CHITINOVOROUS BACTERIA[1]

A PRELIMINARY SURVEY

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Chitin, the flexible but tough insoluble substance which reinforces the cell walls of the fungi and the exoskeletons of the arthropods, was probably first prepared in 1811 by Braconnot. He used the word fungine to designate the insoluble nitrogenous residue obtained by heating mushrooms with alkali. It is now known to be the characteristic framework substance of the fungi, though thus far all attempts to isolate it from the true bacteria have failed. It was first called chitin in 1823 by Odier who got it from the wing covers of May beetles. It occurs in most of the invertebrates, lobster and crab shells being the most convenient source. It was not until about the turn of the century that fungine and chitin were recognized as nearly if not exactly identical; possible physical or chemical differences between animal and vegetable chitin have been the subject of research up to the present day.

Considering the large amounts of this substance constantly built up and broken down in nature, surprisingly little work has been done on the organisms capable of attacking it. So far as is known, the higher animals do not digest it. Abderhalden (1911) calls attention to the fact that the thinning of chitinous shells in the digestive tracts of selachians and fishes is due to the acidity of the gastric juice—the chitin is not destroyed and can be recovered from the lower intestine. Apparently no one has

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considered the possible availability of this compound as a source of carbon, nitrogen or energy to animals. Cellulose, another relatively insoluble polysaccharide, is a source of energy to herbivores after it has been hydrolysed by their intestinal bacteria. Whether chitinovorous bacteria perform an analogous function in the digestive tract of any animal is unknown.

Of even greater importance is the rôle of chitin in the metabolism of microörganisms and consequently in the nitrogen and carbon cycles, both of soil and of water. Evidence has come to light within the past few years that soils contain numerous types undiscovered by pioneer bacteriologists. The cellulose bacteria described by Winogradsky (1929) are morphologically and culturally very different from those previously recognized, and it seems reasonable that modern elective culture methods might yield organisms capable of breaking down other complex organic substances. The, as yet, unpublished work of Henrici on the ecology of water organisms reveals a bewildering variety of types never before investigated. The rigid, relatively insoluble chitinous structures which are constantly disintegrating in soil and water, must obviously be susceptible of attack by microörganisms of some kind.

Zopf (1880) reported that certain fungi parasitic on insects can digest chitin. Benecke (1905) was the first to describe a bacterium which used chitin as a food. He prepared chitin from crabs, added it to water containing 0.03 per cent K_2HPO_4, 0.03 per cent $MgSO_4$, and 1.5 per cent NaCl, and inoculated this medium with rotting plankton from the Keil harbor. After several transfers he isolated an organism which he named *Bacillus chitinovorus*, aerobic, gram-negative, asporogenous, rod-shaped, motile with peritrichous flagella, often aggregated into zoögleae, occasionally in chains. It broke down the chitin without producing any acidity, odor or characteristic end-products. Neither glucosamine nor any other reducing substances appeared in the cultures; the intermediate products of chitinolysis must have been consumed as soon as they were formed. The organism produced acid from glucose and sucrose, and could use lactose as a source of energy though no mention is

made of acid production from the latter. Gelatin was liquefied and nitrate reduced, but starch and cellulose were not attacked. From rotting basidiomycetes he also isolated bacteria which grew well on crab chitin, but he did not describe or name them.

Folpmers (1921) sixteen years later isolated two strains of chitinovorous bacteria from the harbor water at Kiel; one he considered identical with Benecke's organism; the other differed from it only in its inability to attack gelatin. He made the interesting observation that butyric or butylic bacteria added to his cultures caused the chitin to be broken down twice as fast and that gas and butyric and acetic acids were formed by these mixed cultures. He also stated that in the soil chitin is destroyed by bacteria and by actinomycetes, but he did not describe any pure cultures.

Steiner (1931) reports that mixed cultures from the water and mud of an Alpine lake attacked chitin both aerobically and anaerobically. No pure cultures were isolated.

Rammelberg (1931) prepared chitins from fungi and from crabs, and, after having shown that there was little or no chemical difference between them, used each as the sole source of energy in an otherwise mineral medium, which he inoculated with material from a compost heap. The same organism was isolated from both media, and the pure cultures obtained by plating grew equally well on chitin from either source. In practically all the biological characteristics that were determined this organism resembled *B. chitinovorus*. Rammelberg describes in considerable detail a cycle of morphological development which includes rod-forms, filaments, large ovoid and signet-ring forms and symplasms, and emphasizes the fact that Benecke also observed large oval cells as well as bacilli.

Stormer (1908) and Jensen (1932) have both reported chitin destruction by actinomycetes in soil, and Johnson (1931, 1932) isolated and described various myxococci which grew on crab or fungus chitin.

Benecke's organism and the two variants reported by Folpmers and by Rammelberg are apparently the only chitinovorous Eubacteriales thus far described. Consideration of the vast

amount of complex chitin-bearing materials constantly being broken down in a variety of situations in the sea, in fresh water, and on dry land, makes it seem improbable that the ability to elaborate a chitinase should be confined to a single species among the true bacteria. The question also arises, whether the chitinovorous bacteria form a highly specialized, well defined group, or whether chitin destruction in nature is due to ordinary soil or water organisms whose activities in that direction have never been observed. The very real theoretical importance of chitin as a possible source of energy for heterotrophic microörganisms, and as a link in the carbon and nitrogen cycles suggests and justifies further investigation. The work to be described has for its object the isolation of chitinovorous bacteria from a variety of habitats, a study of their morphological and cultural characters and some comparison of these cultures with others previously described.

PROCEDURE

Chitin was prepared as follows: Crab shells were scrubbed as free as possible of flesh and dirt and decalcified in cold one per cent hydrochloric acid, which was changed three times within a period of about a week. The limp leathery shells were washed, cut into pieces of suitable size and shape, and soaked in 2 per cent potassium hydroxide for ten days, during which period they were stirred several times and on three occasions brought to a temperature just below boiling and then allowed to cool. This should remove the protein and other organic matter except chitin; most of the pigment dissolves out in the alkali. As traces of pigment would not render the product unsuitable for this work, while any chemical alteration of the chitin itself should be avoided for theoretical reasons, the often recommended complete bleaching by permanganate and bisulphite was omitted. The material was washed free of alkali, extracted three times in boiling ethyl alcohol, and dried—after which treatment it appears colorless. For purposes of comparison, a small supply of lobster chitin was procured from the Eastman Company. This, according to their statement, was prepared by the same method, except

that the shells were coarsely ground after decalcification and the alcohol extraction was probably more thorough—it was continued "until a day's extraction failed to bring out any solid on evaporation of a portion of the extract."

The home-made crab chitin offered certain practical advantages. In the enrichment and cultivation media it is always advisable, and in the case of some strains actually necessary, that the chitin protrude above the liquid, as the most vigorous growth and the first visible disintegration of the chitin takes place usually at the surface. A strip about 5 to 7 mm. wide and 4 to 5 cm. long placed in a test tube containing about 5 cc. of liquid meets this requirement admirably and comparisons of amount of turbidity and presence of a pellicle or sediment are much easier when the liquid is not filled with a mass of small irregular bits. Moreover, the beginning of chitin destruction is much more easily observed on a piece with a straight entire edge. Crab shells can be bought in the market and are convenient to handle; a number of strips can be cut from each one, and the remaining portions ground fine and used in the plating medium.

The saline fluid used as a basis in all cultures was distilled water containing 0.03 per cent each of K_2HPO_4, $MgSO_4 \cdot 7H_2O$ and NaCl. The tubes used for enrichment and transfer of mixed cultures and for isolation of colonies fished from plates, each contained a strip of chitin nearly covered with the fluid. After autoclave sterilization the pH was 7.6 to 7.8. Obviously any organism which can grow in pure culture in such a medium must derive its energy and carbon from the chitin and also its nitrogen unless it is able to fix this element from the air. A useful variation is the addition of a trace of peptone. In the case of most of the organisms studied, this hastens the growth of pure cultures. It is not advisable to use it for isolations from plates, however, as it tends to favor the persistence of organisms devoid of chitinase. Apparently these almost always accompany the true chitin destroyers, and mixed colonies are the rule rather than the exception on the first platings.

For obvious reasons, the ordinary nutrient agar and gelatin plates used in Rammelberg and Benecke's method of strewing

sterilized ground chitin over the surface of plates, are far from ideal. A plating medium containing finely ground chitin evenly dispersed through a 3 per cent solution of agar in the basic saline solution has proved entirely satisfactory. A dissecting microscope is almost a necessity for fishing colonies, as they are often too small to be seen well with the naked eye, particularly when obscured by chitin particles.

In general, the material to be investigated was inoculated into the simple enrichment medium, transferred to a second tube as soon as growth was evident (usually within ten days if at all), then to a third tube, and then plated out. Further transfers before plating were apparently of no advantage. Representative colonies were fished, incubated and replated until the plates appeared to contain but one type of colony. Transplants from such homogeneous plates were regarded as pure cultures unless more than one morphological type appeared in stained smears. Single cell isolation seems superfluous in this type of work.

Comparison of successive sets of plates often suggested symbiotic relationships in the original mixed cultures. Sometimes the colonies on a crowded plate were larger than those of the next dilution which were well spaced. In a few instances intermediate mixed cultures in which the chitin had noticeably disintegrated showed, on plating, two or more kinds of colonies which grew well together on the same plate, but no one of which would grow alone in the simple enrichment medium. Limitations of time and equipment have prevented the study of these possibly associative types and of the companion types helpful to the true chitinovorous strains. Also, there is always the possibility that there may be organisms capable of attacking chitin only in the presence of an inorganic source of nitrogen. If there were any in the materials investigated, they were missed by this procedure.

Brilliantly colored colonies were numerous on the first plates in the majority of cases. They were usually mixtures of a colorless chitin destroyer and a chromogen which was unable to persist in pure culture on these media. This was true of many beautiful pink and red colonies, most of the green and fluorescent types,

and a very interesting small vibrio which grew in tiny navy-blue colonies in the vicinity of truly chitinovorous colorless ones. Actinomycetes were numerous and varied, and grew luxuriantly on the chitin though they apparently used it very economically as the strips did not disintegrate to any visible extent until months had elapsed. In view of the present status of the taxonomy of the actinomycetes, no attempt has been made to classify them. Turquoise-blue, flesh-pink, salmon, rose, mulberry, green, yellow, brown, and black varieties have been isolated. Molds appeared less often than actinomycetes. They were all discarded.

TYPES ISOLATED

Chitinovorous eubacteriales were isolated with comparative ease from a great variety of sources. About 250 pure cultures have been studied thus far, and classified according to their morphological and biochemical characters, as tabulated (Table I). They are obviously not members of any single species or genus, but rather fall into a number of fairly well-defined types, most of which do not resemble any of the species listed in Bergey's *Manual* (1934). Some were isolated from but one of the sources investigated, others from so many as to make it appear that they are widespread in nature. Fifty or more pure strains are not included in this report because the data are not yet complete; some of them at least are different from any of the types about to be described. It seems likely that as more sources are investigated still more types will be discovered.

Type I (fig. 1) was isolated repeatedly from the water, bottom mud and plankton of Lake Alexander over a period of two months, and also from the mud of Lake Minnetonka, from the intestines of a frog, and from the intestines of 5 bats. The 52 cultures studied attack chitin very vigorously, are strikingly similar, and apparently belong to a well-defined species. The heavy pink growth on potato, often with brownish gas blisters, is extremely distinctive. Hanging drops sometimes exhibit an entertaining peculiarity of behavior. Among the small actively motile bacilli one sees what appear at first glance to be large flexible

TABLE 1

Morphological and cultural characteristics of types

TYPE	GRAM STAIN*	SHAPE	FLAGELLA†	MILK	GELATIN	INDOL	POTATO	GLUCOSE	MALTOSE	MANNITOL	SUCROSE	LACTOSE	STARCH	CELLULOSE‡	NITRATE REDUCTION	PIGMENTATION
I	−	Plump rods	Polar	Neutral, peptonized	+	+	Abundant, pink	AG	AG	AG	AG	−	+	−	+	
II	−	Plump rods	Polar	Neutral, peptonized	+	+	Abundant, putty-color	AG	AG	AG	AG	−	+	−	+	
III	−	Plump rods	Peritrichic	Alkaline, peptonized	+	−	Colorless	AG	AG	AG	AG	−	−	−	+	
IV	−	Rods	Polar	Peptonized	+	+	Colorless	A	A	A	A	−	+	−	+	
V	−	Rods	Peritrichic	Peptonized	+	−	Colorless	A	A	A	A	−	−	−	+	
VI	−	Vibrios	Polar	Slightly alkaline ?	0	−	0	A	A	−	−	−	−	−	+?	
VII	−	Plump rods	Polar	Alkaline	+	−	Brownish	A	A	−	−	−	−	−	+	
VIII	−	Plump rods	Polar	Peptonized	+	+	Slight growth	A	A	A	−	−	+	−	+	
IX	−	Slender tapering rods	Pclar	No change	+?	−	0	0	A	0	A	A	−	−	−	
X	−	Delicate curved rods	Polar	No change	0	0	0	−	−	−	−	−	+	+	−	Yellow on cellulose and starch
XI	−	Straight rods	Motile	Alkaline, peptonized	+	−	Brownish	−	−	−	−	−	−	+	+	
XII	−	Pleomorphic	Polar	Sometimes peptonized	+	−	Blackened, growth yellow	−	−	−	−	−	+	−?	−	Yellow and "tyrosinase"
XIII	−	Pleomorphic	Polar	No change	+?	−	0	−	−	−	−	−	+	−?	+	Yellow and orange on chitin
XIV	−	Small rods	Polar	Alkaline, peptonized	+	−	Brown	−	−	−	−	−	−	−	−	Green, fluorescent
XV	−	Rods	Polar	Alkaline, peptonized	−	−	Yellow	−	−	−	−	−	±	−	−	Pink on chitin only
XVI	+	Rods with square ends	Motile	No change	+	−	Darkened	A	A	A	A	−	−	−	+	
XVII	+	Pleomorphic	Motile	Slightly alkaline	−	−	Eggshell pink	A	A	−	−	−	−	−	+	
Bc	−	Rods	Peritrichic		+			A (G?)			A (G?)		−	−	+	

Bc = *B. chitinovorus Benecke*; 0 = no growth; A = acid; AG = acid and gas; and ± = some strains +, others −.

* Kopeloff and Beerman modification.

† Gray's stain.

‡ Skinner's medium.

granular organisms, writhing and bending. Close scrutiny reveals that these are groups of the small rods, lying side by side in what might be called a "palisade-chain" arrangement. At this stage of the culture's development, there seems to be a definite tendency for actively motile rods which come into close contact to snap together side by side—an individual arriving against a chain does not become attached except at the end. Sometimes a group of 3 or 4 members becomes detached from the end of a chain, and moves off as an independent unit which may take on recruits at either end, or snap into place at the end of some other chain. The whole picture is absurdly suggestive of an attempt at military drill in the midst of a restless crowd.

Nineteen other cultures from the water, bottom mud and plankton of Lake Alexander comprise type II. They differ from type I only in the color of their growth on potato, which is rather yellowish at first but later takes on a neutral putty shade. The "palisade chains" were not observed in any of this group. However, no special search was made for them, and they may occur in cultures of suitable age.

Type III was isolated from the digestive tract of a speckled trout and is decidedly different from the first two types (fig. 2). On liquids it grows in a thick white layer at the surface, which is somewhat slimy, as is the growth on solid media.

These three types all produce acetyl-methyl-carbinol from glucose, but fail to attack lactose. They obviously do not belong to any of the well-recognized species. Type III might possibly be included in the genus Proteus. Types I and II are excluded from that genus by their morphology and from Achromobacter by their active gassy fermentations.

Type IV, which attacks the same sugars, but without gas formation, was isolated from crayfish, from decaying May-fly nymph shells, from the intestines of snipes and of bats, and from sand at the edge of Lake Minnetonka. This type may be related to the genus Pseudomonas, as more than half of the strains studied have exhibited at some time a trace of fluorescent greenish pigment on agar, though never on gelatin, peptone or potato. Visible chitin destruction is not so prompt as in the first three types and

the cultures are more likely to die out after a few weeks. The somewhat greater acidity produced by these strains in the chitin tubes (terminal pH about 5) may be responsible for this fact.

Type V includes a group of cultures isolated from the bottom mud of Lake Alexander, which are peritrichic and fail to digest starch or produce indol. Otherwise they resemble type IV.

Type VI (fig. 3) appeared in tubes inoculated with material from a ravine on the University Campus; leaf mold, soil from the edge of quiet pools, and sand from beneath the rapidly running water of a little stream. They produce a markedly acid reaction in the chitin tubes, so that with Andrade's indicator the chitin strip itself takes on a deep rose color. The growth on agar slant is very delicate, transparent, and spreading. This type does not resemble any of the vibrios listed by Bergey.

From the stomachs of two different bats type VII was isolated. These are straight plump monotrichic rods similar to type VI in their reactions on carbohydrates, but more active in their growth on other media.

Type VIII, isolated from a compost heap, resembles type VII in morphology, but is even more active on culture media. These last two types could be classified as members of the genus Achromobacter, but correspond to no species now listed.

From sand now exposed by the receding water of Lake Minnetonka type IX was isolated, long tapering monotrichic gram-negative rods, with faintly staining oval forms also present in aging cultures (figs. 4 and 5). The sugar reactions were peculiar. When first isolated the organism failed to grow at all in glucose or mannitol broth, but on the disaccharides, maltose, lactose and sucrose it produced a slimy growth and a small amount of acid. After several transfers on artificial media, it now grows on glucose broth but produces little or no acid. Starch and cellulose have never been attacked.

The remaining gram-negative types are characterized by failure to produce acid from mannitol or the four sugars used. Type X is evidently a definite species and as widespread in soil and water as type I. Thirty-four pure cultures were studied, most of them from the bottom mud of Lake Alexander, some from

Lake Minnetonka, the rest from dead crayfishes, from decaying May-fly nymph shells, from cultivated sandy loam, and from compost. They are gram-negative, extremely long and narrow when first isolated, delicately curved, and motile by a single flagellum (figs. 6 and 7). The enrichment medium becomes turbid within a week and the chitin disintegrates at the water surface a few days later. On the ordinary routine media there is little or no activity. Agar slants exhibit a very characteristic, practically invisible growth. On turning the tube toward the light at precisely the proper angle one sees a very faint film on the surface, as if "G" colonies had become confluent. However, if a small amount of water is agitated over the surface of the agar, it becomes turbid and opaque. Stained smears show hundreds of the delicate graceful threads. On starch agar there is a fairly abundant shiny yellow growth and active digestion within two days. In the cellulose medium the paper is promptly covered with a thin lemon-yellow film above and below the water line, and begins to disintegrate in about two weeks. This type, characterized by its requirement of polysaccharides for vigorous growth, as well as its morphology, is possibly related to the Cellvibrio described by Winogradsky.

Type XI also attacks cellulose, but exhibits no yellow pigment and does not digest starch. These straight motile rods were isolated from decaying crayfish and May-fly nymph shells. They do not correspond to any Cellulomonas listed by Bergey.

Type XII appears to be a well defined species, exhibiting striking morphological and cultural peculiarities. It was found in the intestine of a frog, in a sample of Bryozoa from Lake Alexander, and in mud at the waters' edge of Lake of the Isles. It is distinctly pleomorphic (figs. 8 and 9). Stained smears show rods with pointed ends, sometimes bulging to spindle shapes, sometimes elongating into gracefully curved forms. When an individual divides, it seems to pull apart rather than to cut or break sharply in two; thus, one often sees pairs joined end to end by a long thread-like structure. After a few days, pale-staining oval forms also appear. Young cultures of the frog strains are monotrichic, the others appear to be non-motile. A most striking

color effect occurs in media. A brilliant daffodil-yellow insoluble pigment is produced and in addition a brownish black soluble material which diffuses into the medium, causing the so-called "tyrosinase reaction" often seen in cultures of actinomycetes. As a result the colonies in chitin agar are a yellowish-green, and in the enrichment medium there is a thick layer of chartreuse-green slime at the surface. Chitin destruction is prompt. Thus far, no strains have visibly destroyed the paper in cellulose tubes, though they all produce a yellow growth upon it very promptly. Eight other motile strains, culturally and morphologically similar, but lacking pigmentation, should probably be regarded as variants. Three of them blackened the chitin cultures after two or three days.

Type XIII also is conspicuous in color. It was isolated from the stomach of a bat, from crayfish, from the mud at the edge of a stagnant pool, and from sand under running water. In the enrichment medium the chitin strip is colored a clear daffodil-yellow beneath the surface of the liquid. The portion exposed to the air takes on a rich orange shade. Disintegration is delayed, but ultimately takes place. Stained smears from these tubes show small monotrichic rods (fig. 10), but those from agar slants exhibit the fusiform and spindle shapes seen in type XII (figs. 11 and 12). On agar media the growth is yellow. Pellicles form on liquids. Cellulose is not visibly destroyed, though it supports a prompt yellow growth.

A few gram-negative pigmented forms remain to be discussed. Two brilliantly colored strains of Pseudomonas were isolated, one from the stomach of a bat, the other from a crayfish. Pigmentation was typical on all the media. The chitin destruction was rather slow, but these cultures probably merit designation as type XIV.

Four strains from Lake Alexander, characterized by a pinkish color on the chitin strips, but nowhere else, make up type XV. The growth on potato is yellow to orange rather than pink; no pigment appears on any other medium.

Sixteen strains which exhibit consistent yellow or orange pigmentation on all media have not as yet been designated as

special types, for several reasons. Some of them occasionally show morphological similarities to types XII or XIII, others, culturally very similar, never do. Moreover, their power to attack chitin has been lost on successive transfers in most cases.

A striking feature of this investigation is the almost complete absence of gram-positive forms. In the original mixed cultures they are often present, but only two were found capable of attacking chitin when in pure culture. As they are dissimilar in many respects they must be designated as separate types.

Type XVI, then, consists of a single culture isolated from the bottom mud of Lake Alexander. It may belong to the genus Bacillus, for the motile rods are square-ended, and tend to form in chains, and its abundant growth on the surface of media shows that it is probably not a Lactobacillus. No spores have been observed, but no special methods have been used to provoke their formation.

Type XVII, isolated from a crayfish, is a totally different kind of organism. Though it is motile in hanging drops, stained smears show the pleomorphic forms characteristic of the Corynebacteria. It produces a heavy surface growth on liquids, and on solid media a thick opaque, dull growth, which gradually takes on an eggshell pink color.

DISCUSSION

The description given by Benecke is so meager that it is impossible to tell whether any of these cultures belong to the same species as his *B. chitinovorus*. He states that gas was neither abundant nor completely absent in his chitin cultures, but makes no note as to its occurrence in sugar broth tubes. The assurances of Folpmers and Rammelberg that their organisms are identical with his, are apparently based on absence of recorded differences rather than upon convincingly identical positive findings.

No attempt has been made to group the Minnesota types into genera or to name them. It seems reasonably certain that they are too dissimilar, both in morphology and in biochemical activities, to belong to any one genus. The curved and spindle-shaped

pleomorphic forms must be species as unrelated to the simple rod-shaped chitinovores as Cellvibrio and Cytophaga are to Cellulomonas. If the cultures here reported are by any chance a fair sample of the chitinovorous bacteria in nature, all that could be said about the group as a whole is that most of them are gram-negative and monotrichic. To even this simple generalization at least five of the types described would be exceptions. Of all the biochemical properties that can be determined, the nature of the desmolases secreted by an organism, and consequently its type of fermentative action upon available sugars, is perhaps the most fundamental. The general experience seems to be that it is more significant than the particular sugars which an organism can attack. It is gratifying to note that the classification on this basis is entirely compatible with the morphological findings, and in general with the other biochemical characteristics. It is hoped that the data here assembled are sufficient to define the types so that cultures isolated in the future can easily be recognized as belonging to one of them or to a new and different type.

SUMMARY

1. About 250 pure cultures of chitinovorous bacteria have been isolated from a variety of sources, and classified into seventeen types.

2. Most of these types have not previously been described.

3. The types are so widely dissimilar both in morphology and physiology that they obviously cannot belong to any one genus.

4. Some of these types might be introduced as new species into genera now recognized. Others might be combined with some of the cellulose bacteria described by Winogradsky into a new genus characterized by their peculiar morphology and their ability to break down polysaccharides.

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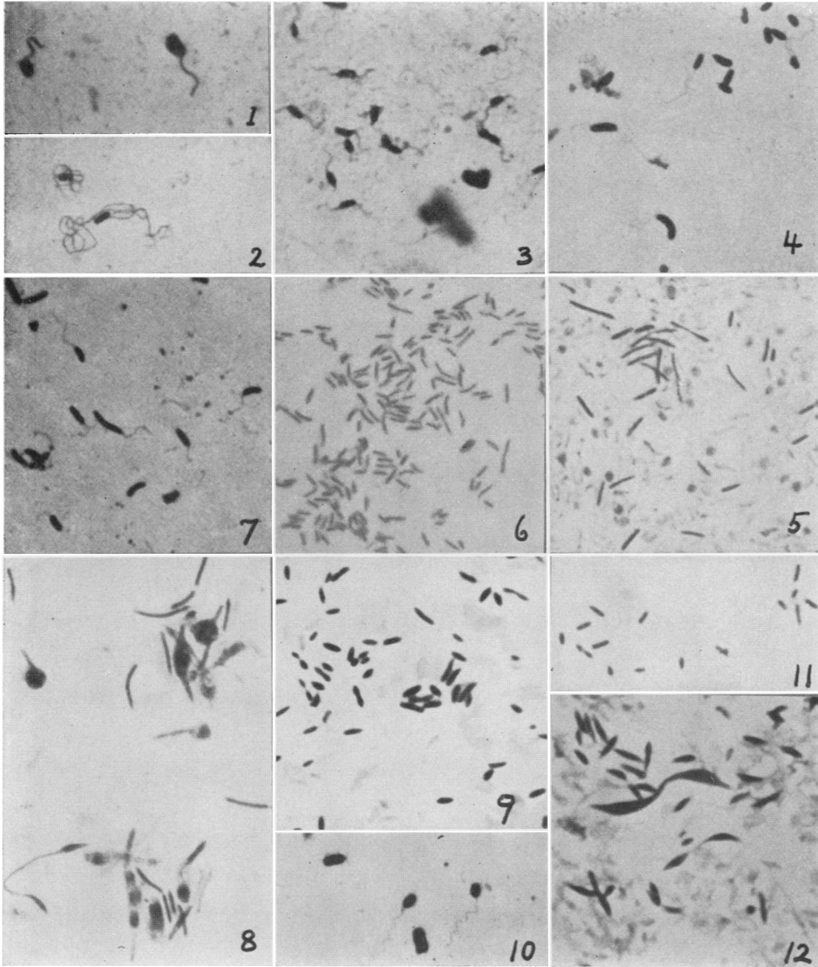
PLATE 1

(Photomicrographs × 2025)

FIG. 1. Type I, flagella stain, young culture on chitin medium
FIG. 2. Type III, flagella stain, young culture on chitin medium
FIG. 3. Type VI, flagella stain, young culture on chitin medium
FIG. 4. Type IX, flagella stain, young culture on chitin medium
FIG. 5. Type IX, Gram stain, twenty-four-hour agar slant culture
FIG. 6. Type X, Gram stain, young agar slant culture
FIG. 7. Type X, flagella stain, young culture on chitin medium
FIG. 8. Type XII, Gram stain, agar slant culture about four days old
FIG. 9. Type XII, Gram stain, agar slant culture twenty-four hours old
FIG. 10. Type XII, flagella stain, young culture on chitin medium
FIG. 11. Type XIII, Gram stain, seventy-four-hour culture on chitin medium
FIG. 12. Type XIII, Gram stain, seventy-four-hour culture on agar slant

Note. The last two were inoculated at the same time from the same inoculum, and incubated together.



(Anne G. Benton: Chitinovorous Bacteria)