ON THE DIFFERENTIATION AND CLASSIFICATION OF WATER BACTERIA.

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When bacteriologists have before them the problem of differentiation and classification of bacteria from water, independent of efforts to isolate with minimum delay some specific organism of disease, they proceed in a manner which is substantially as follows:

1. There is obtained on a given laboratory medium a pure culture of a special bacterium, the environment and life history of which, prior to its isolation at the laboratory, are for the most part unknown.

2. From the pure culture there are seeded a greater or less number of conventional culture media, known to differ in composition somewhat as prepared at different times at the same laboratory, and considerably as prepared by various workers in different laboratories.

3. These cultures are subjected at the laboratory to varying temperatures and periods of development leading up to final descriptions of cultural characteristics.

4. Observations upon and descriptions of the results obtained from the growth of this special bacterium under the above stated conditions are made.

5. Upon a comparison of the records of this bacterium with the limited descriptions of more or less similar forms given by other observers, it is found that they do not coincide, and therefore a new species is recorded.

Practically speaking, the above outline shows briefly and in a general way the conventional custom of bacteriologists in the past fifteen years, and which, as is well known, has brought the subject of species differentiation to a position which is untenable. Owing to the wide recognition of this state of affairs it seems to the writers to be futile to speak farther of the past.

It is the purpose of this paper to refer briefly to a number of points

associated with the above outlined procedure, and call attention to several modifications which experience, in a somewhat extended study of this subject, has led us to believe are of material assistance in the differentiation and classification of water bacteria.

I.—DEBILITATING ENVIRONMENT OF WATER BACTERIA BEFORE ISOLATION, AND THE CONSEQUENT DEGENERATION IN MANY INSTANCES AS NOTED AT THE LABORATORY.

Experience shows that in many cases two members of the same species of water bacterium, isolated at the same time and from the same sample, owing to divergent conditions in their earlier environments and in their life histories, will differ in some specific functions or characters, as studied in the laboratory. We have learned that, by transplanting successively such forms upon a series of media, these differences can, in a great measure, be eliminated. Accordingly, we have adopted the procedure of transferring the organisms after isolation from the pure culture on agar to nutrient broth; from the latter after three days' incubation at 20° C. to gelatine plates; and from a gelatine plate after the same period of incubation back to an agar tube again, from which the conventional media are seeded after the customary three days have elapsed. The value of this course, calculated to lend to the more or less debilitated form a rejuvenating action, and to bring the two members into a more nearly parallel physical condition before diagnostic tests are begun, is illustrated by the representative results given in Table I.

TABLE I.

EFFECT OF PRELIMINARY CULTIVATION ON BACTERIAL DEVELOPMENT.

Name of organism.	Test.	Result of Test.	
		Primary culture.	After preliminary cultivation.
Bacillus fluorescens liquefaciens.	Motility.	Negative.	Positive.
" " "	Nitrate reduction.	"	"
" prodigiosus.	Gas production.	"	"
" coli communis.	Coagulation of milk.	"	"
" ochraceus.	" "	"	"
" similtyphus.	Nitrate reduction.	"	"
" subtilis.	Motility.	"	"
" viridis.	Indol production.	"	"

In each case the temperature and period of development were 20° C. and 10 days, respectively, with the exception of the tests for motility, which feature was observed in cultures two days old.

Attention is particularly called to the last step in the process of preliminary cultivation, as it is important that, once having gotten an organism into a condition from which more favorable results may be obtained, the culture should be allowed to lose none of its regained vitality by being allowed to remain an unnecessarily long time on the agar tube before the various media are inoculated.

If the results of diagnostic tests are to be regarded seriously, and if

TABLE II.

EFFECT OF PRELIMINARY CULTIVATION ON BACTERIAL DEVELOPMENT.

Test.	Percentage constancy of results.	
	Primary cultures.	After preliminary cultivation.
Bacillus—true form,	95	100
Motility,	87	100
Spores—heat test,	93	100
Growth at 37° C.,	98	100
Liquefaction of Gelatine,	98	100
Liquefaction of Casein,	95	100
Liquefaction of Blood serum,	93	100
Fermentation of dextrose broth Gas,	92	100
Fermentation of dextrose broth Turbidity in closed arm,	89	100
Nitrate reduction,	90	100
Indol production,	97	100
Milk coagulated,	80	100
Fluorescence,	95	100
Chromogenesis,	100	100

such results are to lead to classifications which shall be of permanent value, it is obvious that such results must yield a high percentage of constancy. Otherwise they will be misleading and must be eventually discarded.

For the purpose of determining the percentage constancy of the results of prominent diagnostic tests, before and after transplanting the culture upon a series of media as described above, twenty cultures, representing eleven different species of water bacteria, were carried through a series of tests, the results of which appear in Table II.

Each of the media was seeded in triplicate with each culture from which both morphological and biological results were obtained. Development was continued for 10 days at 20° C. before the final results of the respective tests were recorded, although observations were taken day by day.

II.—ON THE COMPOSITION AND PREPARATION OF CULTURE MEDIA WITH ESPECIAL REFERENCE TO LIMITATIONS IN THE USE OF NUTRIENT CARBOHYDRATE SOLUTIONS AS PREPARED AT PRESENT.

In this connection the recommendations of the Bacteriological Committee* leave but little to be desired, so far as is practicable to be obtained at present. And in our species work during the past ten months, it has been our custom in the preparation of media to follow the procedures recommended, with no departure except a limitation in the use of the different carbohydrate solutions as now prepared.

In regard to the use of carbohydrate solutions prepared by present methods, our experience has shown an inconsistency in quantitative results in connection with gas production and the end reaction. In quite an extended study of the accuracy of these tests, the results obtained show the influence of three factors, intimately connected with the inconstant results that were often obtained, which are as follows:

1. The results of growth in a carbohydrate solution are affected somewhat by the possible degeneration of the organism.

2. The results of growth are often affected by the initial reaction of the carbohydrate solution.

3. Irregularities in the composition of the carbohydrate solution as prepared and sterilized, notably in the case of lactose and saccharose, also affect these results.

An illustration of the first factor is presented below in Table III showing representative results upon the quantity of gas and the end reaction in dextrose, lactose and saccharose broths, by six different cultures of Bacillus coli communis isolated on successive days from

* Procedures Recommended for the Study of Bacteria, *Journal of American Public Health Association*, Jan., 1898.

the Ohio River water. The temperature and period of incubation in all cases were 20° C., and ten days, respectively; and the cultures were not subjected to preliminary cultivation.

TABLE III.

REPRESENTATIVE RESULTS OF GROWTHS OF B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS.

Number of organism.	Carbohydrate.	Initial reaction 1.5 per cent Acid.	
		Total gas (per cent).	End reaction (per cent).
1	Dextrose.	77	3.8
2		83	4.1
3		52	3.2
4		41	2.9
5		88	5.0
6		90	4.9
1	Lactose.	91	3.8
2		78	3.6
3		49	5.1
4		80	4.2
5		60	2.6
6		53	3.8
1	Saccharose.	48	3.0
2		0	2.7
3		13	1.9
4		15	0.8
5		2	1.0
6		2	1.6

From what has been stated with regard to the influence of preliminary cultivation, to overcome initial degeneration, as outlined in the last section, it would naturally be expected that such a procedure would eliminate in a measure the discrepancies in the above results. As shown in Table IV this preliminary step as carried out was appreciably helpful, but was incapable within the limits studied of making the quantity of gas of decisive diagnostic value.

Relative to the influence of preliminary cultivation upon the end reaction, it was found to be inadequate to bring about even fairly constant results, as shown in Table IV.

With regard to the second of the above factors it was found that within a fairly close range of initial reactions, as ordinarily employed and reaching from about + 1.5 to —1.5 per cent, its influence upon

TABLE IV.

GROWTH OF B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS, BEFORE AND AFTER PRELIMINARY CULTIVATION.

No. of Organism.	Carbohydrate.	INITIAL REACTIONS.											
		1.5 per cent. Acid.				Neutral.				1.5 per cent. Alkaline.			
		Before.		After.		Before.		After.		Before.		After.	
		Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).
A	Dextrose	32	3.9	68	4.2	43	3.8	57	2.7	52	4.2	68	3.3
B	Dextrose	46	2.7	59	3.7	37	2.9	62	3.8	40	4.4	72	3.9
C	Dextrose	59	3.1	81	5.1	70	4.2	54	4.0	26	5.0	56	5.0
A	Lactose	62	4.2	62	2.9	39	3.4	52	3.3	47	2.9	62	2.9
B	Lactose	27	5.1	48	3.8	64	4.1	69	3.7	38	3.6	51	3.6
C	Lactose	82	3.7	74	3.9	58	3.8	69	4.1	59	2.7	63	3.9
A	Saccharose	10	1.9	83	4.7	12	3.3	64	3.8	0	—1.4	30	2.6
B	Saccharose	0	1.4	4	2.1	0	0.9	1	0.7	0	—1.2	0	—1.4
C	Saccharose	0	1.6	1	1.7	0	0.6	1	0.6	0	—1.1	0	—1.4

the total amount of gas produced and the end reaction does not appear to be very marked. This is especially true if the period of incubation at 20° C. does not exceed 3 or 4 days. When the period of incubation reaches 10 days, then, even within the above stated limits, the initial reaction becomes a factor with reference to the final quantitative results.

But if the initial reaction should be more than about 1.5 per cent from the phenolphthalein neutral point, considerable variations in the results are found, as compared with those obtained with initial reactions within the stated limits. And in most cases it was noticeable that the quantity of gas was greatest in those tubes where the solution was initially most alkaline (—2.0 per cent). This is shown by the results presented in Table V (averages of more than 100 sets of results) with reference to B. coli communis freshly isolated from feces; and when the period and temperature of incubation were 10 days and 20° C. respectively.

TABLE V.

PERCENTAGE OF TOTAL GAS PRODUCTION BY B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS OF DIFFERENT INITIAL REACTIONS.

Initial reaction (per cent).	Carbohydrate.	
	Dextrose.	Lactose.
+2.0	35	28
+1.0	36	34
.0	45	45
—1.0	53	52
—2.0	68	64

In connection with the third factor mentioned at the outset of this section, it will be noted in the foregoing tables that the fermentation data from saccharose solutions, and to a certain degree from lactose solutions, are variable to the point of being erratic. Numerous experiments show them, however, to be representative even when obtained with the same culture. That is to say, there is decisive evidence to show that the variable results were due to the culture solutions and not to the bacteria themselves.

To explain fully these variations involves a thorough knowledge of the chemistry of carbohydrates, and is beyond the scope of this paper. It is sufficient to state that the explanation, in part at least,

appears to be associated with chemical changes (inversion, etc.) which are produced directly by the action of heat. Special efforts for several months were made to obviate the disturbing influence of heat, by using intermittent sterilization of the solution at low temperature, 70° C.; the use of sugars sterilized by dry heat at low temperature for long periods; and by means of adding the pure sugar dissolved in sterile water to the broth after the sterilization of the latter. None of these efforts were successful, and other work caused an abandonment of these studies.

Summing up in brief terms the experience recorded in this section, it may be stated that the quantity of gas formed in carbohydrate solutions and the end reaction of the solution are found to be too indefinite to be of value in the classification of bacteria; and that by the aid of dextrose alone better (more decisive) qualitative evidence is obtained than by the use of dextrose, lactose and saccharose.

III.—THE TEMPERATURE AND PERIOD OF DEVELOPMENT OF WATER BACTERIA DURING CULTIVATION FOR CLASSIFICATION TESTS.

Temperature of Development.—It is undoubtedly true that, for the purposes of prompt differentiation of bacteria intimately associated with the causation of disease, and for detailed comparative studies of races or varieties of a given species, it is necessary that there shall be made comparisons of the results of growths of cultures at substantially 20° and 37° C.; yet, for the classification of water bacteria, it is our experience that cultivation at 20° for each set of tests is sufficient. Of course, it must be learned whether or not the bacterium will grow at 37° C.; and this, according to our present custom, is obtained by means of an agar plate culture.

In explanation of our departure from the custom of employing both 20° and 37° C. for temperatures of development, it is to be stated that such was our practice, in strict accord with the recommendations of the Bacteriological Committee, during the early part of our work at this laboratory. But soon we became satisfied that, for the purposes of classification of water bacteria, the increase in amount of definite information obtained from cultures grown at 37° C. was slight, and to our thought clearly incommensurate with the labor involved. Further-

more, there are substantial grounds for believing that at times the data obtained from the cultivation of water bacteria at 37° C. are misleading, owing to inability by ready means to distinguish uniformly between negative results from positive growths and negative results due to the absence of growth caused by the high temperature. Such data, even if occurring at fairly rare intervals, can lead ultimately only to serious confusion.

Period of Development.—This portion of bacterial procedures has not been standardized to the degree which seems to be imperative for those workers who have in view the object stated in the introduction of this paper. A standard period of development involves the consideration of the following factors:

1. It should not be so short as to exclude a considerable portion of the definiteness of the recorded characters of the given bacterium.

2. It should not be so long as to add unnecessarily to the tediousness of the methods and to difficulties in their applications to practical problems of medical and sanitary science; nor increase the likelihood of complications arising from contamination of the culture or the evaporation of the medium; nor force the constant use of rubber caps to protect the contents of the tubes, as it is found that such a practice affects some growths through the exclusion of oxygen.

3. It must of necessity be an arbitrary limit, lying between the wide extremes now employed by various workers, and filling in a conservative manner an intermediate position with reference to the two factors above stated.

At the outset of our present studies, it was the custom to keep all cultures for four weeks before the final descriptions and tests were made. It was learned, however, after twenty bacteria had been carefully studied, that substantially no changes of specific value were ordinarily obtained after the tenth day; and it was not until this period had elapsed that satisfactory data in several instances could be obtained upon the liquefaction of gelatine, a test which occupies a prominent place in the present bacterial methods.

Accordingly, it was decided to adopt ten days as the standard period of development in our work, and disregard in our classifications any change which by chance might be noted beyond that time, should

the cultures be preserved. It is not to be supposed that the writers are unmindful of the fact that in some instances well-marked changes may take place after the tenth day of cultivation, notably with reference to chemical changes associated with fermentation processes as in the case of milk; but it is our position that under such conditions changes of differential value are seldom obtained, and we are not yet ready to accept that they deal with species rather than with races of bacteria, in the present state of bacterial classification.

IV.—PROCEDURES DIRECTED TO FULFILL THE NECESSITY OF ARRANGING WATER BACTERIA IN GROUPS AS A PRELIMINARY STEP TOWARDS CLASSIFICATION.

Irresistibly bacteriologists working in this field have been drawn towards efforts having for their purpose the arrangement of fairly similar forms into subdivisions, which facilitate the comparative study of closely allied species or races of bacteria. Various workers have referred to their efforts in this direction as "groups," "synopses," "summaries" or "classes" of the bacteria which they studied. The importance of such steps, which has been set forth by a number of writers in the past few years, is probably conceded by substantially all workers in this line. Recently, the time-consuming task of arranging, so far as practicable, the recorded species of bacteria to date into twenty-five classes was completed by Chester* and his contribution has proven to be very helpful to us.

Many bacteriologists in the past, if not at the present time, have been inclined to move with much slowness in efforts to arrange bacteria into groups, on the ground that they could not see their way clear towards a *natural* grouping. It is of course true that with present methods of bacteriology all schemes of grouping bacteria are purely arbitrary; but it is also true that there is no probability of any one ever devising a bacterial grouping which is natural; or, in the immediate present, one which will be permanent and acceptable to all workers.

The arrangement of bacteria into groups is wholly a matter of con-

* A preliminary arrangement of the species of the Genus Bacterium by Frederick D. Chester. From Report of the Delaware College Agricultural Experiment Station, 1897.

venience, undertaken to assist in making comparative studies of similar forms and of data related thereto. While it is true that each bacteriologist might have a different method of grouping, which in his own hands was satisfactory for the purpose to which it was put, and therefore successful, yet it is obvious that a grouping used by many workers in common would lead to more systematic results and more rapid improvement of current methods.

According to our experience the present methods of grouping water bacteria are not wholly satisfactory for the following reasons:

1. The groups are too many in number, and depend for their separation upon results consequent upon immediate environment, and not upon inherent characteristics of a specific nature.

2. Following the views of biologists in other fields the importance attached to morphological data is greater than justifiable, and these data are used to the exclusion of results of more definite physiological tests.

In connection with the second of these points, it may be stated that we have had considerable difficulty in deciding uniformly as to the form (under apparently all ordinary conditions) of those very plump bacilli which at one time were specified as bacteria, and which could quite properly be classed as micrococci. Such forms are quite prevalent in some waters, although they seem to include only a very few species. With regard to spore formation and motility, we feel quite sure of our data upon these points when we make use of our regular custom of applying the heat test for spores, and for motility when we compare preparations in hanging drops of water and of formaldehyde.

The following tests are used at this laboratory to obtain data for the separation of water bacteria into groups to aid in their study and classification.

1. Fluorescence and chromogenesis.
2. Liquefaction of gelatine.
3. Well-marked characteristics of typical gelatine plates.
4. Fermentation of carbohydrates.

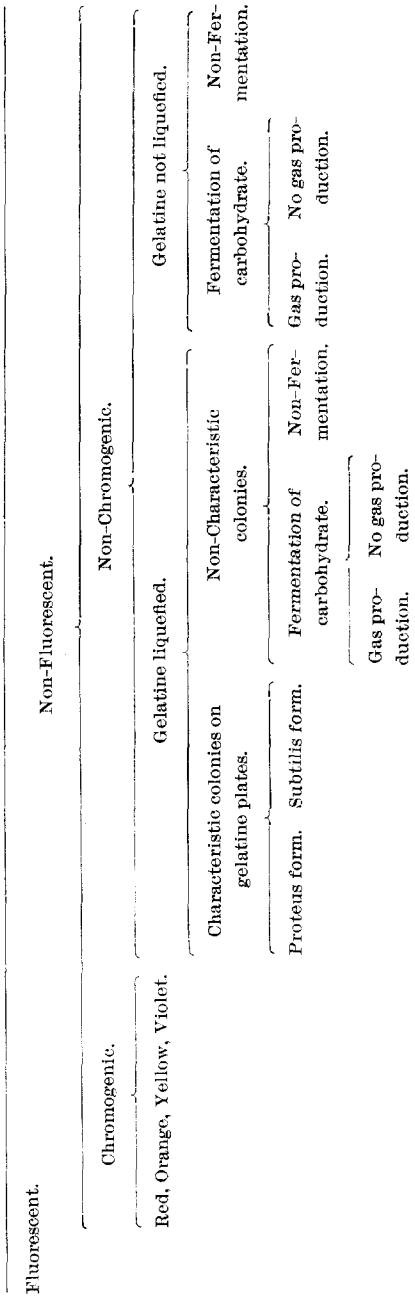
By means of the preliminary cultivation methods described in Section I of this paper, the preparation of media as referred to in Section II, and the standard temperature and period of development as outlined in Section III, the writers have found that from the four

tests above stated a practically constant arrangement of water bacteria may be obtained as indicated by the following outline:

TABLE VI.

DATA FOR ARRANGEMENT OF WATER BACTERIA IN GROUPS.

Water Bacteria.



From the above schedule thirteen groups of water bacteria are obtained as follows:

Group I. All fluorescent forms.

Group II. All red chromogenic forms.

Group III. All orange chromogenic forms.

Group IV. All yellow chromogenic forms.

Group V. All violet chromogenic forms.

Group VII. All non-fluorescent, non-chromogenic, gelatine-liquefying bacteria, forming proteus-like colonies on gelatine.

Group VII. All non-fluorescent, non-chromogenic, gelatine-liquefying bacteria, forming subtilis-like colonies on gelatine.

Group VIII. All non-fluorescent, non-chromogenic, non-proteus- and non-subtilis-like bacteria, which liquefy gelatine and ferment carbohydrate with the production of gas.

Group IX. All bacteria conforming to the specified characteristics of Group VIII, except that fermentation of carbohydrate takes place without the formation of gas.

Group X. All bacteria conforming to the specified characteristics of Group VIII, except that no fermentation of carbohydrate occurs.

Group XI. All non-fluorescent, non-chromogenic, non-gelatine-liquefying bacteria, which ferment carbohydrates with the production of gas.

Group XII. All bacteria conforming to the specified characteristics of Group XI, except that fermentation of carbohydrate takes place without the production of gas.

Group XIII. All bacteria conforming to the specified characteristics of Group XI, except that no fermentation of carbohydrate occurs.

The features upon which the above groups are based we are led to consider, arbitrarily, as "fixed characters." Being looked upon as such, it becomes necessary that certain provisions shall be observed in their consideration in order that the features true of one group may not become diffused into the one closest allied. Chromogenesis, for instance, upon which the separation of four of the above groups are based, rarely appears the same upon two different media owing mainly to differences in their composition. For obvious reasons we have

deemed it advisable to adopt a specific medium (or media) for a given observation and the conditions governing the chief features of the first five groups are therefore briefly given, as follows:

Group I.—Fluorescence should be observed exclusively in agar tube cultures; and, since a given reaction will not under all conditions be found applicable to the production of pigment for all bacteria, it is necessary that agar of three reactions should be used in this test, namely, 1.5 per cent acid, neutral, and 1.5 per cent alkaline, to phenolphthalein.

Groups II, III, IV, and *V.*—The hues or shades of color produced by the growth of certain bacteria upon culture media are well understood to be admixtures of certain colors. Most prominent among these mixed colors is that of yellow and its modifications. While the reds and violets are readily handled, it will require some study to deal effectually with the yellow and orange chromogens. We have learned that colors which are neither yellow nor orange must be set aside and placed among the non-chromogenic forms. Brown and grey-yellow colors frequently observed are instances of this character.

We have found that the most constant results in the study of chromogenic bacteria are obtained from agar tube cultures.

The colors embraced in the above four groups may be briefly explained as follows:

Red.—That color produced by B. prodigiosus on agar.

Yellow.—That color produced by the growth of Sarcina lutea on agar with the dividing line between this and the orange group at the yellow-ochre hue produced by the growth of B. ochraceus on agar.

Orange.—This color begins just below the yellow ochre. The true orange color is the same as that produced by the growth of B. aurantiacus on agar.

Violet.—The same as that color produced by the growth of B. violaceus on agar.

V.—ON THE NECESSITY OF EMPLOYING FOR PURPOSES OF CLASSIFICATION OF WATER BACTERIA DATA OF DEFINITE (POSITIVE OR NEGATIVE) INFORMATION, AND THE EXCLUSION OF THOSE DATA WHICH ARE NOT SHARPLY DEFINED TO A UNIFORM DEGREE.

Upon studying the literature of the differentiation of water bacteria, it is readily apparent to practically all bacteriologists, as was brought out in the instructive paper by Dr. Wyatt Johnston,* that the data now available upon this subject are weak and lacking in two notable ways:

1. Too many of the data, to which a differential value is given, are so indefinite that there is no assurance that the observations could be regularly duplicated even by the original worker; and, accordingly, the records of such work when published not only fail to advance the subject in a substantial manner, but even complicate and confuse matters to a serious degree.

2. There are too few data which may be called definite and which all experienced workers with good technique have reasonable expectations of confirming uniformly.

Among the principal objects of the work of the Bacteriological Committee in setting forth standard procedures was, by the use of uniform and improved methods, to increase the number of definite data by the elimination of certain ones from the first of the above classes and their transposition to the second class.

To what degree these efforts will meet with success can of course be told only after the experience of a large number of workers has been recorded, discussed and brought to a fairly agreeing consensus of opinion.

As a result of our experience of the past ten months in applying the procedures of the Committee, the writers find that in that time their views have become somewhat modified. We have become impressed with the absolute necessity of placing the subject of the classification of water bacteria upon a basis such that the systematization

* On Grouping Water Bacteria, *Journal Amer. Public Health Assn.*, Oct., 1895, p. 445.

shall have for a foundation only such data as can be readily confirmed by all capable workers employing standard methods. Or, expressed in the terms of this paper, there must be secured a foundation for future work that is made up of the results of tests which have substantially 100 for a percentage constancy, when the tests follow promptly a course of preliminary treatment to eliminate the influence of possible initial degeneration.

To some it will occur that such a decision may lead to a classification which would be very crude and fragmentary. In a measure that may be true, but to our thought the procedures of the Committee insure sufficient tests of a percentage constancy of practically 100 to lead to a fairly satisfactory set of data for primary classification, and that the advantage of all bacteriologists having a definitely located datum point in this work far outweighs at present the objection of crudeness.

The future of those tests which from our present evidence have a percentage constancy materially less than 100 is one of much uncertainty. Some of the tests will probably have their technique sufficiently improved to be elevated to the class yielding uniformly definite data. Others will probably have a field of greatest usefulness, after more or less modification in technique, in connection with the prompt identification of disease germs, and studies in the separation of species and varieties, and in tracing the life history of bacteria under different environments and from a purely scientific standpoint. To condemn them for all time seems unjustifiable at present, especially as other workers under other conditions may obtain different results.

With reference to those tests which in our hands at present seem to yield indefinite results (with a percentage constancy of much less than 100) we have no further comment to offer at this time; and list the more important ones below with the statement that from the results of our experience we are not ready to accept them as yielding data of sufficient definiteness to allow them uniformly a place at present in the classification of water bacteria. In every instance in the following list color is regarded as independent of fluorescence and chromogenesis.

LIST OF TESTS RECOMMENDED BY THE BACTERIOLOGICAL COMMITTEE WHICH HAVE BEEN FOUND TO YIELD LESS THAN 100 FOR A PERCENTAGE CONSTANCY.

MORPHOLOGY.

Capsulation. Vacuoles. Staining of spores. Presence of crystals within the cells. Pleomorphism.

BIOLOGY.

Gelatine Plate.—Size and color of colonies and growth under the mica plate.

Agar Plate.—Size, color and margin of colony.

Gelatine Tube.—Color of growth.

Agar Tube.—Extent, shape, margin and surface relief of growth.

Nutrient Broth Tube.—Time elapsing before the appearance of turbidity, deposit and surface pellicle. Color of broth. Appearance after shaking. Color, thickness and structure of pellicle. Color and amount of deposit. Quantitative reaction after a stated period.

Milk.—Color of milk. Formation of gas. Amount of whey. Quantitative reaction.

Fermentation Test. Quantitative.—Reaction of solution. Quantity of gas. Differential value of different sugars.

Lactose Litmus Agar.—Change of color.

Potato.—Color, restriction, shiny appearance and lustre of growth; and formation of gas.

VI.—THE ARRANGEMENT OF DEFINITE DIFFERENTIAL DATA IN THE FORM OF A CHART, AS A MATTER OF CONVENIENCE IN THE CLASSIFICATION OF WATER BACTERIA.

Excluding from the total number of differential tests appearing in the report of the Committee, those which were listed in the last section as insufficiently definite and constant in their value for present purposes, there remain the data from 26 tests. In the light of our experience these results can be recorded as positive or negative, with little or no material loss as to explicitness and with a decided gain as to convenience and uniformity of expression. From our studies of the

literature upon this subject we are convinced that the latter point is no small one, owing to the great tendency of the observer to associate his point of view and his style as a writer with his text descriptions.

The method of recording the results of the leading tests in positive or negative terms allows, of course, the use of plus or minus signs. Early in the work at the laboratory a chart was prepared showing the results of the species work as it advanced, and permitting the main bulk of the work to be presented in a concise and convenient manner.

The heading and arrangement of this chart, together with the records of the differential characters of 42 species of bacteria isolated from the Ohio River water at Cincinnati, are presented at the end of this article. The chart presents in explicit terms the principal records of each species isolated. It also makes simple the task of showing wherein similar species differ from each other. Furthermore, by preparing a chart showing the record of each distant species met with, it is easy to note whether or not any species subsequently found agrees with others previously studied. By making the current records on a chart of the same size and folding it so that the records of the given form under study appears on the top line, a glance of the eye is sufficient to show with which species it is identical, if with any.

In connection with this chart, which is of much assistance in classifying water bacteria according to the reliable methods now available, it of course is not to be understood that it necessarily would serve that purpose as the subject advances, especially as consideration is given to questions of races versus species. It is possible, however, that it may be amplified to serve that purpose for some time.

March, 1899.

NAME OF ORGANISM.	FIRST INVESTIGATOR.	MORPHOLOGY.				CULTURAL F				
						Nutrient broth tube.		Nutrient agar tube.		Gelat plat
		Bacillus.	Diameter greater than 1μ.	Motile.	Spores.	Scum.	Turbidity.	Dull.	Wrinkled.	Characteristic appearance
						Group I.				Fluo
B. fluorescens liquefaciens	Flügge	+	—	+	—	+	+	—	—	+
B. fluorescens non-liquefaciens	Eisenberg	+	—	—	—	+	+	—	—	—
B. viridis	Lesage	+	—	+	—	+	+	—	—	+
B. fluorescens ovalis	Ravenel	+	—	+	—	+	+	—	—	—
B. pyocyaneus	Gessard	+	—	+	—	+	+	—	—	+
B. fluorescens incognitus	Wright	+	—	+	—	+	+	—	—	—
						Group II.				Chromo
B. prodigiosus	Ehrenberg	+	—	+	—	+	+	—	—	+
B. rubidus	Eisenberg	+	—	+	—	+	+	—	—	+
						Group III.				Chromog
B. arborescens	Frankland	+	—	+	—	—	+	—	—	+
B. aurescens	Ravenel	+	—	+	—	—	+	—	—	—
B. fulvus	Zimmermann	+	—	—	—	—	+	—	+	+
B. fuscus	Zimmermann	+	—	—	—	—	+	—	—	—
B. aurantiacus	Frankland	+	—	+	—	—	+	—	—	+
						Group IV.				Chromog
B. desidiosus	Wright	+	—	—	—	+	+	—	—	—
B. ochraceus	Zimmermann	+	+	+	+	—	—	—	+	—
B. flavescens	Pohl	+	—	+	—	—	+	—	—	—
B. lactis erythrogenes	Hueppe	+	—	—	—	+	+	—	+	+
B. subflavus	Zimmermann	+	—	+	—	—	+	—	—	—
Sarcina lutea	Schroeter	—	—	—	—	—	—	—	—	+
						Group V.				Chromog
B. janthinus	Zopf	+	—	+	—	+	+	—	+	+
B. violaceus	Frankland	+	—	+	—	+	+	—	—	+
						Group VI.				P
B. mycoides	Flügge	+	+	+	+	+	—	+	—	+
B. mesentericus vulgatus	Eisenberg	+	+	+	+	+	+	—	+	+
B. proteus fluorescens	Jäger	+	—	+	—	+	+	+	—	+
						Group VII.				S
B. subtilis	Ehrenberg	+	+	+	+	+	—	+	+	+
B. cereus	Frankland	+	+	+	+	+	+	+	+	+
						Group VIII.				
B. cloacæ	Jordan	+	—	+	—	+	+	—	—	—
B. liquefaciens	Eisenberg	+	—	—	—	—	+	—	—	—
						Group IX.				Li
B. liquidus	Frankland	+	—	+	—	—	—	—	—	—
B. antenniformis	Ravenel	+	—	+	—	+	—	—	—	—
						Group X.				Sup
B. superficialis	Jordan	+	—	+	—	+	+	—	—	—
B. annulatus	Wright	+	—	+	—	—	+	—	—	—
B. flexuosus	Wright	+	—	—	—	+	+	—	—	—
B. geniculatus	Wright	+	—	+	—	+	+	—	—	—
B. aquatilis communis	Zimmermann	+	—	+	—	+	+	—	—	—
						Group XI.				
B. coli communis	Escherich	+	—	+	—	+	+	—	—	—
B. aërogenes	Escherich	+	—	—	—	+	+	—	—	—
						Group XII.				T
B. similtyphus		+	—	+	—	+	+	—	—	—
B. solitarius	Ravenel	+	—	+	—	+	+	—	—	—
B. aquatilis sulcatus I	Weichselbaum	+	—	+	—	—	+	—	—	—
B. aquatilis sulcatus V	Weichselbaum	+	—	+	—	—	+	—	—	—
						Group XIII.				C
B. candicans	Frankland	+	—	+	—	—	+	—	—	—

BIOLOGY.

ATURES.			BIOCHEMICAL FEATURES.														PATHOGENESIS.
Potato tube.		Fermentation tube.				Liquefaction.			Gas production.					Nutrient agar tubes.		Mice.	
Visible.	Luxuriant.	Growth in closed arm.	Grows at body temperature.	Facultative anaërobe.	Affected by range of reaction	Gelatine.	Casein.	Blood serum.	Dextrose broth.	Nitrate reduced.	Indol produced.	Milk coagulated.	Fecal odor.	Chromogenesis.	Fluorescence.	Intra-peritoneal inoculation.	
scent Type.																	
+	−	−	−	−	+	+	+	+	−	+	−	+	−	−	+	−	
+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	
+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	
+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	
+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	
+	+	−	+	−	+	−	−	−	−	−	−	−	−	−	+	−	
nic Type.—Red.																	
+	+	+	+	+	+	+	+	+	+	+	−	+	+	crimson	−	−	
+	−	+	−	+	+	+	−	−	−	−	−	−	−	brick red	−	−	
ic Type.—Orange.																	
+	+	+	−	+	+	+	−	+	−	−	−	+	−	orange	−	−	
+	−	−	+	−	+	+	+	+	−	−	−	+	−	"	−	−	
+	−	+	+	+	+	+	−	−	−	−	−	−	−	"	−	−	
+	−	+	+	+	+	−	−	−	−	−	−	−	−	"	−	−	
+	−	−	−	−	+	−	−	−	−	+	−	−	−	"	−	−	
ic Type.—Yellow.																	
+	+	−	−	−	+	+	+	+	−	−	−	+	−	yellow	−	−	
+	−	−	+	−	+	+	−	+	−	+	−	+	−	"	−	−	
+	+	+	−	+	+	−	−	−	−	+	−	−	−	"	−	−	
+	+	−	−	−	+	+	+	+	−	+	−	+	−	"	pink	−	
−	−	−	+	−	+	−	−	−	−	+	−	−	−	"	−	−	
+	−	+	+	+	+	+	−	−	−	−	−	−	−	"	−	−	
ic Type.—Violet.																	
+	+	−	−	−	+	+	+	+	−	+	−	−	−	dark violet	−	−	
+	+	−	−	−	+	+	+	+	−	+	−	+	−	violet	−	−	
eus Type.																	
+	+	+	+	+	+	+	+	+	−	+	−	+	−	−	−	−	
+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	−	−	
+	+	+	+	+	+	+	+	+	−	−	−	−	+	−	+	+	
tilis Type.																	
+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−	
+	+	+	+	+	+	+	+	+	−	+	−	+	−	−	−	−	
bacæ Type.																	
+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	
+	+	+	+	+	+	+	−	−	+	+	−	+	−	−	−	−	
idus Type.																	
+	−	+	−	+	+	+	−	−	−	−	−	+	−	−	−	−	
+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	
icialis Type.																	
+	+	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	
−	−	−	+	+	+	+	+	+	−	+	−	+	−	−	−	−	
+	−	−	+	−	+	+	+	−	−	+	−	+	−	−	−	−	
+	+	−	−	−	+	+	−	−	−	−	−	+	−	−	−	−	
−	−	−	−	−	+	+	−	−	−	+	−	−	−	−	−	−	
lon Type.																	
+	+	+	+	+	+	−	−	−	+	+	+	+	+	−	−	+	
+	+	+	+	+	+	−	−	−	+	+	−	+	−	−	−	−	
hosus Type.																	
+	−	+	+	+	+	−	−	−	−	+	−	−	+	−	−	−	
+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	
+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	
+	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	
dicans Type.																	
+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	