BACTERIA DECOMPOSING ALGINIC ACID

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Received for publication February 9, 1934

Alginic acid is a complex carbohydrate belonging to the group of polyuronides; these yield, on hydrolysis with mineral acids, sugar acids, known as uronic acids, or mixtures of these acids with hexose or pentose sugars; purified alginic acid yields on hydrolysis only mannuronic acid. Alginic acid occurs abundantly in various marine algae, to the extent of 20 to 30 per cent of the total algal material. Although algal residues serve as food for a great variety of animals living both in water and on land, a large part of the residues are decomposed in nature by the action of microorganisms. The processes of their decomposition, especially the fate of the alginic acid complex, still remain to be determined.

In the course of a study of the decomposition of marine algal material, a number of bacteria capable of decomposing alginic acid were isolated from sea water, from phytoplankton and from the sea bottom. The decomposition of this compound was also studied in the case of land soils and several bacteria isolated; these differed, however, in certain of their physiological characteristics from the marine bacteria. It is important to add here that bacteria are the all-important agents concerned in the decomposition of alginic acid in nature. Even in soil, which is such a favorable medium for the growth of numerous fungi and actinomyces, the decomposition was carried out almost entirely by bacteria. The most active cellulose- and hemicellulose-decomposing fungi

\[1\] Contribution No. 43 of the Woods Hole Oceanographic Institution and Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Soil Microbiology.
present in the soil were found to attack the alginic acid only to a limited extent. This was further substantiated by the fact that while active specific enzyme preparations were obtained from the bacteria, fungus enzymes, such as taka-diastase and pectolytic enzymes, had no effect upon the alginic acid.

The following methods were used in the isolation of the bacteria from their respective habitats. One per cent solution of purified alginic acid (Schmidt and Vocke, 1926) prepared from different species of Fucus, largely *F. vesiculosus*, was dissolved in NaOH or KOH solution and the reaction adjusted to pH 7.0 to 7.2. Sodium nitrate and salts, in concentrations used in Czapek's solution were added. For the isolation of the marine bacteria, sea water or 3.5 per cent NaCl solution was used; for soil bacteria, distilled water.

The liquid media were inoculated with sea water, a piece of algal growth, phytoplankton, marine mud or sand, or with land soil. After bacterial development took place, as shown by the formation of turbidity in the medium, by pellicle or gas formation, the crude cultures were plated out on the corresponding agar media, and the bacteria isolated. A number of cultures were obtained; these were tested for their ability to decompose alginic acid, and only the most active forms selected for further study.

The determination of the decomposition of alginic acid by bacteria presents no difficulty in the case of distilled water or salt water media; the residual alginic acid is precipitated by means of a mineral acid or by addition of a solution of a calcium salt. In the case of the sea water medium, a large part of the alginic acid is precipitated in the medium by the calcium and magnesium of the water; as a result of the development of specific bacteria, the precipitate gradually disappears, due both to the action of the organisms and to that of the enzyme, alginase, which they produce abundantly.

The various alginic-acid-decomposing bacteria so far isolated both from the sea and from soil were found to be Gram-negative, non-spore-forming rods; they vary, however, considerably in their physiological properties. Some are more specific than others: some are able to attack, in addition to alginic acid, starch
and other carbohydrates and even agar-agar, while others are more strictly limited to alginic acid as the chief source of energy. The soil bacterium described here produces gas actively from alginic acid, while the marine bacteria do not. Some of the bacteria produce pigments, while others do not. They also vary in the rapidity of alginic acid decomposition. All the bacteria stain better with aqueous fuchsin or gentian violet than with carbol erythrosin; with the latter stain, the cells appeared much smaller and more coccoid.

A description of four types of bacteria selected as representing the alginic-acid-decomposing organisms in nature is reported here. The forms most closely related to this group are the agar-liquefying bacteria. The B. gelaticus of Gran and especially the form later described by Lundestad (1928), under the name of B. grani, seem to be related to the first organism, namely Bact. alginovorum. However, the growth characteristics of the two organisms are sufficiently different and the activity of the latter in decomposing alginic acid is so marked that a detailed description of this form is justified. The Bact. fucicola, with its specific characteristics and pigment production, has evidently not been described before. Bact. aliginicum may have been referred to in the various descriptions of marine, especially agar-liquefying, bacteria, such as those of Angst (1929), but because of the fact that these descriptions are so imperfect, it was felt best to describe this form as well.

BACTERIUM ALGINOVORUM N. SP.

Rods with rounded to almost elliptical ends, especially when single. Occurring frequently in twos and even in chains, 1.5 to 2.0 by 0.75 to 1.2 microns in size. Non-spore-forming. Actively motile. Gram-negative. Capsule-forming. Stains well with aqueous fuchsin. Appears as a short coccoid form in carbol erythrosin stain.

Alginic acid plate: colony large, white in appearance with coarse granular center, entire margin. Clears up turbidity caused by the alginic acid on plate. No odor.

Alginic acid liquid medium: heavy pellicle formation. Active
production of an enzyme, alginase, which brings about the disappearance of the alginic acid precipitate in sea water medium. On salt water medium, a slimy pellicle of a highly tenacious nature is produced, the whole medium later turning to a soft jelly.

Sea water gelatin: active and rapid liquefaction in two to six days, at 18°C.; highly turbid throughout the liquefied zone.

Sea water glucose broth: abundant uniform turbidity, with surface pellicle; some strains give heavier turbidity and others heavier pellicle.

Litmus milk containing 3.5 per cent salt: no apparent growth. Potato moistened with sea water: moist, spreading growth, ivory-colored; heavy sediment in free liquid at bottom.

Starch plate: abundant, cream-colored slimy growth; extensive diastase production.

Agar liquefaction: extensive softening of agar, no free liquid. Aerobic, although able to grow under reduced oxygen tension.

Optimum temperature: 20°C.

Habitat: isolated from sea water, sea bottom sediments and from surface of algal growth in the sea. Very common in the sea.

**Bacterium Alginicum N. sp.**

Rods short to almost spherical, 0.6 to 1.0 micron in diameter. Non-spore-forming. Sluggishly motile. Gram-negative. Capsule-forming. When stained with aqueous fuchsin, the organism is found to be definitely a rod with rounded ends, occurring in twos. In carbol erythrosin stain, it is distinctly coccoid.

Alginic acid plate: white, finely granulated colonies, with entire margin. Does not clear up the turbidity in plate, due to limited production of the enzyme alginase. Odor formed, resembling that of old potatoes.

Alginic acid liquid medium: thin pellicle, weak alginase formation. Grows less rapidly and decomposes alginic acid less actively than previous organism.

Sea water gelatin: thin growth throughout gelatin stab, no liquefaction in seven days at 18°C.

Sea water glucose broth: uniform but very limited turbidity; no pellicle; no sediment.
Litmus milk containing salt: no apparent growth.
Potato moistened with sea water: moist, spreading growth, cream-colored; heavy sediment in free liquid at bottom.
Starch plate: limited, pale blue growth; no diastase.
Agar liquefaction: none.
Aerobic.
Optimum temperature: 20°C.
Habitat: isolated from sea water, and from surface of algal growth. A common form.

**BACTERIUM FUCICOLA N. SP.**

Rods, short, with ends rounded to almost coccoid; slightly curved; occurs singly and in twos. Does not stain evenly, 1.0 to 1.5 by 0.6 to 1 micron in size. Gram-negative. Actively motile, with twirling motion.
Alginic acid plate: colonies finely granular, entire; at first whitish, turning in three to five days brown, and later almost black, producing a deep brown soluble pigment.
Alginic acid liquid medium: limited growth on surface in the form of a pellicle. Frequently produces no growth at all.
Sea water gelatin: active liquefaction; no growth in stab; thin, fluorescent growth throughout liquefied zone.
Sea water glucose broth: faint turbidity; no pellicle, no sediment.
Litmus milk containing salt: no apparent growth.
Potato moistened with sea water: no growth.
Starch plate: no growth.
Agar liquefaction: positive, although limited; only softening of agar.
Aerobic.
Optimum temperature: 20°C.
Habitat: isolated from sea water taken from George's Bank, near the surface of the sand bottom. Rare occurrence.

**BACTERIUM TERRESTRALGINICUM N. SP.**

Long rods, with somewhat rounded ends, occurring usually single, but also in twos, and occasionally in chains of shorter rods,
1.5 to 2.5 by 1.0 to 1.5 microns. Gram-negative. Motile. Granular.

Alginic acid plate: colonies small, whitish in appearance with a slight metallic sheen.

Alginic acid liquid: medium at first clouded. Later, a pellicle is formed on surface of medium, which is soon broken up due to active gas formation. Reaction of medium becomes slightly alkaline.

Gelatin medium: slow growth throughout stab; slow liquefaction at surface of medium, at 18°C.

Glucose broth: abundant turbidity; some sediment; no pellicle; slightly fluorescent.

Litmus milk: acid; milk coagulated; only limited digestion of coagulum.

Potato: abundant, pinkish-colored, compact, dry growth on surface of plug, the rest of plug becoming grey, with tendency to darkening.

Starch plate: limited growth along streak; no diastase.

Agar liquefaction: none.

Aerobic to facultative anaerobic.

Optimum temperature: 30°C.

Habitat: soil.

In order to illustrate the action of the different bacteria upon alginic acid and upon crude algin, certain data are reported in tables 1 to 3. The term algin is applied to the washed acid precipitate of the dilute ammonium hydroxide extract of the algal material. The crude algin preparation contained 51.8 per cent uronic acid, 2.1 per cent ash and 1.66 per cent nitrogen.

In the first experiment, 100-cc. portions of sea water medium containing 1 gram of the air-dry alginic acid preparation, in the form of a potassium salt, were placed in flasks. A stream of CO₂-free air was passed above the cultures. The CO₂ produced by the organisms was absorbed in standard Ba(OH)₂ solution and the excess of the latter titrated with standard oxalic acid. Decomposition of the alginic acid was allowed to proceed, at room temperature, for twenty-one days. The results (table 1) show that the purified alginic acid is decomposed by the various bac-
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However, the extent of decomposition of the alginic acid in the crude algin preparations was much less, with greater varia-

**TABLE 1**

*Decomposition of purified alginic acid by marine bacteria*

<table>
<thead>
<tr>
<th>BACTERIAL CULTURE</th>
<th>CO₂ GIVEN OFF</th>
<th>RESIDUAL ASH-FREE ALGINIC ACID</th>
<th>ALGINIC ACID DECOMPOSED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.15</td>
<td>935</td>
<td></td>
</tr>
<tr>
<td><em>Bact. alginicum</em></td>
<td>113.93</td>
<td>308</td>
<td>627</td>
</tr>
<tr>
<td><em>Bact. alginovorum, No. 4</em></td>
<td>98.83</td>
<td>160</td>
<td>775</td>
</tr>
<tr>
<td><em>Bact. alginovorum, No. 10</em></td>
<td>101.56</td>
<td>150</td>
<td>785</td>
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<tr>
<td><em>Bact. fucicola</em></td>
<td>122.13</td>
<td>175</td>
<td>760</td>
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</table>

**TABLE 2**

*Decomposition of crude algin by marine bacteria*

<table>
<thead>
<tr>
<th>BACTERIAL CULTURE</th>
<th>CO₂ GIVEN OFF</th>
<th>RESIDUAL ASH-FREE ALGIN</th>
<th>ALGIN DECOMPOSED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.40</td>
<td>266</td>
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<tr>
<td><em>Bact. alginicum</em></td>
<td>43.21</td>
<td>181</td>
<td>85</td>
</tr>
<tr>
<td><em>Bact. alginovorum, No. 4</em></td>
<td>56.86</td>
<td>118</td>
<td>148</td>
</tr>
<tr>
<td><em>Bact. alginovorum, No. 10</em></td>
<td>44.92</td>
<td>146</td>
<td>120</td>
</tr>
<tr>
<td><em>Bact. fucicola</em></td>
<td>46.26</td>
<td>155</td>
<td>110</td>
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</table>

**TABLE 3**

*Decomposition of alginic acid by Bact. terrestralginicum*

<table>
<thead>
<tr>
<th>URONIC ACID ANHYDRIDE</th>
<th>REACTION OF CULTURE</th>
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<tr>
<td>Left</td>
<td>Decomposed</td>
</tr>
<tr>
<td></td>
<td>pH</td>
</tr>
<tr>
<td>Control</td>
<td>443.5</td>
</tr>
<tr>
<td>Bacterium</td>
<td>343.3</td>
</tr>
</tbody>
</table>

In the last experiment, 300-mgm. portions of the algin preparation in the form of the potassium salt were used in 100-cc. portions of medium.
The rapidity of decomposition of the alginic acid by the soil organism was then tested. Fifty-cubic centimeters of distilled water medium, each containing 0.5 gram of the air-dry alginic acid preparation, in the form of a sodium salt, were inoculated with the bacterium and incubated at 28°C for twenty days. No attempt was made to measure the CO₂ given off in the growth of this organism. The amount of uronic acid anhydride decomposed by the culture was determined. However, the decrease in total uronic acid content cannot be taken as a true index of decomposition of the original alginic acid, since considerable hydrolysis of complex to smaller mannuronic acid units took place, without complete decomposition. The growth of the soil organism was accompanied by active gas evolution.

The various bacteria, both of marine and soil origin, were also found capable of decomposing alginic acid in the original Fucus material.

SUMMARY

The decomposition of alginic acid, a polyuronide occurring abundantly in marine algae, was found to be brought about largely by the action of certain specific bacteria, and only to a very limited extent by other microorganisms, such as fungi. Specific bacteria capable of decomposing alginic acid occur abundantly in sea water, in marine plankton, in the sea bottom and in land soils.

Four species of bacteria are described in this paper, three of which were isolated from the sea and one from the soil.

These bacteria decompose alginic acid actively, not only in purified preparations, but also in the original algal material.

REFERENCES

