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DISTRIBUTION OF CHITINOLYTIC BACTERIA IN VARIOUS AQUATIC SYSTEMS: A COMPARATIVE STUDY

Steve A. Konings*
Robert J. Wolff
and
Gerrit D. Van Dyke
Trinity Christian College
6601 West College Drive
Palos Heights, Illinois 60463

ABSTRACT

Chitin is a natural, abundant, resistant aminopolysaccharide. Samples taken from several aquatic ecosystems indicate that chitinolytic bacteria seem to be most abundant in habitats with high levels of dissolved oxygen, high levels of total organic carbon, and agitation of the water. This correlates with the high levels of chitinolytic bacteria found in streams and may be due in part to soil humus in runoff. Bacteria identified as chitinolytic included species of *Klebsiella*, *Proteus*, *Serratia*, and *Pseudomonas*. Species of *Klebsiella* and *Proteus* have not previously been recorded as chitinolytic.

INTRODUCTION

Chitin is a stable, unbranched, aminopolysaccharide (Aspinall, 1970) found in the exoskeleton of arthropods, in cell walls of fungi, in some green algae, in nematode eggs, and the covering of fecal pellets of many zooplankton (Gray and Williams, 1971 and Rheinheimer, 1980). It is the second most abundant polymer in nature; only cellulose is more abundant (Rawls, 1984).

Because of its abundance there is a potential for chitin to accumulate in both aquatic and terrestrial systems. The potential for accumulation is enhanced by the fact that the degradation of chitin is a slow process (Gray and Williams, 1971).

*Current address: Department of Biological Sciences, University of Wisconsin-Milwaukee, P.O. Box 413, Milwaukee, WI 53201. Reprint requests and correspondence should be sent to RJW.

The degradation of chitin is important, not only to avoid its accumulation, but also to return carbon and nitrogen to their respective nutrient cycles (Warnes and Randles, 1977 and 1980).

Chitin is aerobically broken down by the bacterial exoenzyme chitinase. The limiting step in this degradation is the cleavage of chitin into N-acetyl-D-glucosamine (NAGA) fragments by chitinase. Chitinase degradation of chitin produces small amount of NAGA along with large amounts of chitobiose and chitotriose. Chitobiose and chitotriose are rapidly degraded by the enzyme chitobiase into NAGA units which eventually produces primarily glucose and ammonia (Gray and Williams, 1971 and Rheinheimer, 1980).

Sixteen genera of bacteria that occur in fresh water contain species known to degrade chitin. These include *Achromobacter*, *Aeromonas*, *Alcaligenes*, *Bacillus*, *Beneckea*, *Chromobacterium*, *Cytophaga*, *Flavobacterium*, *Micrococcus*, *Micromonospora*, *Norcardia*, *Pseudomonas*, *Serratia*, *Streptomyces*, *Vibrio*, and *Zymomonas* (Kuznetsov, 1970; Gray and Williams, 1971; and Srikantaiah and Mohankumar, 1980).

The purpose of this research was to identify the chitinolytic bacteria in various aquatic systems.

MATERIALS AND METHODS

In this study nine different aquatic systems were sampled. A single sample was taken one meter above the bottom or at the midpoint of the water column if the depth was less that one meter. Two hundred ml water samples were collected using a sterile sampler and transferred to a sterile glass bottle with a ground glass stopper. The samples were brought back to the lab and processed within one hour. Visual evidence of water agitation was recorded. Dissolved oxygen was determined in the field using a Hach kit (Model OX-DT). The pH was determined using a Beckman Century SS pH meter.

Total organic carbon was measured by placing fifty ml subsamples in preweighed evaporation dishes. The water was evaporated in a drying oven at 110 C. The dishes were cooled in a desiccator, and weighed to ascertain total solids. They were then placed in a muffle oven at 500 C for twenty-four hours. The dishes were again cooled in a desiccator and weighed. The difference between the weight of the evaporation dish after evaporation and the weight after combustion represents the total volatile compounds and approximates the total organic carbon in the sample. The grams of organic carbon divided by the amount of water sample yields the grams of total organic carbon per liter of water sampled.

Total organic carbon was measured to see whether it correlates with the density of chitinolytic bacteria. Such a correlation seems logical since organic carbon is necessary as an energy source for bacterial growth and some of the organic carbon can be expected to be in the form of chitin, a substrate for the growth of chitinolytic bacteria.

To test for the presence of chitinolytic bacteria in the water samples, chitin agar was prepared by using 5/6 strength Difco Nutrient Agar enriched with one-half percent purified chitin powder from crab shells which was purchased from Sigma chemical company. A one ml subsample from each water sample was mixed with 5 milliliters of melted and cooled (48 C) chitin agar and was poured as an overlay on

top of a normal strength nutrient agar base in a sterile petri plate. Serial dilutions were conducted for each sample and treated in the same manner.

The plates were checked at twelve and twenty-four hours for evidence of total bacterial numbers as colony forming units (CFU) and chitinolytic bacterial activity (CFU's) as indicated by a zone of clearing in the chitin around the colonies. Observations for chitinolytic activity were continued daily for three weeks. Numbers of colonies for each sample were recorded from the undiluted sample or the dilution which gave 30 to 300 colonies per plate.

Colonies which appeared to be chitinolytic were streaked on chitin agar plates to ensure chitinolytic activity of pure cultures. Standard tests were conducted to identify each colony to species.

Five lentic systems, lakes and ponds, and four lotic systems, including three streams and a slow flowing canal were sampled. Lentic systems were sampled under two different conditions. First, on calm or windless days, when there were no waves and the water was little agitated. And second, on windy days with considerable wave action at the surface of the lake and agitation via undercurrents. Two types of lotic sytems were sampled. One was very slow flowing, while the second was flowing at a moderate rate.

RESULTS AND DISCUSSION

The bacteria in the samples which showed chitinolytic activity included three species of the family Enterobacteriaceae which were identified as *Klebsiella ozaenae*, *Proteus inconstans*, and *Serratia liquefacians*. No species from either *Klebsiella* or *Proteus* have been previously reported to degrade chitin. Three strains of *Pseudomonas* were also distinguished.

Abundance and distribution of chitinolytic bacteria are given in Table 1. Population levels of both total bacteria and chitinolytic bacteria seem to be positively related to the degree of agitation of the water. The lentic systems sampled on calm days had low numbers of total bacteria and chitinolytic bacteria per ml of water, except for the very shallow and rich segment of the old canal. Slightly agitated systems, the lentic system sampled on a windy day and slow flowing lotic sytems had more total bacteria and chitinolytic bacteria than the lentic systems sampled on calm days. The highest populations of both total and chitinolytic bacteria were found in the moderately flowing lotic systems.

The degree of agitation apparently affects the location of chitin and chitinolytic bacteria in the system. In calm waters the heavy chitin likely settles out of the water column into the bottom sediments. When the water is sufficiently agitated, the chitin probably becomes and remains suspended in the water column.

High populations of chitinolytic bacteria also seemed to demand high levels of organic carbon. However, some systems which had a high level of organic carbon per gram of water supported no detectable chitinolytic bacteria. Saganashkee Slough had about the same suspended organic carbon per liter of water as Tampier Creek. Saganashkee Slough had no detectable chitinolytic bacteria while Tampier Creek supported up to 120,000 per ml of water. Thus, although high suspended organic carbon may be necessary for high levels of chitinolytic bacteria, it does not ensure a high population of chitinolytic bacteria. It appears that in addition to high levels of suspended organics, agitation may also help keep chitin and bacteria in suspension.

Aerobic chitinolytic activity is well known. Anaerobic chitinolytic activity, but at a much slower rate, has been suggested by Warnes and Randles (1977 and 1980). Therefore, the relationship of chitinolytic activity to the dissolved oxygen concentrations was examined. Crooked Creek, with a dissolved oxygen concentration of 13.1 mg/ml, supported about 190,000 chitinolytic bacteria per ml of water. Those systems with little oxygen, such as the Cal-Sag Canal, with 1.2 mg/ml, had no detectable chitinolytic bacteria. However, a high dissolved oxygen concentration alone is not sufficient to support a high density of chitinolytic bacteria. Stormy, well agitated Lake Michigan, with a dissolved oxygen concentration of 13.7 mg/ml supported a population of only 13 chitinolytic bacteria per ml. This probably relates to the fact Lake Michigan was also lowest in total organic carbon per L of water.

We also tested our four species of chitinolytic bacteria in the Enterobacteriaceae for chitinolytic activity under anaerobic conditions. Each of the four species was inoculated on chitin agar plates as described previously. These plates were placed in a candle jar and kept in anaerobic conditions for 21 days. No chitinolytic activity occurred in this time period. Although the data of Warnes and Randles (1980) indicates the possibility of chitinolytic activity in anaerobic sediments, this needs further investigation.

Streams which were high for all three parameters, agitation, total organic carbon, and dissolved oxygen, had the highest populations. This is probably due at least in part to soil humus which is carried into streams with surface run-off. Soil humus not only contains soil bacteria, but also decaying fungi, nematode eggs, arthropod exoskeletons, and other sources of chitin.

The pH of the samples ranged from 7.3 to 8.7. Bacterial growth, both total and chitinolytic, seemed to be independent of pH in this range.

SUMMARY

The data reported in this paper suggestes a relationship between dissolved oxygen levels, total organic carbon concentration, agitation of the water and chitinolytic bacterial levels. When all three were high, high numbers of chitinolytic bacteria were present. High total organic carbon and high dissolved oxygen levels were related to agitation. Streams which were high for all three parameters had the highest populations. This is probably due at least in part to soil humus which is carried into streams with surface run-off. Soil humus not only contains soil bacteria, but also decaying fungi, nematode eggs, arthropod exoskeletons, and other sources of chitin. Future studies measuring actual levels of chitin and the ratio of chitin to other suspended organics would be useful.

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Table 1. List of sample sites, dissolved oxygen in mg/ml, total organic carbon (TOC) in mg/ml, pH, colony forming units of bacteria per milliliter (CFU) and chitinolytic bacteria per milliliter (as CFU).

Sample	Dissolved Oxygen	TOC (mg/ml)	pH	CFU of Bacteria	Chitinolytic Bacteria
Lentic, Calm Day					
Saganashkee Slough	6.5	106	7.6	2000	0
Maple Lake	10.8	71	8.4	2000	0
Canal Segment	7.8	118	7.6	10300	100
Lentic, Windy Day					
Lake Michigan	13.7	35	8.3	282	13
Limestone Quarry	13.8	88	8.7	19100	100
Lotic, Little Flow					
Cal-Sag Canal	1.2	78	7.3	17400	0
Lotic, Moderate					
Stoney Creek	9.1	213	7.7	TNTC*	140000
Crooked Creek	13.1	144	7.9	TNTC	190000
Tampier Creek	12.1	102	8.3	TNTC	120000

*Too Numerous To Count