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MARINE ALGAE OF VIRGINIA AS A SOURCE OF AGAR AND AGAROIDS

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Introduction

Along the Eastern Shore of Virginia and in the lower Chesapeake Bay there are five species of red algae in abundance that are known to produce agar or an agar-like polysaccharide of actual or potential economic value. The abundance of two of these species is such at certain times of the year that they may be present in sufficient quantities to make it worthwhile for fishermen to harvest and dry them for sale to a processing factory. But there are many problems yet to be solved before Virginia's seaweed resources are likely to be an item of commerce. Of fundamental importance is a more precise knowledge of their distribution and abundance during their best growing season so that statements concerning amounts available can be something more than conjecture, as are those made above. Another problem is the need for a better understanding of the physical properties and chemical nature of their extractives in order that there be a sound basis for determining the uses for which they are best adapted.

Chemical Nature and Properties of Extractives of the Red Algae

The cell walls of the red algae, in addition to a thin layer of cellulose, are made up of a thick layer of a polysaccharide or polysaccharides. Some of these substances are soluble in cold fresh water but most of them are soluble only in hot water. They form colloidal solutions and most of them form a thermally reversible gel when cooled to a certain degree if the pH of the sol is maintained between 5.5 and 8.5. At a pH of 4.5 or lower they usually hydrolyze rapidly when heated and a hexose monosaccharide, galactose, is the principal product. Apparently all the cell wall polysaccharides of the red algae are sulfate esters of linear galactans, and they seem to constitute a family of compounds produced among living things only by the red algae and possibly also by the bluegreen algae (Phylum Cyanophyta). The chemical complexity of these polysaccharides is such that there is little likelihood that they will be synthesized in the near future despite the economic value of certain of them. Thus our sole source of these remarkable sea plant extractives that find their way into hundreds of foods, cosmetics, pharmaceuticals, and other products of commerce will continue to be the red algae.

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Agar.-- One of the earliest and most important uses for an extractive of the red algae was in bacteriological culture media as a gelling agent. This use was first established by the famous German medical bacteriologist Robert Koch about 1881 (Hitchins and Leikind 1939). Because of its importance in bacteriology and the very specific requirements as to physical properties, the term agar has been more precisely defined in recent years: an extractive of certain red algae, especially of the genera Gelidium and Pterocladia, which in 1.0% solution in pure water will form a firm gel between 42 and 37° C. and which exhibits a relatively low viscosity as a sol. Though its importance in culture media cannot be denied, only about 10% of the agar used in the United States goes into bacteriological media.

Recent analyses on the chemical nature of agar have shown it to consist of at least two distinct polysaccharides which have been termed agarose and agaropectin. Agarose is the principal gel-forming constituent of agar. It can in turn be separated into two fractions: (1) 1,3-linked b-D-galactopyranose and (2) 1,4-linked 3,6 anhydro-a-L-galactopyranose.

In order to conserve the term agar for those commercially-prepared extractives that meet the requirements for bacteriological media, the general term agaroid may be applied to all other extractives of the red algae. Thus "agar" and "agaroid" are both terms of convenience and neither refers to a precise chemical entity.

Agaroids.-- The term agaroid may be used to refer to the wide variety of extractives of the red algae that are not agar in the strict sense. Most of the polysaccharides, like agar, form a thermally reversible gel though sometimes a weak one. A few do not gel at all, merely increasing in viscosity with reduction of temperature.

The most famous agaroid is carrageenin, the extractive of "Irish moss" (Chondrus crispus Stackhouse) which grows in abundance along the New England coast and the provinces of Prince Edward Island and Nova Scotia. Its range is from New Jersey to Newfoundland and probably to Labrador. Carrageenin is the most important seaweed extractive produced commercially along the Atlantic coast of the United States.

In the past, the term carrageenin has been used to refer exclusively to the whole extractive of Irish moss. In recent years, however, analyses of the polysaccharides from Irish moss by Yaphe (1955, 1957, 1959) have shown that it is a polymer of a-D-galactopyranoside-4-sulfate and that it can be fractionated with salts, such as KCl, into two components, one of which forms a gel and the other of which does not. Yaphe has termed the gel-forming

constituent "kappa carrageenin" and the other "lambda carrageenin." He has also shown that these same constituents occur in several other red algae and indications are that they may be widespread as cell wall constituents among the red algae which do not produce true agar. The extractive of the red alga Hypnea seems to be principally kappa carrageenin on the basis of bacteriological tests and physical properties. The extractive of Gigartina acicularis (Wulfen) Lamouroux, on the other hand, may be entirely lambda carrageenin. While no one has yet reported finding a mixture of the two agar constituents with those of carrageenin, such mixtures are to be expected and may occur in the genus Gracilaria.

The American Agar and Agaroid Industry

Before World War II, Japan held virtually a world monopoly on the production of agar. This had come about as a result of the extensive use of algae in Japan for hundreds of years, the abundance of excellent raw material along the coast, and the low cost of labor. With the advent of war, our importation of Japanese agar was much curtailed and it was one of the first commodities to be declared a critical war material by the War Production Board. Several agar factories were established in California using Gelidium cartilagineum (L.) Gaill. in 1942 and 1943 and they produced agar somewhat superior to that from Japan because of its uniformity and high gel strength. All but one of these factories closed down after the war as a result of resumption of full-scale importation from Japan.

An agar factory was established at Beaufort, N. C., in 1942 using Gracilaria verrucosa (Hudson) Papenfuss, G. foliifera (Forsskal) Børgesen, and Hypnea musciformis (Wulfen) Lamouroux as raw material for agar production (Humm 1951a). This factory ceased processing seaweeds in 1947. For a shorter period of time during the war a small factory at Jensen, Florida, made agar from G. foliifera from the Indian River. Under normal conditions, it is extremely difficult for a domestic producer of agar to compete with the Japanese product.

The history of the agaroid industry in the United States is quite different. Several firms were extracting and purifying the polysaccharides from Chondrus crispus (Irish moss) and Gigartina stellata (Stackhouse) Batters from Atlantic coast waters before World War II. This industry has continued to make steady growth since the war, for Japanese agar does not compete with their products. Instead of agar, they make a wide variety of sea plant colloids for which there is little or no foreign competition. Fundamentally, these highly purified products serve as stabilizers in a host of products,

stabilizers of emulsions, of moisture, of texture, of consistency and of viscosity in foods, cosmetics, pharmaceuticals, and many other products of commerce (Humm 1947; 1951b).

The algae of Virginia, if any occur in sufficient abundance, would be of economic value as agaroids, not as agar.

The Species in Virginia of Possible Value

One species each of three genera and two of a fourth, all of which are known for their polysaccharide content, occur in Virginia waters. Their abundance along the Eastern Shore and in the more saline waters of the Chesapeake Bay has never been carefully estimated.

The five species and their classification areas follows:

Phylum Rhodophyta
  Class Rhodophyceae
    Subclass Florideae
      Order Gelidiales
        Family Gelidiaceae
          Gelidium crinale (Turner) Lamouroux

      Order Gigartinales
        Family Solieriaceae
          Agardhiella tenera (J. Agardh) Schmitz
        Family Hypneaceae
          Hypnea musciformis (Wulfen) Lamouroux
        Family Gracilariaceae
          Gracilaria verrucosa (Hudson) Papenfuss
          Gracilaria foliifera (Forsskål) Børgesen

Gelidium crinale is the only one of the four species that produces agar. However, the plants are so small that it is inconceivable that this species could ever serve as a commercial source of agar. The species is abundant on oyster shells from 4-6 inches above mean low tide down to a foot or more below low tide. They are about one inch tall, slender, wiry, purplish black in color, and firmly attached. The branching is pinnate and this is the easiest way to distinguish them from a similar species, Gymnogongrus griffithsiae (Turner) Martius, which is mostly dichotomously branched and is somewhat coarser. This latter species certainly produces a polysaccharide, but evidently it has never been studied. It is less abundant in Virginia than is Gelidium.

While having no potential commercial value (unless it produces an antibiotic or has some unique value), Gelidium crinale can be gathered in sufficient quantity for use in demonstrating an agar source in the laboratory.

Agardhiella tenera is probably the most abundant of any species of red alga in Virginia waters, especially in the spring and summer. It grows just below mean low tide and is usually attached to oyster shells. It produces bushy-branched plants 10-18 inches in height and rose red in color and it resembles Gracilaria verrucosa, but it can be distinguished positively from Gracilaria by microscopic examination of a cross section. In Agardhiella the branches are fundamentally hollow in the center but the central area is partially filled with colorless filaments; in Gracilaria the branches are cellular throughout although the cells in the center are quite large.

Agardhiella tenera produces a cold-water-soluble agaroid (carrageenin ?) with a very low gel strength.

Hypnea musciformis is abundant in Virginia waters in certain areas from mid-summer until fall where the salinity rarely falls below 15 °/oo. It is also a bushy-branched plant but it is never red in color. It varies from olive green to purplish green. The ultimate branches are finer than those of Agardhiella or Gracilaria and are spine-like in appearance but not in ridigity. The tips of many branches may be hooked or recurved. The branching is never dichotomous. The tetraspores, always borne on the ultimate branches near their base, are zonate. In Agardhiella the tetrasporangia are scattered on nearly all branches and are also zonate.

The polysaccharide obtainable from Hypnea has remarkable properties. As indicated above, it may be principally kappa carrageenin.

Gracilaria verrucosa often becomes abundant in quiet bays, especially along the Eastern Shore in late summer and fall. It is loosely bushy-branched and resembles Agardhiella but is a little more slender and is parenchymatous-cellular throughout. It grows attached to oyster and clam shells, developing in the spring when the water temperature is consistently above 16° C. and disappearing in the fall or winter when the water temperature is again below this point. How it persists during the winter months has never been determined; possibly its holdfasts remain viable.

G. verrucosa (formerly referred to as G. confervoides (L.) Greville) reaches its best development in the area of Beaufort, N. C. (Humm 1942), and served as the principal raw material for the agar industry there during World War II (DeLoach et. al. 1946a). Its polysaccharide closely resembles agar and it may be a mixture of agarose and carrageenin. There is good evidence that the proportions of the fractions of its extractive vary with season and possibly habitat so that the physical properties are not constant.

G. foliifera (G. multipartita (Clemete) J. Agardh; G. lacinulata (Vahl) Børgesen?) is never red in color but always olive green to greenish purple. The branches are only moderately dense and they are often flattened, in contrast to G. verrucosa, and there is much dichotomous branching. In all species of Gracilaria the tetrasporangia are scattered on all branches and are tetrapartite rather than zonate as in Hypnea and Agardhiella. In Gracilaria the cystocarps (which occur only on female plants) are on the surface and much raised whereas in Agardhiella they are immersed and only slightly protruding.

The polysaccharide of G. foliifera is sometimes agar-like, sometimes carrageenin-like. Its variation among samples from widely separated habitats including the Hampton Roads area was studied by Kim (1959).

A comparison of characteristics of the Virginia species of algae known to produce useful polysaccharides is given in Table 1. Some of the data in this table are based upon observations on these species from other areas, some are estimates and subject to revision and correction. Where a range is given, the indications are that there is a variation in this characteristic in relation to season of the year, circumstances of extraction, or other substances present. Sources include DeLoach et al. 1946a, 1946b; Humm 1944; Humm and Williams 1948; and previously unpublished data.

Laboratory Methods of Extracting the Polysaccharides

Drying and Bleaching. -- Freshly collected algae should be rinsed thoroughly with fresh water and then spread to dry on a concrete surface in the sun or in some other favorable drying situation. When dry, the algae should be washed in fresh water with agitation or squeezing to remove silt and salt from inside the cells. With Agardhiella, the washing should be very brief as the polysaccharide is cold-water-soluble. With Hypnea washing should be no longer than five minutes for once the salt is removed from the cells the polysaccharide becomes cold-water-soluble. With Gelidium and Gracilaria washing can be continued as long as there appears to be need for it.

After washing, the seaweed should be spread thinly to dry a second time. This time considerable bleaching will occur and this helps to eliminate pigments from the extractive. If further bleaching is desired, the seaweed should be turned over and simply wet again with fresh water. With the thir drying, the material should be bleached to a pale straw color, especially if drying occurs in good sunlight.

Table 1. Characteristics of the five species of Virginia algae

	Agardhiella tenera	Gracilaria foliifera	Gracilaria confevoides	Hynea musciformis	Gelidium crinale
Known range, Atlantic coast of N. America	Tropics to New Hampshire	Tropics to New Hampshire	Florida to P.E. Island	Tropics to Cape Cod	Florida to St. Lawrence
Season of most abundance in Virginia waters	Summer	Summer	Fall	Summer	Year-round
Usual color of plants in their natural habitat	rose-red	purplish-green	rose-red	purplish-green	purple-black
Wet wt./dry wt. ratio of raw material	20:1	15:1	18:1	20:1	12:1
Polysaccharide* content, % of bone-dry wt.	80	40	25-45	45-60	?
Approx. gel** strengths or range of a 2% solution	10-20	20-80	75-125	0-200	100
Temperature of gelation, °C.	?	45-60	40-63	adjustable	38-42
Approx. viscosity of melted solutions	high	moderate to high	moderate	very low to high	low

*These figures represent the approximate total. In the laboratory, half or more is usually obtained as a yield after one or two extractions. Factory yields, however, are lower and also are based upon air-dried material, which contains considerably more moisture than "bone-dry" raw material. The latter is usually dried to a constant weight at 50°C.

**The gel strength of agar from Gelidium crinale was arbitrarily rated as 100 as a basis of comparison. It is probably quite similar in gel strength to agar of commerce.

Gelidium crinale. -- To one liter of distilled water add 40 g. of thoroughly dry Gelidium. Adjust the pH to 5.5 and steam pressure cook at 15 pounds for 15 minutes. Filter through paper. This procedure is best done by placing a pyrex funnel with folded filter paper in a tripod over a beaker into the autoclave and holding the steam pressure at 5-15 pounds for about 30 minutes. Under steam pressure the viscosity of the agar is at a minimum and filtration is more rapid. Reduce the volume of the extract by one-third or one-half and then pour into a sterile petri dish if it is desired to demonstrate the use of this agar as a bacterial culture medium. Addition of nutrients is unnecessary as sugars and protein break-down products from inside the cells are present in sufficient amounts to support good bacterial growth. Expose to the air for about 20 minutes and keep at room temperature for 10 days.

To dehydrate and purify the agar, pour the filtered solution (without reducing the original volume) into a container suitable for placing in a refrigerator freezing compartment or deep freeze. Crush the agar ice and allow it to thaw on a screen. As much as 85% of the water of hydration will run free and remove with it the same proportion of soluble impurities (salts, sugars, amino acids, pigments) that were extracted with the agar. Air dry the wet, spongy agar that remains. Dissolution of the dry agar may be difficult because of its thickness. This procedure, however, demonstrates dramatically what happens when frozen foods, especially meats, are thawed and refrozen, and why they lose flavor and become tough. As the agar solution (or any hydrophilic colloid) freezes, there is an almost complete separation of water from the colloidal micelles as the water becomes ice. As thawing occurs, only that amount of water that the colloid is capable of absorbing (rehydration) will go back into the colloid, the rest running free with its content of substances in true solution. Agaroids often reabsorb large amounts of the water as thawing occurs, or even go into solution completely, hence freezing and thawing with such types does not accomplish dehydration and purification.

Agardhiella tenera.-- Most of the polysaccharide of this species is cold-water-soluble. Extraction occurs by simply soaking the dry seaweed in pure water at room temperature. There is also a gel-forming component and apparently this is obtained only by heating, but there seems to be no literature on the nature of the extractive. It behaves as if it were composed of a mixture of mainly lambda carrageenin and a small portion of agaropectin, but an undescribed fraction may be involved.

Sea plant colloids with these properties are of special value for a wide variety of uses.

Hypnea musciformis. -- A truly remarkable sea plant colloid is that obtained from Hypnea. Briefly, it is cold-water-soluble in the absence (or very low concentration) of solutes but soluble only in hot water and forming a thermally reversible gel in the presence of solutes. Furthermore, the properties of the extractive vary with different kinds and different amounts of solutes. The solutes can be various salts, sugars, alcohols, or some other type of organic compound. The physical properties affected include temperature of gelation and melting (hysteresis range), gel strength, viscosity of the sol phase, gel elasticity, light transmission, syneresis. Since many of the important physical properties can be controlled more or less independently of each other, it is possible to prepare an agar-like gel with any desired temperature of gelation and gel strength, within certain limitations (Humm 1948; DeLoach et al. 1946b; Humm and Williams 1948).

Gel strength can be controlled primarily by concentration of Hypnea extractive, secondarily by nature and amount of solute. Temperature of gelation can be controlled mainly by the amount of solute. With some solutes, the gel strength in relation to concentration of Hypnea extractive will be much greater than is ever obtained with agar of commerce.

Extractives of Gelidium, Gracilaria, and Agardhiella are only slightly affected by the presence of solutes, hence gels from these prepared in pure water are near maximum strength. In the case of carrageenin from Irish moss, one fraction (kappa) is strongly influenced by solutes.

Because of the influence of solutes either of two procedures may be used in the preparation of Hypnea extractive. Well-washed, dry Hypnea can be extracted at room temperature in distilled water for 6-12 hours or overnight. (A longer period results in too much bacterial activity.) Or it can be boiled or pressure-cooked as recommended for Gelidium.

Dehydration can be accomplished by freezing and thawing if about 0.2% KCl is added to the filtered and concentrated extractive. Since some KCl will remain in the dry extractive, only about 0.1% need be used when reconstituting the gel.

Pavlov and Engel'shtein (1936) studied the effect of cations and anions on the strength of agar and gelatin gels. They found that the effect of gel strength of cations in decreasing order is: caesium, rubidium, ammonium, potassium, sodium, lithium, calcium, barium, and strontium; for anions the decreasing order is nitrate, bromide, sulfate, chloride, iodide, and acetate. Studies of the effect of these ions on Hypnea extractive gel strength indicate a similar but not identical order of influence. Potassium, for example,

was found to be of greater influence than ammonium (unpublished data). These differences may be due to the difficulty of measuring accurately the slight influences upon agar.

For use in bacteriological media, Micara (1946) found the addition of 0.3% KCl to be the optimum amount with 1.0 to 1.5% concentration of Hypnea extractive.

Gracilaria foliifera and G. verrucosa. -- All species of Gracilaria are best extracted by 15 pounds steam pressure for 15 minutes using a ratio of about 40g dry weight to one liter of water. The pH should be adjusted to about 5.5. An increase in pH will occur during extraction.

Optimum extraction will occur if the raw material is soaked for several hours or overnight in water of pH 5.0. This is then poured off and extraction water at pH 5.5 is added. It is advantageous to buffer the extraction water.

Ordinarily considerable dehydration and purification can be accomplished by the freezing and thawing process, though it is not as effective with Gracilaria as it is with Gelidium or with Hypnea and 2.0% KCl. Some samples of G. foliifera lose very little water upon thawing.

An alternative method of dehydrating the extractive is to pour it into a shallow pan (preferably enamelware) to a depth of about one-fourth to one-half inch after it has been filtered but without reduction of its volume. Place the pan of agar in the sun or in a favorable drying situation until evaporation has left a coating of dry agar resembling varnish. Flood the pan with tap water for about 15 minutes, pour off the water and carefully lift up the margin of the agar film at one end of the pan. It is often possible to peel the wet agar film from the bottom of the pan in one intact sheet. Soak the sheet in one or two changes of distilled water to bleach out the soluble substances and pigments and then hang it up to dry again. The resulting sheet of agar will resemble cellophane or sheet plastic, and if it is thin it will dissolve readily in hot or boiling water in 1.0 or 2.0% solution.

Future Work

Of primary importance in an investigation of the economic possibilities of the algae of Virginia is a survey to determine approximate quantities available at the most favorable time of the year of Agardhiella tenera and the two species of Gracilaria. Careful laboratory analyses should be made of these and other species with reference to yield, methods of processing,

chemical nature and physical properties of the polysaccharides, methods of separating the components, and methods of altering and controlling the physical properties. Comparisons should be made with raw material obtained from different habitats and from the same habitat at different times of the year.

Ecological studies are needed to determine the principal factors of the environment influencing the growth and reproduction of each species. Where it appears that the abundance of a species of interest is proportional to the solid substrate available, such as scattered shells, an experimental program should be initiated to determine the effect upon abundance of the addition of shells, brick, or other solid material to the habitat at different times of the year. Growth rates should be determined in relation to water temperature and salinity, and experimental impounded areas should be established to determine the growth of loose plants added to such areas.

Summary

Five species of red algae are known to occur along the coastline of Virginia from which agar or agar-like polysaccharides can be obtained: Gelidium crinale, Agardhiella tenera, Hypnea musciformis, Gracilaria verrucosa, and G. foliifera. Vegetative characteristics, methods of extracting and dehydrating the polysaccharides, and the principal physical properties of these extractives are given. Their potential economic value is indicated in relation to the history of the seaweed industry, with reference to the red algae, of the Atlantic coast of the United States. The basic research essential to an evaluation of Virginia marine algae is proposed.

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