tr>
<th>Product Inoculation</th>
<th>Before 72 Hours</th>
<th>After 72 Hours</th>
<th>% of Original Absorbency After 72 Hours</th>
<th>Clarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water Blank:</td>
<td>0.000</td>
<td>0.000</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Control Broth:</td>
<td>0.135</td>
<td>0.102</td>
<td>75%</td>
<td>NA</td>
</tr>
<tr>
<td>Pond Saver:</td>
<td>0.125</td>
<td>2.418</td>
<td>1934%</td>
<td>-</td>
</tr>
<tr>
<td>Pond Clarifier:</td>
<td>0.235</td>
<td>0.424</td>
<td>180%</td>
<td>-</td>
</tr>
<tr>
<td>Bacta-Pur Klear:</td>
<td>0.223</td>
<td>0.361</td>
<td>162%</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 2.** C. pyrenoidosa culture absorbency at 664 nm with extreme product dosage.

<table>
<thead>
<tr>
<th>Product Inoculation</th>
<th>Before 72 Hours</th>
<th>After 72 Hours</th>
<th>% of Original Absorbency After 72 Hours</th>
<th>Clarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water Blank:</td>
<td>0.000</td>
<td>0.000</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Control Broth:</td>
<td>0.013</td>
<td>0.024</td>
<td>185%</td>
<td>NA</td>
</tr>
<tr>
<td>Pond Saver:</td>
<td>0.012</td>
<td>0.268</td>
<td>2233%</td>
<td>-</td>
</tr>
<tr>
<td>Pond Clarifier:</td>
<td>0.013</td>
<td>0.024</td>
<td>185%</td>
<td>-</td>
</tr>
<tr>
<td>Bacta-Pur Klear:</td>
<td>0.012</td>
<td>0.209</td>
<td>1742%</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. *Anabaena* culture absorbency at 664 nm with recommended first product dosage.

<table>
<thead>
<tr>
<th>Product Inoculation</th>
<th>Before Inoculation</th>
<th>After 48 Hours</th>
<th>% of Original Absorbency After 72 Hours</th>
<th>Clarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water Blank:</td>
<td>0.000</td>
<td>0.000</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Control Broth:</td>
<td>0.225</td>
<td>0.235</td>
<td>104%</td>
<td>NA</td>
</tr>
<tr>
<td>Pond Saver:</td>
<td>0.109</td>
<td>0.092</td>
<td>84%</td>
<td>+</td>
</tr>
<tr>
<td>Pond Clarifier:</td>
<td>0.072</td>
<td>0.058</td>
<td>81%</td>
<td>+</td>
</tr>
<tr>
<td>Bacta-Pur Klear:</td>
<td>0.250</td>
<td>0.160</td>
<td>64%</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 4. *C. pyrenoidosa* culture absorbency at 664 nm with recommended first product dosage

<table>
<thead>
<tr>
<th>Product Inoculation</th>
<th>Before Inoculation</th>
<th>After 48 Hours</th>
<th>% of Original Absorbency After 72 Hours</th>
<th>Clarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Slant with no algae:</td>
<td>0.000</td>
<td>0.000</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Control Slant:</td>
<td>0.815</td>
<td>0.825</td>
<td>101%</td>
<td>NA</td>
</tr>
<tr>
<td>Pond Saver:</td>
<td>0.955</td>
<td>0.770</td>
<td>81%</td>
<td>+</td>
</tr>
<tr>
<td>Pond Clarifier:</td>
<td>0.518</td>
<td>0.642</td>
<td>124%</td>
<td>-</td>
</tr>
<tr>
<td>Bacta-Pur Klear:</td>
<td>0.429</td>
<td>0.666</td>
<td>155%</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5. Total phosphorous and nitrate-nitrites for products

<table>
<thead>
<tr>
<th>Product</th>
<th>Total Phosphorous</th>
<th>Nitrate+Nitrites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond Saver</td>
<td>3830mg/kg</td>
<td>.06g/kg</td>
</tr>
<tr>
<td>Pond Clarifier</td>
<td>210mg/kg</td>
<td>.17g/kg</td>
</tr>
<tr>
<td>Bacta-Pur Klear</td>
<td>310mg/liter</td>
<td>1.13g/liter</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSIONS

**Identification of Species**

As discussed in Ford (1993), remarkably little is known about the bacteria of fresh water. While many of the bacteria found in sediments and in water are known, it is not clear that the ones most easily cultured and most often observed are the most active in the aquatic environment (Ford, 1993). In three separate iterations of culturing the three products in this study, the noted bacteria were consistently observed. Other smaller colonies may well have contained other species not noted as different. It is possible that some species of bacteria found in the formulations did not culture on any of the three
media used, although those media were chosen because they are reputed to ensure the best possible growth.

DNA advances have changed the taxonomy of bacteria, complicating identification of those that are algicidal. As noted earlier in this paper, many of the bacteria involved have been reclassified. Although the translation between current taxonomy and the older names has been made in most cases, some of the old names have no known (to the author) correspondence (e.g., *Cellvibrio* spp.). Additionally, current volumes of “Bergey’s” (Kreig and Holt, 1984; Sneath, 1986; Staley, J. T., 1989; Williams, 1989) associate characteristics with candidate species opposite to those described in the original literature (e.g., Gram staining results for *Arthobacter* sp. and oxidase reaction results for *Flavobacterium* spp.).

**Pour and Spread Plate Techniques.**

The probable cause of spread plate growth outperforming pour plate growth was the heat sensitivity of the aquatic microorganisms involved although strict aerobic requirements may have been responsible for some of the differences observed.

The Drygalski spatula technique worked best in this investigation, but is problematic. The spatula tended to overheat with continued use and flaming. The weight of the spatula tended to abrade the surface of the medium if the media was soft, making colony counting tedious. Using the spatula left the plate much more exposed to air currents – and contaminants (e.g., the *Penicillium* sp. cultured) than when using a bacteriological loop. This was due to its size and the requirement to spread the droplet across the entire media surface. If moisture was present inside the petri plate, the addition of the 0.1ml innoculum would cause drops to fall off the media onto the petri plate cover when the plate was inverted. This removed product from contact with the media and resulted in lowered CFU counts.

**CFUs Claimed.** An immediate judgment might be made disputing the veracity of advertising claims of four billion CFUs per gram for Pond Saver and 400 billion CFUs per pound for Pond Clarifier; however, difficulties growing aquatic bacteria on nutrient rich data are well documented (Ford, 1993 after Buck, 1979). Additionally, some of the bacteria cultured grew aggressively on the media while others barely showed their presence in 48 hours. When attempts were made to count plates at 72 hours, lower CFU counts resulted due to the aggressive bacteria growing over/around the slower growing bacteria. This research was done to validate advertised claims. CFU counts were performed repeatedly to overcome issues such as those experienced using the spread plate technique discussed above. It is unfortunate that none of the purveyors of products claiming to provide bacterial suppression of aquatic algae would share with the BFS their methodologies for culturing and testing their products (Lord, unpubl; Albright, 1998). It can only be concluded that CFU count claims cannot be verified.
Media Performance

Bacterial Media. NWRI was, by far, the most difficult media to prepare of the three used. Preparation of the others was trivial by comparison. Counting colonies with NWRI was easier than with any other media. Its clarity made counting straightforward. PCA was the darkest tinted, but tended to be softer causing problems with the Drygalski spatula technique. NWRI media did not produce more colonies than the other media contrary to APHA, AWWA, WPCF (1992). In spite of its second best performance in culturing Pond Saver bacteria, BHI nutrient agar had to be used on all isolated bacterial types to obtain cell and colony morphologies that were consistent with that found in most of the literature.

Algae Media. Anabaena sp. tended to grow slowly and was clumpy in all media, but most consistently in proteose peptone supplemented Bristol’s solution. It failed to grow on the Ward’s supplied Proteose Agar slants. C. pyrenoidosa would grow in a variety of media, but most consistently on the Proteose Agar slants followed by the soil-water supplemented modified Bristol’s solution.

Product Impact on Tested Algae. In recommended dosages, Pond Saver appears to curtail the growth of C. pyrenoidosa and Anabaena sp. Both Pond Clarifier and Bacta-Pur Klear appear to curtail Anabaena sp. while aiding the growth of C. pyrenoidosa. Excessive doses of all products appear to cloud the water tested while inhibiting algae. Time limitations and a paucity of cultured algae prevented testing in replicate.

Most Likely Product Origins. If made totally soluble, the total phosphorous content of a kg of Pond Saver is enough to produce a “nuisance algal bloom” (Mackenthun 1965) in a 255,333 liter pond using the criteria outlined in Mackenthun (1965) and Sawyer (1969). For Pond Clarifier, the size pond so polluted would be 14,000 liters and for Bacta-Pur Klear the size would be 20,667 liters. Similarly, the nitrate-nitrite component will support algae blooms. Saferman and Morris (1963) noted that algicidal bacteria were much more common in “bottom and bank muds” than in effluent “pond water”. Given the sand, phosphorus and nitrate-nitrite content of Pond Saver and Pond Clarifier, it is likely that they are extractions from sand filters used to clarify sewage treatment effluent (Other researchers cited herein [e.g., Reim, et al., 1974; Daft et al., 1973] also collected their bacteria in ponds associated with sewage treatment.) That being said, Ford (1993) notes that billions of bacteria can be found in every cubic centimeter of sediment. This makes the products’ claims appear reasonable – and the products’ contents unremarkable. Bacta-Pur Klear origins are much less apparent, but it could be produced by settling and filtering the waters outputted from such sand filters.

Duration of effect. As noted in Martin (1976), Delucca and McCracken (1977), Cole (1982), and Burnham (1984), and as is apparent in other research (Berger, 1979), certain bacteria have been found to favor one algae over another. This could lead to the apparent suppression of algae in a body of water when, in fact, the improved transparency is actually visible evidence of algae succession facilitated by the introduction of the
bacteria. The long-term impact of the introduction of certain bacteria (e.g., *Pseudomonas* sp., *Flavobacterium* sp., and *Xanthomonas* sp.) may actually be to lessen transparency. Additionally, researchers have noted that certain algae lysing bacteria are “good parasites” (Bukovsan, 1998) in that they either fail to kill all their hosts (Code and Starr, 1978) or that they have no impact on heterocysts which would ensure future blooms (Shilo, 1970). Other researchers have noted that the bacilli exude their algae killing products while creating spores in their postlogrithmic phase (Reim *et al*., 1974) which implies a sine wave predator-prey model. Daft *et al.* (1973) note that algae and bacteria numbers fluctuated in “a similar manner” while Burnham *et al.* (1984) note predator-prey cycles of about nine days.

**Recommendations regarding use.** While these products may suppress a particular algae bloom, they do so in a manner that leaves a pond susceptible to blooms involving other algae species while facilitating subsequent blooms with increased nutrients. The long-term health of most ponds would be facilitated with a dose of alum (Anon., 1990) as opposed to the products evaluated here.

**Recommended future research.**

- Bacteria in these compounds should be identified to species if possible to confirm or refute suspected origins and to identify potential pathogens.
- Product impact on algae should be tested in replicate.
- Soluble phosphorous content of these products, in recommended usage concentrations, should be evaluated.
- If vendor production and CFU counting procedures become available, further attempts should be made to validate or correct CFU counts claimed.
- Given the suspected source of these products, they should be tested for the presence of heavy metals.

**CONCLUSION**

As a result of the foregoing, it can be concluded that these products probably can offer ephemeral relief from certain types of pond algae blooms. However, nutrients will increase which will exacerbate future blooms. Additional work is recommended to fully identify product contents.

**REFERENCES**


Burnham, J. C. 1984. A completion report for the grant entitled “Use of *Myxococcus PCO2* to control aquatic algae. Ohio Water Resources Center, Ohio State University, Columbus, Ohio. Project completion report number B-086-OHIO.


Galbraith, J. C. and Hayward, A. C. 1984b. The potential of indigenous microorganisms in the biological control of water hyacinth in Australia. AWRC research project 80/132 final report, Australia Department of Resources and Energy. 84 pp.


Marr, C. 1999. Personal communication. SUNY Oneonta, Oneonta, NY.


Appendix A:
Bacteria taxa identified as likely components of commercial microbial products.

1. *Achromobacter* sp. (Mamkaeva, 1966; Kreig and Holt, 1984) formerly used name for some *Alcaligenes* and some *Acinetobacter*;
3. *Alcaligenes* sp. (Martin, 1976);
4. *Arthrobacter* sp. (Berger, et al., 1979);
5. *Bdellovibrio bacteriovorus* (Burnham, 1973; Burnham et al., 1976);
6. *Cellvibrio* spp. (Granhall and Berg, 1972);
7. *Flavobacterium* spp. (Mamkaeva, 1966; Delucca and McCraken, 1977; Mayfield and Inniss, 1978);
8. *Flavobacterium breve* (Kreig and Holt, 1984) formerly known as *Bacillus brevis* (Reim et al., 1974);
9. Myxobacteriales spp., possibly *Myxococcus* spp. or *Polyangium* spp. (Shilo, 1970; Daft, et al., 1973);
11. *Myxococcus fulvus* (Burnham, 1984);
12. *Myxococcus xanthus* (Burnham et al., 1981 and Burnham, et al., 1984);
13. *Pseudomonas* sp. (Blasco, 1965; Mamkaeva, 1966; Burnham, 1973; Delucca and McCraken, 1977; Mayfield and Inniss, 1978);
14. *Serratia* sp. (Mamkaeva, 1966);
15. *Serratia marcescens* (Darveau and Lynch, 1977);
16. *Vampirovibrio chlorellavorus* (Kreig and Holt, 1984) formerly known as *Bdellovibrio chlorellavorus* (Code and Starr, 1978);
Appendix B: Dilution Calculations

Table 1. Pond Saver CFU-Dilution conversions for 100 ml bottles.

4 billion CFUs per gram

<table>
<thead>
<tr>
<th>Blank #</th>
<th>Inserted</th>
<th>Advertised CFUs/Bottle</th>
<th>Advertised CFUs/0.1ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution 0</td>
<td>1 gram</td>
<td>4,000,000,000</td>
<td>4,000,000</td>
</tr>
<tr>
<td>Dilution 1</td>
<td>1 ml from 0</td>
<td>40,000,000</td>
<td>40,000</td>
</tr>
<tr>
<td>Dilution 2</td>
<td>1 ml from 1</td>
<td>400,000</td>
<td>400</td>
</tr>
<tr>
<td>Dilution 3</td>
<td>0.1 ml from 1</td>
<td>40,000</td>
<td>40</td>
</tr>
<tr>
<td>Dilution 4</td>
<td>1 ml from 2</td>
<td>4,000</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2. Pond Clarifier CFU-Dilution Conversions for 100 ml bottles.

400 billion CFUs per pound

<table>
<thead>
<tr>
<th>Blank #</th>
<th>Inserted</th>
<th>Advertised CFUs/Bottle</th>
<th>Advertised CFUs/0.1ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution 0</td>
<td>10 grams</td>
<td>8,820,001</td>
<td>8,820</td>
</tr>
<tr>
<td>Dilution 1</td>
<td>1 ml from 0</td>
<td>88,200</td>
<td>88</td>
</tr>
<tr>
<td>Dilution 2</td>
<td>0.1 ml from 0</td>
<td>8,820</td>
<td>9</td>
</tr>
<tr>
<td>Dilution 3</td>
<td>1 ml from 1</td>
<td>882</td>
<td>1</td>
</tr>
<tr>
<td>Dilution 4</td>
<td>0.1 ml from 1</td>
<td>88</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Bacta-Pur Klear Application Conversions.

Rate:

100 ml per 1000 liters = 0.1 ml/liter

Table 4. Pond Saver Application Conversions

liters = 1.057*gallons*4 kg = 2.2046 pounds

3 pounds = 6.6138 Kg

325,000 gallons = 1,374,100 liters

Rate:

3 pounds per 325,000 gallons = 4.81319E-06 kg/liter

0.004813187 g/liter

19,252,747 CFUs/liter
Table 5. Initial Estimates for Bacta-Pur Klear CFU-Dilution Conversions.

No claim to CFUs per volume
Assuming CFUs per treatment close to Pond Saver:

<table>
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<tr>
<th>Blank #</th>
<th>Inserted</th>
<th>Estimated CFUs/Bottle</th>
<th>Estimated CFUs/0.1ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution 0</td>
<td>0.1ml Bacta-Pur</td>
<td>19,252,747</td>
<td>19,253</td>
</tr>
<tr>
<td>Dilution 1</td>
<td>1ml from 0</td>
<td>192,527</td>
<td>193</td>
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<tr>
<td>Dilution 2</td>
<td>0.1ml from 0</td>
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<td>19</td>
</tr>
<tr>
<td>Dilution 3</td>
<td>1ml from 1</td>
<td>1,925</td>
<td>2</td>
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Table 6. Revised Dilutions for Bacta-Pur Klear.

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</tr>
</thead>
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</tr>
<tr>
<td>Dilution 1</td>
<td>0.1ml Bacta-Pur</td>
</tr>
<tr>
<td>Dilution 2</td>
<td>1ml from 0</td>
</tr>
<tr>
<td>Dilution 3</td>
<td>0.1ml from 0</td>
</tr>
</tbody>
</table>
Appendix C:

Characteristics of bacteria taxa identified as likely components of commercial microbial products.

- **Actinomycetes**
  - Form: hyphae with branching (Holt, 1977).
  - Various spore types but not endospores (Holt, 1977).
  - Gram positive rods.
    - Irregular staining often presents beaded appearance (Sneath, 1986).
  - Non sporing (Sneath, 1986).
  - Straight or wavy slender branching filaments (Sneath, 1986).
  - Faculative anaerobic (Sneath, 1986).
  - Mature colonies (7-14 days) are either
    - Rough and dry, or
    - Smooth and soft to mucoid.
    - White to gray-white or creamy white (Sneath, 1986).
  - Glucose fermenter (Sneath, 1986).
  - Indole negative (Sneath, 1986).

- **Alcaligenes** sp.
  - Gram negative rods (1 x 3-4 um) singly or paired.
  - “Four ‘degenerately peritrichous’ flagella with subpolar or lateral insertion.”
  - Aerobic.
  - Colonies: “white, round, smooth, and convex.”
  - Negative for acid production from: arabinose, fructose, galactose, glucose, inulin, lactose, maltose, mannitol, salicin, sorbitol, sucrose, and trehalose.
  - Positive for Nitrate → Nitrite and motility.
  - Reduces litmus milk.
  - Negative for catalase, indole, methyl red, Voges-Proskauer, H2S, urease production, starch hydrolysis, gelatin liquefaction, and citrate (Martin, 1976).
    - Gram negative rod.
    - 0.5 x 2.0 – 3.0 um.
    - Flagellate.
    - Oxidase positive.

- **Arthrobacter** sp.
  - Hydroxylamine production positive.
  - Cell morphology changes markedly during life cycle (Holt, 1977; Sneath, 1986).
  - Gram positive, but may
    - Appear Gram negative with Gram positive granules (Holt, 1977).
    - Become Gram positive coccus in growth cycle (Sneath, 1986).
  - No fermentation (Holt, 1977).
  - Catalase positive (Holt, 1977; Sneath, 1986).
  - Strict aerobe (Holt, 1977).
  - No endospore (Sneath, 1986).
• Stationary phase: typically large coccus: 0.6 – 1.0μm (Sneath, 1986).

- **Bdellovibrio bacteriovorus.**
  - Burnham et al. (1976) does not provide identifying characteristics.
  - Fermentation: negative (Holt, 1977).
  - Aerobic (Holt, 1977).
  - Most often parasitic (Holt, 1977).
  - Catalase positive (Holt, 1977).
  - Colonies on yeast extract and peptone:
    - Small to pinpoint (Holt, 1977).
    - Pale yellow to orange; (Krieg and Holt, 1984).

- **Cellvibrio spp.**
  - Granhall and Berg (1972) do not provide identifying characteristics.
  - Not noted in Volumes 1-4 of “Bergey’s” (Kreig and Holt, 1984; Sneath, 1986; Staley, J. T., 1989; Williams, 1989).
  - Noted in Hatt (1982) which notes two species: *C. gilvus* and *C. polyotrophicus* but provides no identifying characteristics.
  - Noted in Gibbons et al. (1981) which notes four species: *C. flavescens*, *C. gilvus*, *C. polyotrophicus*, and *C. violacea* but provides no identifying characteristics.

- **Cytophaga sp.** AKA myxobacter e.g., *Myxococcus fulvus* and *Myxococcus xanthus* (Burnham, 1984).
  - Noted to be closely related to *Flavobacterium*, but not described in Kreig and Holt (1984).

- **Flavobacterium** spp.
  - Gram negative (Deluca and McCraken, 1977).
  - Short rods (Deluca and McCraken, 1977).
    - Typically 0.5 x 1.0 x 3.0 μm long (Kreig and Holt, 1984).
  - No endospores (Kreig and Holt, 1984).
  - Aerobic (Kreig and Holt, 1984).
  - Colonies:
    - Smooth, flat with entire margins (Deluca and McCraken, 1977).
    - Typically yellow to orange; usually translucent; circular; smooth and shiny with entire edges (Kreig and Holt, 1984).
  - Sucrose: negative (Deluca and McCraken, 1977).
  - Manitol: negative (Deluca and McCraken, 1977).
  - Saccharose: negative (Deluca and McCraken, 1977).
  - Citrate: negative (Deluca and McCraken, 1977).
  - Indole: negative (Deluca and McCraken, 1977).
  - H₂S Production: positive (Deluca and McCraken, 1977).
  - Oxidase:
    - Negative (Deluca and McCraken, 1977).
• Positive (Krieg and Holt, 1984).
• Catalase positive (Krieg and Holt, 1984).
• Phophotase positive (Krieg and Holt, 1984).
• Mayfield and Inniss, 1978 does not provide identifying characteristics.

Flavobacterium breve.
• Gram negative (Krieg and Holt, 1984).
• Rods ~0.5 x 1.0 – 2.0 μm (Krieg and Holt, 1984).
• Colonies on nutrient agar:
  • Entire edge.
  • Convex.
  • Convex.
  • Circular.
  • Smooth.
  • Shining.
  • Pinpoint to 2.5mm.
• Nonhemolytic (Krieg and Holt, 1984).
• Spores central and oval (Reim et al., 1974).
• Mannitol acid positive (Reim et al., 1974).
• Deamination of phenylalanine negative (Reim et al., 1974).
• Decomposition of tyrosine positive (Reim et al., 1974).
• Glucose acid positive (Reim et al., 1974).
• Growth in 5% NaCl negative (Reim et al., 1974).
• Width: 0.6-0.9 μm; length: 1.5-4.0 μm.
• Gram: variable.
• Nonmotile (Krieg and Holt, 1984).
• Starch hydrolysis: negative.
• Volges-Pros.: positive.
• Indole: negative.
• Decomposition of casein: negative (Holt, 1977).

Myxobacterium CP-1
• Gram:
  • Negative rods (Daft, et al., 1984).
  • Weak staining (Sneath, 1986).
  • Strong acid fast (Sneath, 1986).
  • “Aflagellate” (Daft, et al., 1984).
• Colonies:
  • Yellow pigmented (Daft, et al., 1984).
  • Not visible for two days (Sneath, 1986).
• “Strictly Aerobic” (Daft, et al., 1984; Sneath, 1986).
• Non motile (Sneath, 1986).
• Catalase positive (Sneath, 1986).
• Ammonia as sole nitrogen source: negative (Daft, et al., 1984).
• Nitrate as sole nitrogen source: negative (Daft, et al., 1984).
• Hydrolyze starch: positive (Sneath, 1986).
• Liquefy gelatin: positive (Sneath, 1986).
• Glucose as sole carbon: negative (Sneath, 1986).
• Starch as sole carbon: negative (Sneath, 1986).
Myxococcus spp.
- Rods with rounded ends (Holt, 1977).
- Myxospores: refractile and spherical or ellipsoidal (Holt, 1977).
- Motile (Holt, 1977).
- Strict aerobes (Holt, 1977).

Myxococcus fulvus
- Burnham (1984) does not provide identifying characteristics.
- Colony flesh to reddish orange (Holt, 1977).
- Oxidase negative (Holt, 1977).

Myxococcus xanthus
- Yellowish to yellowish orange pigment (Burnham et al., 1981; Holt, 1977).
- Myxospore diameter of 1.8 μm (Burnham et al., 1981).
- Forms small fruiting bodies without a stalk (Burnham et al., 1981).
- Oxidase negative (Holt, 1977).
- Burnham, et al., 1984 does not provide identifying characteristics.

Pseudomonas spp.
- Rods.
  - Short (Delucca and McCraken, 1977).
  - Straight or slightly curved (Kreig and Holt, 1984).
  - 0.5 – 1.0 x 1.5 – 5.0 μm (Kreig and Holt, 1984).
- White circular colonies (Delucca and McCraken, 1977).
- Aerobic or nitrate users (Kreig and Holt, 1984).
- Normally fail to grow in acid conditions (Kreig and Holt, 1984).
- Widely distributed in nature (Kreig and Holt, 1984).
- Glucose: positive (Delucca and McCraken, 1977).
- Sucrose: negative (Delucca and McCraken, 1977).
- Saccharose: negative (Delucca and McCraken, 1977).
- Citrate: positive (Delucca and McCraken, 1977).
- Indole: negative (Delucca and McCraken, 1977).
- Motile:
  - Positive (Delucca and McCraken, 1977).
  - With polar flagella (Kreig and Holt, 1984).
- H₂S Production: positive (Delucca and McCraken, 1977).
- Oxidase:
  - Positive (Delucca and McCraken, 1977).
  - Usually positive (Kreig and Holt, 1984).
- Catalase: positive (Kreig and Holt, 1984).
- Mayfield and Inniss, 1978 does not provide identifying characteristics.

Serratia marcescens
- Darveau and Lynch, 1977 does not provide identifying characteristics.
- Gram: negative.
• Rods:
  • Straight.
  • 0.5 – 0.8 x 0.9 – 2.0 μm.
• Facultative anaerobic.
• Motile.
• Colonies:
  • Pink, white, or red.
  • Opaque, somewhat iridescent.
  • 1.5 – 2.0 mm diameter colonies overnight on nutrient agar.
• Catalase positive (Krieg and Holt, 1984).

□ Vampirovibrio chlorellavorous
  • Rods and cocci (Krieg and Holt, 1984).
  • Pleomorphic: vibrios 0.3 μm wide & cocci 0.6 μm wide (Code and Starr, 1978).
• Flagella: single, unsheathed (Code and Starr, 1978).
• Unable to grow in any media other than live algae cells (Code and Starr, 1978).

□ Xanthomonas sp.
• Gram negative (Krieg and Holt, 1984).
• Rods:
  • Short (Delucca and McCraken, 1977).
  • Straight (Krieg and Holt, 1984).
  • 0.4 – 0.7 x 0.7 – 18 μm (Krieg and Holt, 1984).
  • Usually singles (Krieg and Holt, 1984).
• Colonies: yellow and smooth (Krieg and Holt, 1984).
• Aerobic (Krieg and Holt, 1984).
• Catalase positive (Krieg and Holt, 1984).
• Lactose fermentation: negative (Delucca and McCraken, 1977).
• Colonies:
  • Yellow to orange pigmentation (Delucca and McCraken, 1977).
  • “Punctiform which were raised with entire margins” (Delucca and McCraken, 1977).
• Glucose: positive (Delucca and McCraken, 1977).
• Sucrose: negative (Delucca and McCraken, 1977).
• Lactose: negative (Delucca and McCraken, 1977).
• Manitol: negative (Delucca and McCraken, 1977).
• Saccharose: negative (Delucca and McCraken, 1977).
• Maltose: negative (Delucca and McCraken, 1977).
• Citrate: negative (Delucca and McCraken, 1977).
• Indole: negative (Delucca and McCraken, 1977).
• Motile:
  • Positive (Delucca and McCraken, 1977; Krieg and Holt, 1984).
  • By single flagella (Krieg and Holt, 1984).
• H₂S Production: positive (Delucca and McCraken, 1977).
• Oxidase:
  • Negative or weak (Krieg and Holt, 1984).
  • Negative (Delucca and McCraken, 1977).
Appendix D: Calculations for equivalent recommended first dosages.

Table 1. Pond Saver Conversions for Algae Inoculation.

Recommended First Application: 3# per acre/foot

1 pound = 2.205**-1 kg

3 pounds = (3/2.205) kg = 1.360544218 kg

1 acre/foot = 325,000 gallons

1 gallon = 3.785 x 10**-3 cubic meters

325,000 gallons = 3.785 * 325 = 1230.125 cubic meters

3# per acre/foot = 1.36kg/1230.13cu meters =

Moving 1 ml with Each Dilution:

Dilution Bottle 0: 0.01106 g/100ml
Dilution Bottle 1: 0.000111 g/1 ml
Dilution Bottle 2: 0.000111 g/100ml

Table 2. Pond Clarifier Conversions for Algae Inoculation.

Recommended First Application: 8# per acre/foot

1 pound = 2.205**-1 kg

8 pounds = (8/2.205) kg = 3.628117914 kg

1 acre/foot = 325,000 gallons

1 gallon = 3.785 x 10**-3 cubic meters

325,000 gallons = 3.785 * 325 = 1230.125 cubic meters

8# per acre/foot = 3.63kg/1230.13cu meters =

Moving 1 ml with Each Dilution:

Dilution Bottle 0: 0.00294939 kg/cu meters =
Dilution Bottle 1: 0.0010602E-06 kg/liters =
Dilution Bottle 2: 0.00110602 g/100ml
0.00294939 g/liters = mg/ml
2.94939E-06 g/ml
0.000294939 g/100ml

Moving 1 ml with Each Dilution:

<table>
<thead>
<tr>
<th>Dilution Bottle 0:</th>
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<td>Dilution Bottle 2:</td>
<td>0.000295 g/100ml</td>
</tr>
</tbody>
</table>

Table 3. Bacta-Pur KIear Conversions for Algae Inoculation.

Recommended First Application: 100 ml product / 1000 liters water

100 ml / 1000 liters = 0.0001 ml/ml
0.01 ml/100ml

Moving 1 ml with Each Dilution:

<table>
<thead>
<tr>
<th>Dilution Bottle 0:</th>
<th>1 ml/100ml</th>
</tr>
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<tbody>
<tr>
<td>Dilution Bottle 1:</td>
<td>0.01 ml/1ml</td>
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<tr>
<td>Dilution Bottle 2:</td>
<td>0.01 ml/100ml</td>
</tr>
</tbody>
</table>
Appendix E: CFU Counts.

Table 1. Pond Saver 24 Hour CFU Count.

<table>
<thead>
<tr>
<th>Dilution</th>
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<th>BHI Count</th>
<th>PCA Count</th>
<th>NWRI Count</th>
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<tr>
<td>0</td>
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<td>Lawn</td>
<td>Lawn</td>
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<td>Lawn</td>
<td>Lawn</td>
</tr>
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<td>1</td>
<td>b</td>
<td>Lawn</td>
<td>Lawn</td>
<td>Lawn</td>
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<td>1</td>
<td>c</td>
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<tr>
<td>4</td>
<td>c</td>
<td>2</td>
<td>3 *</td>
<td>1</td>
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* broken agar

Table 2. Pond Saver 48 Hour CFU Count.

<table>
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<th>BHI Count</th>
<th>PCA Count</th>
<th>NWRI Count</th>
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* broken agar
Table 3. Pond Clarifier 24 Hour CFU Count.

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Table 4. Pond Clarifier 48 Hour CFU Count.

<table>
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<th>PCA Count</th>
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* broken agar
Table 5. Bacta-Pur Klear 24 Hour CFU Count.

<table>
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<tr>
<th>Dilution</th>
<th>Replicate</th>
<th>BHI Count</th>
<th>PCA Count</th>
<th>NWRI Count</th>
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Table 6. Bacta-Pur Klear 48 Hour CFU Count.

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<th>PCA Count</th>
<th>NWRI Count</th>
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<td>1</td>
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<td>a</td>
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<td>0</td>
<td>0</td>
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<tr>
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<td>b</td>
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<td>0</td>
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<td>c</td>
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<td>0</td>
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<td>0</td>
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<tr>
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<td>c</td>
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<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* broken agar
Appendix F: Microorganisms cultured from products.

- **BPK-A:**
  - Source: Bacta-Pur Klear.
  - Circular colony with raised yellowish-cream center with an entire cream "halo" edge.
  - Gram negative rods ~1.5 \( \mu \text{m} \times .75 \mu \text{m} \).

- **BPK-B:**
  - Source: Bacta-Pur Klear.
  - Circular white flat colony with serrate-lobate edge.
  - Gram negative rods ~4.5 \( \mu \text{m} \times .75 \mu \text{m} \).

- **BPK-B₂:**
  - Source: Bacta-Pur Klear.
  - Circular white flat colony with serrate-lobate edge.
  - Gram positive rods with endospores towards one end.
  - Identified by as *Bacillus* sp. (Bukovsan, 1999).

- **BPK-C:**
  - Source: Bacta-Pur Klear.
  - Irregular white flat colony with lobate edge.
  - Gram positive rods ~7.0 - 8.0 \( \mu \text{m} \times .50 \mu \text{m} \) with no obvious endospores.
  - Potentially: *Achromobacter* sp., *Actinomycetes*, *Arthrobacter* sp., *Bdellovibrio bacteriovorus*, *Cellvibrio* spp., or *Myxobacterales* spp.

- **PC-A:**
  - Source: Pond Clarifier.
  - Black irregular raised colony with lobate edge.
  - Gram negative rods ~1.5\( \mu \text{m} \times .75 \mu \text{m} \).
  - Potentially: *Achromobacter* sp., *Arthrobacter* sp., *Bdellovibrio bacteriovorus*, *Cellvibrio* spp., or *Myxobacterales* spp.

- **PC-B:**
  - Source: Pond Clarifier.
  - Cream circular colony with articulate umbonate ridges (often looks like a dry, partially collapsed blister) and a lobate edge.
  - Gram negative sheathed rods.

- **PC-C:**
  - Source: Pond Clarifier.
  - Yellowish-cream colony with a filamentous edge.
  - Gram negative rods ~2.25 - 3.75 \( \mu \text{m} \times .75 \mu \text{m} \).
- Potentially: *Achromobacter* sp., *Arthrobacter* sp., *Bdellovibrio bacteriovorus*, *Cellvibrio* sp., *Myxobacterium CP-1*, *Myxobacterales* sp., *Myxococcus fulvus*, *Myxococcus xanthus*, *Serratia* sp., or *Xanthomonas* sp.

**PC-D:**
- Source: Pond Clarifier.
- As BPK-A: Circular colony with raised yellowish-cream center with an entire cream "halo" edge.
- Gram negative rods ~7.5 - 6.0 \( \mu m \) x .75 \( \mu m \).
- Potentially: *Achromobacter* sp., *Arthrobacter* sp., *Bdellovibrio bacteriovorus*, *Cellvibrio* sp., *Flavobacterium* sp., *Myxobacterium CP-1*, *Myxobacterales* sp., *Myxococcus fulvus*, *Myxococcus xanthus*, *Serratia* sp., or *Xanthomonas* sp.

**PC-E:**
- Source: Pond Clarifier.
- Small circular white granular colony with alobate edge.
- Gram negative rods ~1.0 \( \mu m \) x .2 \( \mu m \).
- Potentially: *Achromobacter* sp., *Arthrobacter* sp., *Bdellovibrio bacteriovorus*, *Cellvibrio* sp., *Myxobacterales* sp., *Serratia* sp., or *Serratia marcescens*.

**PS-A:**
- Source: Pond Saver.
- Circular colony with a pink center and bottom with fuzzy appearing gray surrounding the center and a yellowish fuzzy edge.
- Identified as *Penecillium* sp. (Marr, 1999).

**PS-B:**
- Source: Pond Saver.
- As BPK-B: Circular white flat colony with serrate-lobate edge.
- Gram negative rods ~3.0 \( \mu m \) x .75 \( \mu m \).
- Potentially: *Achromobacter* sp., *Arthrobacter* sp., *Bdellovibrio bacteriovorus*, *Cellvibrio* sp., *Myxobacterales* sp., *Pseudomonas* sp., or *Serratia* sp.

**PS-C:**
- Source: Pond Saver.
- Smaller but as PC-B: Cream circular colony with articulate umbonate ridges (often looks like a dry, partially collapsed blister) and a lobeate edge.
- Gram negative rods ~3.0 \( \mu m \) x .5 \( \mu m \).
- Potentially: *Achromobacter* sp., *Arthrobacter* sp., *Bdellovibrio bacteriovorus*, *Cellvibrio* sp., *Myxobacterium CP-1*, *Myxobacterales* sp., *Myxococcus fulvus*, *Myxococcus xanthus*, *Pseudomonas* sp., *Serratia* sp., or *Xanthomonas* sp.

**PS-D:**
- Source: Pond Saver.
- Irregular white flat colony with lobate edge.
- Gram positive rods in chains with endospores ~3.0 \( \mu m \) x 1.0 \( \mu m \).
- Potentially: *Actinomycetes*, *Arthrobacter* sp., *Bdellovibrio bacteriovorus*, *Cellvibrio* sp., or *Myxobacterales* sp.