DIFFERENTIATION OF MEMBERS OF THE GENUS CHROMOBACTERIUM BERGONZINI

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DIFFERENTIATION OF MEMBERS OF THE

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By

Robert J. Hans

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VITA

Robert J. Haas

candidate for the degree of

Doctor of Philosophy

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Dissertation: Differentiation of Members of the Genus <u>Chromobacterium</u> Bergonzini

Outline of Studies:
- Major Subject: Microbiology
- Minor Subject: Mycology

Biographical Items:

Born December 17, 1928 in Detroit, Michigan

Undergraduate Studies: University of Michigan, 1946-47, 1948-51
Dearborn Junior College, 1947-48

Graduate Studies: Wayne University, 1951-53
University of Munich (Germany), 1954-55

Experience: Teaching Assistant at the Dental School, University of Detroit, 1951-53; Research Assistant at Parke, Davis and Company, summers of 1953, 1954; Research Assistant at Michigan State University, 1953-54, 1955-56.

Affiliations: Society of American Bacteriologists
Society of the Sigma Xi

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ABSTRACT

A search was made for biochemical characteristics useful in differentiating members of the Genus Chromobacterium Bergonzini. It was shown that these organisms are alkaligenic in peptone media, so special emphasis was placed upon utilization of various carbohydrates in two peptone free media.

The organisms were found to be very sharply divided into two groups by growth temperature ranges and optimum temperatures. These characteristics were closely correlated with fermentation of four carbohydrates in peptone media, the utilization of 11 carbohydrates in peptone free media, hydrogen cyanide production, pigmentation, and gelatin liquefaction. Methylene blue thiocyanate reduction, nitrate reduction, ammonia production, tryptophane utilization, MRVP reactions, hydrogen sulfide production, and urea hydrolysis were found of no differential value.

Specific names are suggested for the two groups; one mesophilic and the other psychrophilic.

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INTRODUCTION

In the United States the Genus Chromobacterium Bergonzini is considered to consist of the gram negative bacteria producing a non-photosynthetic violet pigment. These organisms are aerobic, motile, non-spore forming, occasionally pathogenic rods, and occur in water, soil and pathological processes in both man and domestic animals.

Relatively ignored and in a confused taxonomic state ten years ago, these bacteria have received considerable attention this past decade. These recent investigations have dealt with taxonomy, physiology, and pathogenicity, and the pigment *per se*; but those dealing with the taxonomy and physiology have been little more than repetitions of previous studies.

Bacterial classification is based largely upon biochemical characteristics, chief among which on the species level is the production or the lack of production of acid or acid and gas from various carbohydrates. It has been shown, however, that members of the Genus Chromobacterium, like many other Gram negative bacilli, produce alkaline reactions in peptone media. Despite this fact, investigators have continued to employ such media in attempts to arrive at suitable taxonomic criteria. In this study much attention is given to carbohydrate utilization in peptone-free media, using a much larger number of strains and isolates than examined by any single worker to date. Additionally, such other biochemical determinations were made as seemed duplicable in other laboratories.

HISTORICAL REVIEW

Many authors have described supposedly new species of the violet pigmented organisms since the original description of Schröter in 1872. Many of these differed only in motility, apparent flagellation, size, morphology, gelatin liquefaction, action on litmus milk, pigmentation on various media and similar unreliable criteria. These characters are recognized today as highly variable and depend both upon the cultural conditions and upon the method employed. In addition, some have even been described as different by virtue of spore formation, but recent evidence indicates that these supposed spores were artifacts.

Accordingly, little is to be gained here from an exhaustive description of all existing species descriptions. Instead, only those will be discussed which have been seriously considered and widely accepted at one time or another.

A gram negative, non-motile organism isolated by Schröter in 1872 and named Bacteridium cyaneum produced a water soluble violet pigment and is thus not a member of the genus Chromobacterium. Both organisms isolated and described as Micrococcus cyaneus by Cohn (1872) and "Blauer Coccus" by Maschek (1887) have been considered identical to Bacteridium cyaneum Schröter (Migula, 1900; Godfrin, 1934).

In 1872, Schröter isolated and described a bacterium, Bacteridium violaceum, producing a water insoluble violet pigment (Breed, Murray and Hitchens, 1948). This is perhaps the first true member of the Genus Chromobacterium to be described, and it is so accepted by Bergey's Manual of Determinative Bacteriology (Breed, Murray and Hitchens, 1948);

viz., as Chromobacterium violaceum (Schröter) Bergonzini.

Also in 1872, Cohn described a Micrococcus violaceus, now considered to have been the same as Bacteridium violaceum Schröter (Breed, Murray and Hitchens, 1948).

Bergonzini (1881) isolated a gram negative rod producing a chromoparous violet pigment from an egg white solution and designated it Cromobacterium violaceum (sic). This is the origin of the generic name Chromobacterium.

In 1885, Zopf characterized Bacterium janthinum (sic), a supposedly indol and hydrogen sulfide producing, non-motile gelatin liquefying organism isolated from "pieces of pig's bladder floating in badly contaminated water."

In 1886, Schröter again isolated a violet organism, designated Bacillus violaceus (Breed et al., 1948), said to be identical with Bacterium janthinum Zopf (Schröter, 1886; Lehmann and Neumann, 1896).

Schröter (1889) and Toni and Trevisan (1889) described Bacillus lacmus from fresh greenhouse paint and Streptococcus violaceus from water respectively, both said to be the same by Godfrin (1934). Breed et al. (1948) consider this Streptococcus violaceus Trevisan the same as Bacillus violaceus Schröter, Bacteridium violaceum Schröter, and Chromobacterium violaceum Bergonzini.

Organisms subsequently isolated and designated Bacillus violaceus Mace (1887), Bacillus violaceus Frankland and Frankland (1889), and Bacillus violaceus Laurentius Jordan (1890) were considered the same as Bacterium ianthinum Zopf by Chester (1901). Godfrin confirmed this in so far as he wrote Bacteridium violaceum Schröter, Bacillus violaceus

Frankland and Frankland, and Bacillus violaceus Mace are probably the same species.

Migula (1900) stated that Bacillus violaceus Mace and Bacillus violaceus lutentiensis Kruse (Flugge, 1886) show no significant differences, and along with Bacillus violaceus Berolinensis (sic) Kruse, are identical with Bacteridium violaceum Schröter. Chester (1901) likewise pointed out that Bacillus violaceus Berolinensis and Bacillus violaceus lutentensis Kruse are similar.

Thirey (1900), like Chester, felt that Bacillus violaceus Mace and Bacillus ianthinus Zopf were similar, as were organisms described as Bacillus lividus Plagge and Proskauer (1887) and Bacillus membranaceus amethystinus Eisenberg (1891).

Lehmann and Neumann (1912) regarded Bacteridium violaceum Schröter the first of its type to be described and considered it to be the same as Bacterium ianthinum Zopf. They further stated that Bacillus violaceous Mace, Bacillus violaceus Laurentius Jordan (1890) and Bacillus violaceus Berlinensis Kruse differ very little and agree with Zopf (1884) that the latter is identical with Bacillus lividus Plagge and Proskauer. Finally, they stated that Bacillus membranaceus amethystinus Eisenberg (1891) and Bacillus membranaceus amethystinus mobilis Germano (1892) are, along with the "sometimes motile and sometimes non-motile" organism isolated from the Thames by Ward (1898), closely related to those just mentioned.

These relationships are perhaps best seen in Table I.

It should be noted here that it was a custom of the time to apply trinomials and tetranomials to organisms which were considered slightly different from previously described binomial species (Novy, 1953). Accordingly, much of the above synonomy, such as between the various forms of Bacillus violaceus, is understandable and gains even more significance when viewed in the light of modern day knowledge of variability in bacteria.

In 1890, Claessen described an indigo blue pigment producing bacterium designated Bacillus indigonaceus. The pigment of this organism was soluble in both water and chloroform, however, and it is therefore not a member of the Genus Chromobacterium.

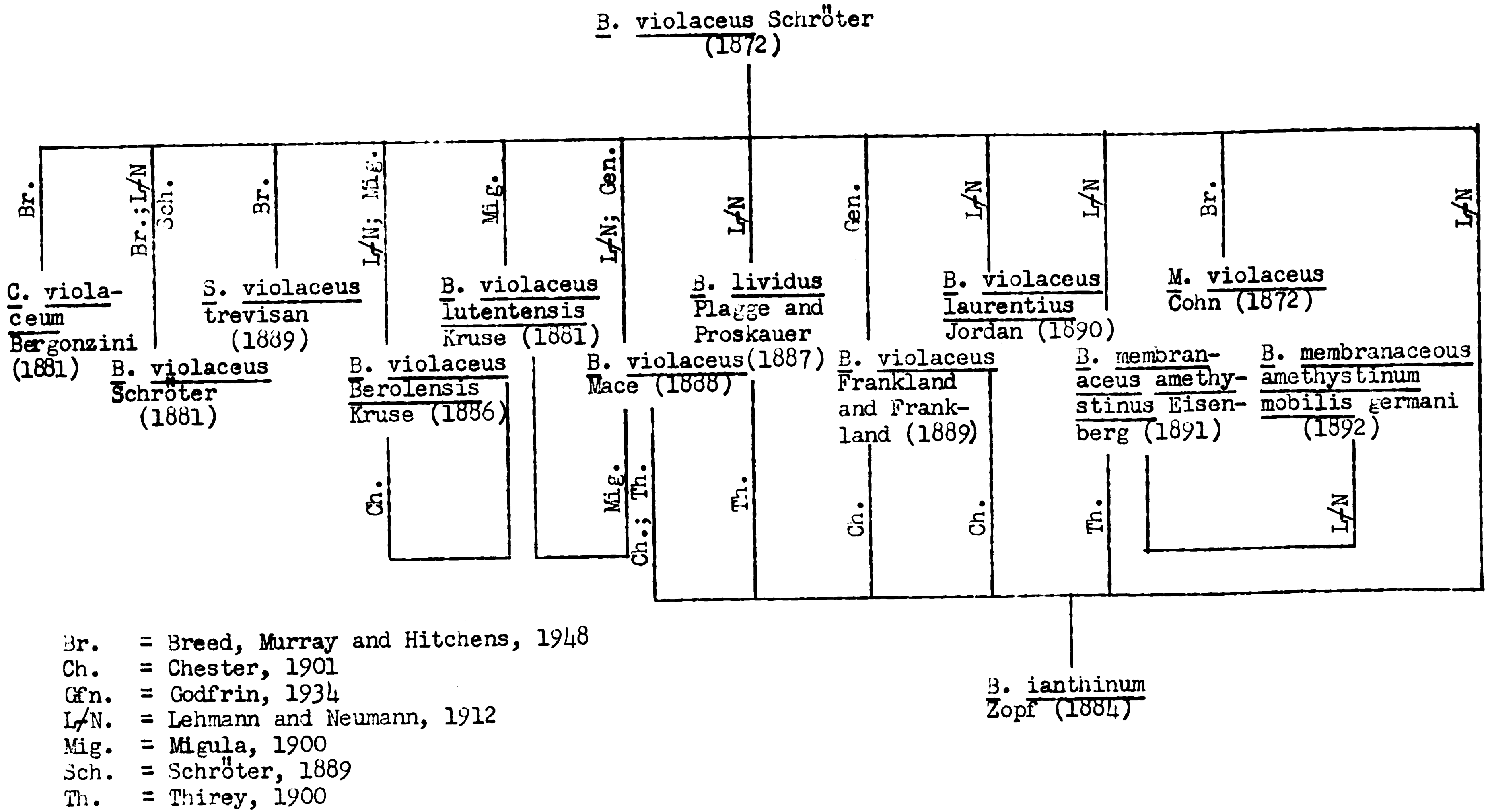
Beijerinck (1891, 1892) isolated from mucilage and described a motile, water soluble blue pigment producing bacterium which was also found to produce a condition of cheese in Holland known as "bleu." The natural habitat, however, is said to be soil and water (Godfrin, 1934). Since the pigment is water soluble, neither is this organism a member of the Genus Chromobacterium.

Voges (1893) and Smith (1887) described almost blue-black pigment producing bacilli under the name Bacillus coeruleus; but the two organisms were substantially different. Most significant of these differences is the water soluble pigment of Bacillus coeruleus Smith, while that of Voges' organism is insoluble in water.

Also in 1893, Voges described a supposedly spore forming, violet pigmented organism designated Bacillus indigoferus, isolated from water at Kiel. It is significant that the organism was also reported by Voges to be killed by exposure to 60°C for 15 minutes. Obviously, some structure had been mistaken for spores.

TABLE I

Taxonomic relationships of some early described *Chromobacterium* species



Lustig (1893) isolated an organism which he called "Bacille Bleu Indigo" which according to Godfrin is essentially the same as Bacillus indigonaceus Claessen.

Godfrin (1934) briefly described a Bacillus pavoninus Forster, studied by Kraal (1899), Bowhill (1899), Thomas (1930) and Van der Slenn (1894), which produced opaque colonies, blue by transmitted light. This cannot be considered a violet pigment producing organism.

Jobling and Wooley (Wooley, 1905) were the first to isolate a violet organism from a lesion. They isolated Bacillus violaceus manilae from the lymph glands of carabaos dying of fatal septicemia in the Philippine Islands. This organism was pathogenic for guinea pigs and rabbits and produced lesions which healed in dogs, cats and calves. No soluble toxin could be demonstrated. Subsequently, Gaudecheau (1907) and Minnett (1911) isolated strains, pathogenic for animals, from water supplies in Indo-China and British Guiana. More recently, Anderbaud et al. (1954) reported infection of the liver in the monkey Cercopethecus cephus, and Sippel et al. (1954) reported fatal infection of cattle and swine. Lesslar (1927), Martin (1931), da Silva (Sneath, 1953), Black and Shahan (1938), Soule (1939), Schattenberg and Harris (1941), Hetherington (Sneath, 1953) and Sneath (1953) reported twelve cases of human infection, seven terminating fatally and two reported as recovered, with the termination not recorded in the other three cases. Symptoms of these various infections included pyaemia, regional adenitis, liver or cutaneous abscesses, septicemia, urinary infection, rectal bleeding, and mild diarrhea.

In 1905, Harrison and Barlow described Bacillus violaceus viscofucatus (or Bacterium viscofucatum) from water, now thought to be a

Pseudomonas sp. by Tobie (Breed, Murray and Hitchens, 1948).

Breaudat (1906) isolated and named Bacillus violareus acetonicus (sic) from water in Saigon. This organism was said to produce acetone in a peptone-sucrose-potassium carbonate medium.

Bampton (1913) studied 18 strains of Bacillus violaceus and 4 strains of Bacillus membranaceus amethystinus, considered by him to be the two main types in the Genus Chromobacterium. Of the latter species, he designated his strains Bacillus membranaceus amethystinus I, II, III and IV.

Mace, in 1913, isolated and described Bacillus lilacinus.

In 1920, a Committee of the Society of American Bacteriologists, headed by C. -E. A. Winslow, proposed that the generic name Chromobacterium Bergonzini be accepted for the group of violet pigmented, non-photosynthetic bacteria.

Cholkevitch (1922) isolated Bacterium cristillino violaceum from peat near Leningrad. This organism was reported to produce violet, yellow or red crystalline pigment(s) and is obviously not a member of the genus under consideration.

In 1927, Creuss-Callaghan and Gorman described a Bacterium violaceum amethystinum, now considered to be identical with Bacillus membranaceus amethystinus Rosenberg (Breed, Murray and Hitchens, 1948); and Grimes (1927) described but did not name a violet bacterium from butter now designated Chromobacterium viscosum (Breed, Murray and Hitchens, 1948). This organism was shown by Hans (1953), Gilman (1953) and Sneath (1956) not to be a member of the Genus Chromobacterium.

Grimes, in 1930, described Chromobacterium hibernicum and Chromobacterium cohaerens from well water.

Creuss-Callaghan and Gorman (1935) examined 24 strains of violet bacilli and concluded that they represented 3 species; viz, Bacterium membranaceum amethystinum Eisenberg, Bacterium violaceum Schröter and Bacterium ianthinum Zopf.

Waeldele (1938) reported Bacillus violaceus sartoryi, a supposed spore-former from dental pus.

The following year, Davis (1939) isolated and described Chromobacterium iodinum from milk, shown by Tobie (1939) to be probably a Pseudomonas.

Chromobacterium maris-mortui was isolated from the Dead Sea by Elzari-Volcani (1940).

The last two organisms to be placed in this genus were Chromobacterium chocolatum Knutsen and a variant of this, Chromobacterium orangium Knutsen, both isolated and named by Knutsen (Lasseur and Giabicani, 1942-44; Lasseur, Dupaix-Lasseur and Celcion, 1942-44a, 1942-44b, 1942-44c). These two organisms have been shown not to be members of the Genus Chromobacterium (Hans, 1953; Gilman, 1953).

Thus it is obvious that the taxonomy of this group is confused. From the table on page 8, however, one might suspect that a single true species, by virtue of the frequent encounters of these mutually similar organisms, is represented by that group. Chromobacterium violaceum Schröter appears to be the logical type species for this group; and this name is used in Bergey's Manual of Determinative Bacteriology (Breed, Murray and Hitchens, 1948).

Then, from the investigations of Bampton (1913) and Creuss-Callaghan and Gorman (1935) one might conclude that a second species is represented by Chromobacterium amethystinum Eisenberg. Chromobacterium ianthinum Zopf, thought by Creuss-Callaghan and Gorman to be a third species, has been too often likened to Chromobacterium violaceum Schröter to be considered significantly different. The "Chromobacterium ianthinum" of Gilman (1953) was later found not to be Chromobacterium ianthinum (Gilman, personal communication).

This theory, viz., that the Genus Chromobacterium consists of two distinctly different species, is largely proved in the present study.

GENERAL METHODS AND MATERIALS

Source of Cultures

The cultures for this investigation included both strains from established laboratory collections and isolates obtained by the author from soil in the United States and Europe.

Purification and Maintenance of Cultures

All cultures were tested for purity by streaking on nutrient agar plates containing 3 per cent yeast extract (Difco). No contaminated cultures were received from cooperating laboratories.

Each stock culture was maintained by weekly transfer from a typical isolated colony on a nutrient agar streak plate. Plates were incubated 48 hours at room temperature (25°C) and were then stored at 4°C.

Isolation Method

The rice enrichment method of Corpe (1951) was used for obtaining isolates from soil. Five grams of soil in a sterile petri dish was covered with sterile distilled water. Sterile precooked rice grains (Minute Maid) were sprinkled on the surface, and the plates were incubated at room temperature for five days. Approximately 50 per cent of the plates prepared showed one or more areas of violet bacteria growing on the rice grains after incubation. These grains were transferred to a mortar with a small amount of sand and 5 ml. of distilled

water and were mascerated. One loopful of this suspension was then streaked on each of two nutrient agar plates, subsequently incubated at room temperature for 48 hours or more, until violet pigmented colonies appeared. In some instances it was necessary to return to the original soil sample and repeat the isolation procedure when no violet colonies appeared on the initial agar plates.

Biochemical Methods

Media for biochemical determinations were employed in 13 by 100 mm., cotton plugged test tubes. With the exception of media containing carbohydrates, ammonium hydrogen phosphate, ammonium sulfate, or urea, sterilization was effected at 121°C in 15 minutes. Carbohydrates (except aesculin and dextrin), ammonium hydrogen phosphate, ammonium sulfate, and urea were sterilized in concentrated solution by filtration through ultra fine sintered glass filters. These concentrates were aseptically added to flasks of autoclaved basal medium, and this was aseptically distributed to dry heat sterilized, cotton plugged test tubes. Aesculin and dextrin, because of their poor solubility, were added to the basal medium before autoclaving. Non-peptone containing media were employed in 2.5 ml. amounts and peptone media in 3.5 ml. amounts. All media were incubated 48 hours at room temperature to assure sterility.

pH determinations were made with a Beckman Model G meter standardized with pH 7.0 phosphate buffer.

All inorganic reagents were of C.P. grade. Organic materials were products of well known concerns.

Inoculum

Inoculum for solid media was obtained from typical isolated colonies on nutrient agar plates, incubated at room temperature (25°C) for 48 hours. Inoculum for liquid media consisted of 0.1 ml. of a 70 per cent transmission saline suspension of cells from 48 hour old nutrient agar slants. Seventy per cent transmission was measured on a 6 volt, Cenco-Sheard-Stanford Photolometer, Industrial Type B-2, employing the blue filter and operating on a 6 volt Sears Roebuck motorcycle battery.

Plate counts on 14 such 70 per cent transmission suspensions were made in nutrient agar to determine the approximate number of viable cells being employed.

Incubation

Tests were incubated at room temperature (25°C), and readings, unless otherwise stated below, were recorded every 24 hours for one week.

Carbohydrates

To illustrate the alkaligenic character of the organisms under consideration, all strains were tested in Phenol Red Broth Base (Difco), containing 1 per cent glucose, mannose, maltose, sucrose, and lactose. The final pH of these cultures after 7 days incubation was determined and recorded.

To show, additionally, that this alkaligenic reaction could not

be avoided by employing a semi-solid medium to allow partial anaerobic fermentation of the carbohydrates, all organisms were grown in agar stab cultures of carbohydrate media. The basal medium consisted of Proteose Peptone, 5 gm; NaCl 5 gm; carbohydrate, 5 gm; agar, 3 gm; and phenol red, 0.024 gm per liter. Carbohydrates employed were: adonitol, aesculin, arabinose, cellobiose, dextrin, dulcitol, fructose, galactose, glucose, inulin, lactose, maltose, mannitol, mannose, melebiose, melezitose, raffinose, ribose, rhamnose, salicin, sorbitol, sorbose, soluble starch, sucrose, trehalose and xylose.

Only new culture tubes were employed to avoid mistakes as to presence or absence of growth in the non-peptone containing media. In addition, only those tubes were selected which gave 100 per cent transmission when filled with distilled water. These were compared to a tube selected as a standard in the Cenco Photometer employing the blue filter, as described above. This was necessary since it was hoped to detect both cases of slow carbohydrate utilization and cases of enzyme adaptation to carbohydrates in a reasonable period of time, viz. 7 days. Ultimately, however, instances of utilization or failure to utilize a given carbohydrate were in most cases more easily distinguishable than had been expected.

Modifications of the basal media of Ayers, Rupp and Johnson (1919) and of Elrod and Braun (1942) were employed. Determinations were first made in the first mentioned medium, containing $(NH_4)_2SO_4$, 2.0 gm; K_2HPO_4, 0.2 gm; $MgSO_4 \cdot 7H_2O$, 0.2 gm; carbohydrate, 5.0 gm; distilled water, 1 liter; final pH adjusted to 6.8 with 1 N NaOH. The nitrogen source was accidentally omitted in the Elrod and Braun publication, but it is to be found in that of Liu (1952). Calcium

chloride and sodium chloride included in this medium by Elrod and Braun and by Liu were not found to be beneficial in this study and were thus omitted.

All determinations were then repeated in the medium of Ayers, Rupp and Johnson, containing $NH_4H_2PO_4$, 1.0 gm; $MgSO_4 \cdot 7H_2O$, 0.2 gm; carbohydrate, 5.0 gm; and distilled water, 1 liter; final pH adjusted to 6.8 with 1 N NaOH. The 0.2 gm per liter potassium chloride included by Ayres, Rupp and Johnson was found experimentally to be of no value and was omitted.

Carbohydrates used in these two media included: adonitol, aesculin, arabinose, cellobiose, dextrin, dulcitol, fructose, galactose, glucose, inulin, lactose, maltose, mannitol, mannose, melebiose, melezitose, raffinose, ribose, rhamnose, salicin, sorbitol, sorbose, sucrose, trehalose and xylose.

Hydrogen Cyanide Production

Hydrogen cyanide production by members of this genus was first reported by Clawson and Young (1913). In the present study, production of this gas was determined by the inclusion, in nutrient broth cultures, of filter paper strips soaked in picric acid and sodium carbonate as described by Guidnard (1905, 1906).

Methylene Blue Thiocyanate Reduction

One ml. of a 1-250 (w/v) aqueous solution of methylene blue thiocyanate was added to the cultures remaining from the hydrogen cyanide

determinations after 48 hours incubation. The tubes were observed for reduction of the indicator.

Nitrate Reduction and Ammonia Production

Nitrate utilization and production of ammonia was determined in both Nitrate Peptone Broth and in Dimmick (1947) Nitrate Solution after 48 hours and 7 days incubation. Nitrate Peptone Broth contains Beef Extract, 3gm; Bacto Peptone, 5 gm; KNO , 1 gm; and distilled water, 1 liter. Dimmick Nitrate Solution contains K_2HPO_4, 0.5 gm; $MgSO_4 \cdot 7H_2O$, 0.2 gm; $NaNO_3$, 0.2 gm; glucose, 10.0 gm; and distilled water, 1 liter. Sulfanilic acid and alpha-napthylamine were employed to detect nitrite ion as recommended in the Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists (1946). Nessler's Reagent was used to detect ammonia. Negative nitrite tests were checked for the presence of nitrate ion by reduction with powdered zinc (ZoBell, 1932).

Gelatin Destruction

Gelatin liquefaction determinations were made by two methods. Tubes of Nutrient Gelatin Medium (Difco) were inoculated by stab and were observed at 72 hours and 14 days for liquefaction. The tubes were incubated at room temperature and were refrigerated one hour at 4°C before observations were made.

Secondly, nutrient agar plates containing 0.4 per cent gelatin were streaked in duplicate to obtain isolated colonies. Employing

Smith's Modification (1946) of Frazier's Method (1926), single plates were flooded at 72 hours and 7 days with a solution containing 15 gm. mercuric chloride and 20 ml concentrated hydrochloric acid in 100 ml. of distilled water. Gelatin liquefaction, when present, was seen as clear zones around each colony, formed by the surrounding precipitate of the intact gelatin.

Tryptophane Utilization and MRVP Reactions

Tryptophane utilization and the MRVP Reactions were performed on 7-day old cultures according to the methods recommended in the Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists (1946). Kovac's Reagent was used for the indol determinations, and alpha-napthol and potassium hydroxide in the Voges Proskauer determination.

Motility and Hydrogen Sulfide Production

Motility and hydrogen sulfide production determinations were performed in Motility Sulfide Medium (Difco). In addition, motility was observed in hanging drop preparations of 24-hour Nutrient Broth cultures incubated at room temperature, and hydrogen sulfice determinations were made in Lead Acetate Agar (Difco).

Urea Hydrolysis

Urea hydrolysis was investigated in Urea Broth prepared according to the formula of Difco (1953), containing filter-sterilized urea.

Stains

Smears for staining were prepared from 48-hour old cultures in nutrient broth grown at the optimum temperature for each organism. Gram stains and Löffler's alkaline methylene blue stains were prepared by the usual bacteriological techniques.

Pigmentation

Pigmentation was observed on nutrient agar plates after 7 days incubation at room temperature.

Temperature Requirements

The ability of each organism to grow at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48°C was determined in a nutrient broth containing: Bacto Peptone, 10.0 gm; Yeast Extract, 3.0 gm; glucose, 5.0 gm; and distilled water, 1 liter. (In this case the carbohydrate was autoclaved in the complete medium). 0°C was achieved in a large Dewar flask containing distilled water and distilled water ice cubes. A refrigerator was used for 4°C. 8°C through 24°C were obtained in refrigerated incubators, and the higher temperatures in regular bacteriological incubators. Temperatures were accurate within 0.5°C in the range 0°C through 24°C and within 1.0°C at temperatures above that. Increasing turbidity, in the optically uniform tubes, where present, was determined photometrically with the Cenco Photometer as described above for peptone-free carbohydrate media, and was recorded at each 24 hours for 7 days.

Additionally, plate counts were made in nutrient agar of each 10 per cent transmission from 10 to 90 per cent to determine the approximate number of cells represented by each reading made above. Forty-eight hour cultures in nutrient broth containing 0.3 per cent Yeast Extract (Difco), incubated at the optimum temperature, were used, and dilution was made with the same medium. Five cultures were randomly selected from each of two species apparently represented in this study for use in this experiment.

RESULTS

Cultures

A complete list of the cultures studied is to be seen in Table II. The addresses of contributing investigators are to be found in the Appendix.

Biochemical Methods

For convenience in reporting results, the cultures must be divided at this point into two groups found to be present; viz. mesophiles and psychrophiles.

Inoculum

Nutrient Agar plate counts of 70 per cent transmission saline suspensions of fourteen representative organisms are shown in Table III. The average count for the seven mesophilic organism suspensions is 6.20×10^8, and that for the psychrophiles 6.41×10^8 viable cells per ml.

Carbohydrates

Phenol Red Broth Base media showed highly variable reactions and little correlation was possible.

Acid but no gas in glucose was formed by all mesophiles except strain N.C.T.C. 7917, the average final pH of these cultures being 6.10.

TABLE II

Sources of *Chromobacterium* spp. cultures used in the present study

Accepted Designation	Contributor	Original Investigator	Source	Location	Date of Isolation
MESOPHILES					
A.T.C.C. 553 (Rettger 4/22; N.C.T.C. 8685)	Parke, Davis & Co.	Rettger			
A.T.C.C. 6357 (Shahan; N.C.T.C. 8684)	Sneath	Shahan(1)	Human Infection	Florida, U.S.A.	1937
A.T.C.C. 7461 (Lewitus; N.C.T.C. 8683)	Sneath				
A.T.C.C. 12472 (Mentekab; N.C.T.C. 9757)	Sneath	Whelan	Water	Malaya	1952
Birch	Sneath	Sneath(2)	Human Urine	Malaya	1951
Brown	Sneath	Sneath(2)	Fatal Human Infection	Malaya	1952
Cambridge	Sneath				
Frazier's Hill	Sneath	Whelan	Water	Malaya	1952
Institut Pasteur 532	Thibault				
Lake Garden	Sneath	Whelan	Water	Malaya	1952
Metropolitan Water Board	Sneath	Thomas	Water	England	
N.C.T.C. 7917 (Collins Strain 2)	Sneath	Collins	Lake Water	England	1935

TABLE II (continued)

Sources of *Chromobacterium* spp. cultures used in the present study

Accepted Designation	Contributor	Original Investigator	Source	Location	Date of Isolation
N.R.R.L. B-1085	Haynes		Soil		
Reeves	Sippel	Sippel(3)	Bovine Infection	Georgia, U.S.A.	1952
Sealey	Sippel	Sippel(3)	Swine Infection	Georgia, U.S.A.	1953
University of Michigan	Sneath				
University of Nancy	Marchal				
University of Pennsylvania	Morton		Water	Pennsyl-vania	1955
PSYCHROPHILES					
A.T.C.C. 11104	Kluyver				
A.T.C.C. 12473 (Hans 24; N.C.T.C. 9796)		Hans	Soil	Detroit	1954
Berlin 16; 19; 30; V-1; V-8; R5/2	Bortels	Bortels	Soil	Berlin	1955
Corpe 4-A	Corpe	Corpe	Soil	U.S.A.	
Creuss-Callaghan Strain 16 (May be A.T.C.C. 6915)	Kluyver	Creuss-Callaghan and Gorman(4)	River Maas	Rotterdam, Holland	

TABLE II (continued)

Sources of **Chromobacterium** spp. cultures used in the present study

Accepted Designation	Contributor	Original Investigator	Source	Location	Date of Isolation
England I	Burman		River Lea at Chingford Mill	England	1954
England II	Burman		Rye Common Well	England	1954
England III	Burman		River Lea at New Gauge	England	1954
English Garden		Hans	Soil	Munich, Germany	1955
Futa Pass		Hans	Soil	Futa Pass, Italy	1955
Herrenchiemsee		Hans	Soil	Herrenchiem-see Island, Germany	1955
Hornstein		Hans	Soil	Grüningen, Germany	1955
H-4; H-11; H-20; H-23; H-25; H-27; H-29; H-30; H-31; H-33; H-34; H-35; H-36; H-39; H-58		Hans	Soil	Detroit, Michigan	1952
Indiana University X	McClung				
Institut Pasteur 52227	Thibault		Water	France	1952

TABLE II (continued)

Sources of *Chromobacterium* spp. cultures used in the present study

Accepted Designation	Contributor	Original Investigator	Source	Location	Date of Isolation
Lichtenstein		Hans	Soil	Valduz, Lichtenstein	1955
M.G. 2.1	van Niel	van Niel	Contaminated Culture	California	
M.G. 2.2	van Niel				
M.W.B. 25	Burman		Water Filter	England	1955
M.W.B. 27	Burman		Water Filter	England	1955
Munich		Hans	Soil	Munich, Germany	1955
N.R.R.L. B-1020 (N.C.T.C. 7916)	Haynes				
N.R.R.L. 468; N.R.R.L. 469; N.R.R.L. 470; N.R.R.L. 471	Haynes		River Water	California	1955
Sneath RU; Sneath DA; Sneath GA; Sneath NC	Sneath	Sneath	Soil	England	1955
Traunstein		Hans	Soil	Traunstein, Germany	1955
Ulm		Hans	Soil	Ulm, Germany	1955
Veer	Kluyver	Veer	Cocoanut	Delft, Holland	19
6-1; 6-4; 6-5; 6-10; 6-13 6-15; 6-16; 6-20; 6-21; 6-22		Hans	Soil	East Lansing Michigan	1953

TABLE III

Viable *Chromobacterium* spp. cells per ml. of 70 per cent transmission saline suspensions inoculum as determined by Nutrient Agar plate counts

Strain	Count per ml. ($x10^8$)
MESOPHILES:	
Frazer's Hill	10.30
Cambridge Univ.	3.00
N.R.R.L. B-1085	7.20
Sealey	8.70
Univ. Nancy	7.50
Univ. Michigan	5.30
Valley	1.40
PSYCHROPHILES:	
Eng. III	3.50
H-20	6.00
H-27	5.70
H-29	6.50
N.R.R.L. 471	6.50
Smith DA	7.90

Twenty-five per cent of the psychrophiles also produced varying degrees of acid from glucose in the lower half of each tube, with an alkaline reaction above. The remaining 75 per cent produced an alkaline reaction throughout the media. The average final pH for the psychrophilic cultures was 7.55 (range 7.20 to 8.00).

An alkaline reaction was shown by 66 per cent, an acid reaction by 7 per cent, and no reaction by 27 per cent of the cultures in maltose. No correlation between psychrophiles and mesophiles could be seen; but the pH average in this medium for the former was 7.75 (range 7.20 to 8.35), and for the latter it was 8.45 (almost uniformly).

One hundred per cent of the cultures showed only an alkaline reaction in both lactose and mannose media. The average pH was 8.30 for the psychrophiles and 8.45 for the mesophiles.

In sucrose, an acid reaction was produced by 15 per cent, an alkaline reaction by 45 per cent, and no reaction by 40 per cent of the cultures. The average final pH for the psychrophilic cultures was 7.85, and that for the mesophilic cultures was 8.45 (range 6.10 to 8.45).

In the semi-solid carbohydrate agar, all psychrophiles showed an alkaline surface with all carbohydrates within 48 hours incubation. No acid formation was evident. The mesophiles showed varying degrees of acid production with fructose, galactose, glucose, glycerol, mannose, sorbose, sucrose, trehalose, and xylose, often accompanied by an alkaline surface reaction. All except strains Lewitus and Shahan in mannose showed acid in glucose, fructose, mannose and trehalose. This group showed either no reaction, or merely an alkaline surface in arabinose, adonitol, aesculin, cellobiose, dexrin, dulcitol, inositol, inulin,

lactose, maltose, mannitol, melebiose, melezitose, raffinose, rhamnose, salicin, sorbitol, and sorbose.

Carbohydrate utilization in the peptone-free media of Ayres, Rupp and Johnson, and of Elrod and Braun is shown in Table IV. A plus sign indicates an increase in turbidity during the seven day incubation period, and a negative sign indicates a lack of increase. General reactions for the psychrophiles and mesophiles are indicated at the head of each group. Only cultures in each group which did not behave as their type are indicated in the remainder of the table (i.e. the exceptions).

Growth of the mesophiles in fructose, galactose, glucose, glycerol, mannitol, and xylose was not as decisive as might be desired. In most cases the turbidity of different organisms in various of these carbohydrates barely reached 10 per cent transmission. On the other hand, the differentially useful arabinose, inositol, maltose, salicin, sorbitol, sucrose, trehalose, and xylose gave large turbidity increases with the psychrophiles and absolutely none with the mesophiles.

Lactose was utilized slowly, increased turbidities being first evident in most cases only after 5 days incubation.

Hydrogen Cyanide Production

Detectable hydrogen cyanide was produced within 48 hours of incubation, often within 24 hours, by all mesophiles except strains Frazer's Hill and Univ. Michigan. None was detected from the psychrophiles. The negative reactions with strains Frazer's Hill and Univ. Michigan recurred upon repetition.

TABLE IV

Carbohydrate utilization by Chromobacterium spp. in peptone free media

	Monosaccharides									Di-saccharides						Poly-saccharides				Alcohols						Glucocides	
	Arabinose	Fructose	Galactose	Glucose	Mannose	Ribose	Rhamnose	Sorbose	Xylose	Cellobiose	Lactose	Maltose	Melebiose	Sucrose	Trehalose	Dextrin	Inulin	Melezitose	Raffinose	Adonitol	Dulcitol	Glycerol	Inositol	Mannitol	Sorbitol	Aesculin	Salicin
MESOPHILIC TYPE REACTIONS:	-	+	+	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-
Exceptions:																											
A.T.C.C. 553			-																			-		-			
A.T.C.C. 7461																						-					
Birch														+													
Lake Garden														+													
N.C.T.C. 7917															-												
Reeves			-											+								-					
Sealey														+										-			
University Michigan			-												-							-		-			
Valley		-	-	-		-									-	-						-		-			
PSYCHROPHILIC TYPE REACTIONS:	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	+	-	+
Exceptions:																											
A.T.C.C. 11104							+										+		+								
England I							+										+		+								
England II							+										+		+								
Hornstein							+										+		+								
Lichtenstein							+										+		+								
M.G. 2.1		+					+										+		+								
M.G. 2.2							+										+		+								
Met. Water Bd. 27							+										+		+								
Munich							+										+		+								

TABLE IV (Continued)

Carbohydrate utilization by Chromobacterium spp. in peptone free media

	Monosaccharides									Di-saccharides						Poly-saccharides				Alcohols						Gluco-cides	
	Arabinose	Fructose	Galactose	Glucose	Mannose	Ribose	Rhamnose	Sorbose	Xylose	Cellobiose	Lactose	Maltose	Melebiose	Sucrose	Trehalose	Dextrin	Inulin	Melezitose	Raffinose	Adonitol	Dulcitol	Glycerol	Inositol	Mannitol	Sorbitol	Aesculin	Salicin
PSYCHROPHILIC TYPE REACTIONS:	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	+	-	+
Exceptions:																											
Smith DA							+												+								
Smith RU							+												+								
Indiana Univ. X																			+								
Inst. Pasteur 52227																			+								
Met. Water Bd. 25																			+								
6-1																			+								
6-10																			+								
6-13																			+								
H-35														-					+								
N.R.R.L. B-1020														-					+								
N.R.R.L. 469														-					+								
Berlin R5/2														-					+								
Creuss Callaghan Str. 16																											
England III																											
Futa Pass																											
H-11																											
H-20																											
H-23																											
H-29																											
H-31																											
H-36																											
H-39																											
H-58																											
Munich																											

TABLE IV (Continued)

Carbohydrate utilization by Chromobacterium spp. in peptone free media

	Monosaccharides									Di-saccharides						Poly-saccharides				Alcohols						Glucocides	
	Arabinose	Fructose	Galactose	Glucose	Mannose	Ribose	Rhamnose	Sorbose	Xylose	Cellobiose	Lactose	Maltose	Melebiose	Sucrose	Trehalose	Dextrin	Inulin	Melezitose	Raffinose	Adonitol	Dulcitol	Glycerol	Inositol	Mannitol	Sorbitol	Aesculin	Salicin
PSYCHROPHILIC TYPE REACTIONS:	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	+	-	+
Exceptions: Met. Water Bd. 25 Sneath GA 6-18 6-20 6-22																											

+ = utilization; - = no utilization

Methylene Blue Thiocyanate Reduction

Methylene blue thiocyanate was completely reduced by all strains within 10 minutes.

Nitrate Reduction and Ammonia Production

Only strains Veer, N.R.R.L. B-1020, N.R.R.L. B-1085 and Ind. Univ. showed no nitrate utilization in Nitrate Peptone Broth at 48 hours. By 7 days, only N.R.R.L. strains B-1020 and B-1085 had failed to reduce nitrate in this medium.

At 48 hours, most mesophiles showed no nitrate reduction in Dimmick Nitrate Solution while most psychrophiles did do so. After 7 days, only strains N.R.R.L. B-1085, Valley, Mentekab, Lake Garden, and Lewitus were negative.

Ammonia was produced by all strains in both media within 48 hours.

Gelatin Destruction

Gelatin stab cultures showed significant liquefaction by all mesophiles after 72 hours incubation. All psychrophiles showed little or no liquefaction at this time. By 14 days, the mesophiles had all liquefied at least two-thirds of the medium. Only a few psychrophiles (15 per cent) had produced any degree of liquefaction within 14 days, and in no case did it exceed one-quarter of the medium.

All mesophiles showed a zone of gelatin destruction on Frazier Plates after 72 hours incubation, while no psychrophiles did so. After 7 days, only nine mesophiles (14 per cent) showed no zone of gelatin destruction.

Tryptophane Utilization and MRVP Reactions

Indol and acetylmethylcarbinol were not formed by any of the cultures, and the MR reaction was negative in all cases.

Motility and Hydrogen Sulfide Production

Motility of all strains was apparent near the surface of the medium within 24 hours. It was also observed microscopically in all cases.

Hydrogen sulfide had not been produced by any strain in either medium after 14 days incubation.

Urea Hydrolysis

Urea was not hydrolyzed by any strain.

Stains

All cultures were gram negative. The mesophiles averaged 0.75 x 2 microns in size and the psychrophiles 1.0 x 3.5 microns.

With Löffler's alkaline methylene blue stain, the mesophiles usually showed bipolar staining, while the psychrophiles often showed metachromatic granules.

Pigment

All mesophiles were constantly dark violet pigmented on Nutrient Agar (Difco), Nutrient Gelatin (Difco) and glycerinated potatoes. The

psychrophiles, on the other hand, ranged in color from blackish violet to cream with or without scattered traces of bluish pigmentation.

Temperature Requirements

The temperature range and optimum temperature for each strain examined are shown in Table V.

The average results of plate counts with broth cultures of 10 strains diluted to each 10 per cent transmission from 10 to 90 per cent are shown in Table VI. These data can be used to calibrate Figure 1.

The positive readings for all psychrophiles and then those for all mesophiles at each 10 per cent transmission from 10 to 90 per cent after 48 hours incubation were averaged to yield data for the curves in Figure 1.

TABLE V

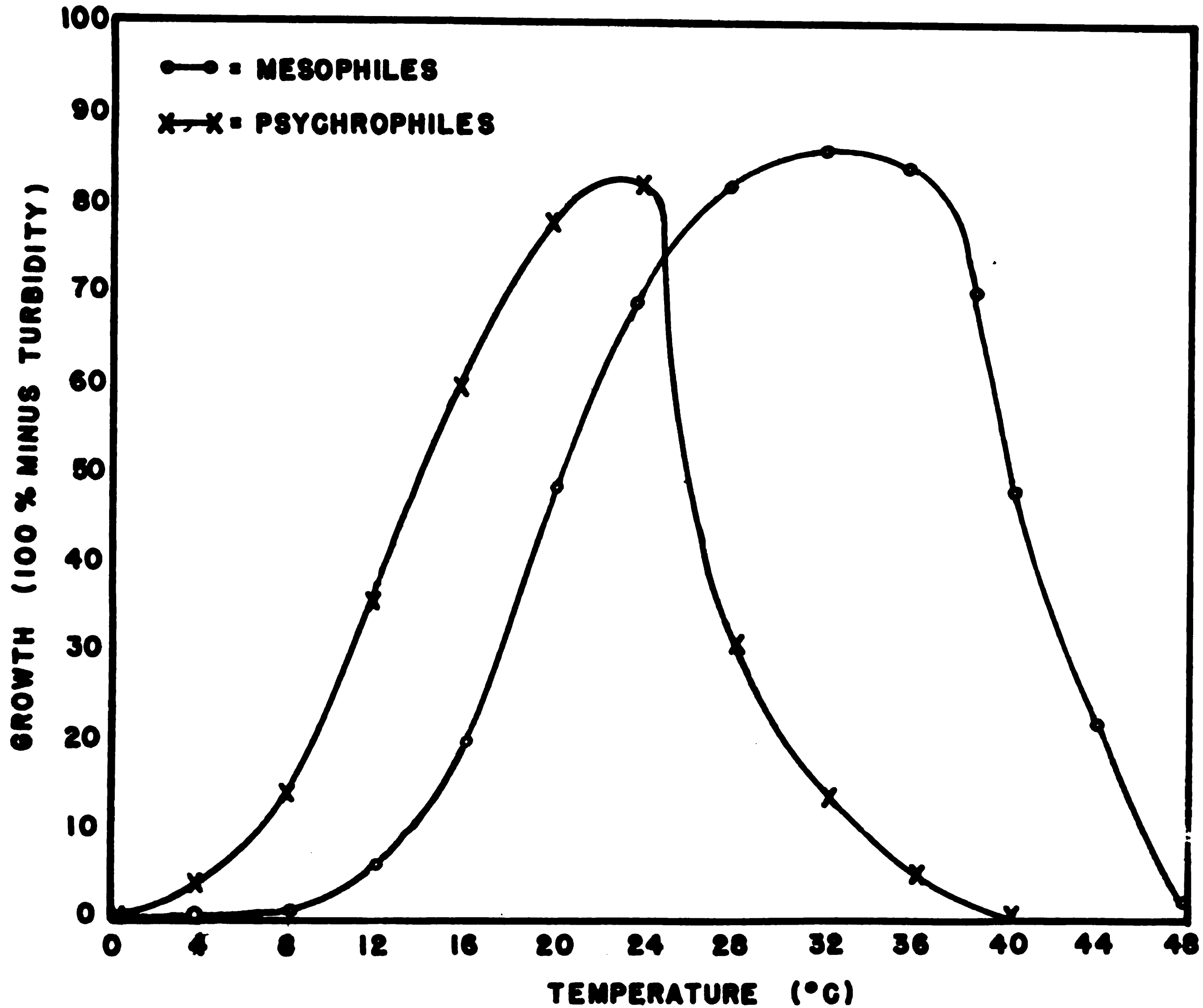
Temperature optima and ranges for
<u>Chromobacterium</u> spp. strains examined

Strain	Range (°C)	Optimum (°C)
MESOPHILES:		
A.T.C.C. 553	8-36	28-36
A.T.C.C. 6357	8-36	32-36
A.T.C.C. 7461	12-36	28-32
A.T.C.C. 12472	12-44	28-36
Birch	8-36	28-36
Brown	8-36	28-36
Cambridge	8-36	28-36
Frazer's Hill	8-48	32-36
Inst. Pasteur 532	8-36	28-36
Lake Garden	12-44	28-36
Met. Water Bd.	12-36	32-36
N.C.T.C. 7917	4-36	28-36
N.R.R.L. B-1085	12-36	28-32
Reeves	16-48	28-36
Sealey	12-44	28-36
Univ. Michigan	12-44	32-36
Univ. Nancy	16-36	32-36
Univ. Pennsylvania	8-36	32
Valley	8-36	28-36
PSYCHROPHILES:		
A.T.C.C. 11104	0-36	24
A.T.C.C. 12473	0-36	24
Berlin 16	0-36	20
Berlin 18	0-32	24
Berlin BO	0-32	24
Berlin V-1	0-32	24
Berlin V-8	0-32	24
Berlin R5/2	0-32	24
Corpe 4-A	0-32	24
Creuss-Callaghan Strain 16	0-32	24
England I	0-36	24
England II	0-32	24
England III	0-28	24
English Garden	0-32	24
Futa Pass	0-28	24
H-4	0-32	24
H-11	0-32	24
H-20	0-32	24
H-25	0-32	24

TABLE V (continued)

Strain	Range (°C)	Optimum (°C)
H-27	0-36	24
H-29	0-32	24
H-30	0-32	24
H-31	0-28	20-24
H-33	0-32	20-24
H-34	0-28	24
H-35	0-32	24
H-36	0-28	24
H-39	0-32	24
H-58	0-32	24
Herrenchiemsee	0-32	24
Hornstein	0-36	24
Ind. Univ. X	0-32	24
Inst. Past. 52227	0-32	24
Lichtenstein	0-32	24
M.G. 2.1	0-32	20-24
M.G. 2.2	0-32	24
M.W.B. 25	0-32	24
M.W.B. 27	0-36	24
Munich	0-32	24
N.R.R.L. B-1020	0-32	20-24
N.R.R.L. 468	0-36	20-28
N.R.R.L. 469	0-32	24
N.R.R.L. 470	0-36	24
N.R.R.L. 471	0-36	24
Sneath DA	0-32	24
Sneath GA	0-32	24
Sneath NC	0-32	24
Sneath RU	0-36	24
Traunstein	0-32	24
Ulm	0-36	24
Veer	4-32	24
6-1	0-36	24
6-4	0-36	24
6-5	0-36	24
6-10	0-36	24
6-13	0-36	24
6-15	0-32	24
6-18	0-32	24
6-20	0-32	24
6-21	0-36	24
6-22	0-32	20

FIGURE I



AVERAGE GROWTH OF ALL MESOPHILIC AND ALL PSYCHROPHILIC STRAINS OF CHROMOBACTERIUM SPP. AT EACH 4° FROM 0 TO 48°C AFTER 48 HOURS INCUBATION

TABLE VI

Average viable cell counts at each 10 per cent transmission from 10 to 90 per cent of five psychrophilic and five mesophilic *Chromobacterium* spp. strains grown in broth culture, determined by nutrient agar plate counts

Percent Transmission	Count per ml. ($x10^8$) Mesophiles	Psychrophiles
10	.32	.57
20	.75	.99
30	1.03	1.58
40	1.86	1.88
50	3.20	2.68
60	5.73	4.18
70	7.00	6.44
80	15.77	12.80
90	22.87	16.15

DISCUSSION

Carbohydrate utilization in peptone-free media, acid production in peptone containing media, hydrogen cyanide production, temperature range, gelatin digestion, and pigmentation are useful differential characters in this genus.

Carbohydrate Utilization

Apparently the mesophilic and psychrophilic groups can be distinguished by the production of acid by the mesophiles from fructose, glucose, mannose and trehalose in solid peptone containing media, while the psychrophiles fail to do so.

Failure of the psychrophiles to utilize carbohydrates in agar solidified peptone media may well be due to their reluctance or failure to grow under the microaerophilic or practically anaerobic conditions in the depth of the medium. The strongly alkaline reaction produced by these strains at the aerobic surface, however, precludes any formation of an acid reaction here.

These reactions are summarized as follows:

TABLE VII

Acid production from glucose, fructose, mannose and trehalose by mesophilic and psychrophilic Chromobacterium spp. in peptone containing media

	Glucose	Fructose	Mannose	Trehalose
Mesophiles	+	+	+(-)	+
Psychrophiles	-	-	-	-

+ = acid; (-) = acid rarely produced; - = no acid

In addition, utilization in peptone-free media of adonitol, arabinose, inositol, lactose, maltose, rhamnose, salicin, sorbitol, and sucrose can also be used as distinguishing characteristics.

As can be seen from Table IV, the mesophilic organisms are able to utilize fructose, galactose, glucose, glycerol, mannitol, ribose and trehalose as sole carbon sources. Fermentation of mannose and xylose in the semi-solid peptone medium would indicate these two carbohydrates can be fermented but cannot be used as the sole source of carbon.

This is strikingly parallel to the findings of Liu (1952) with Pseudomonas aeruginosa. Liu found P. aeruginosa able to utilize the same carbohydrates as sole carbon sources, and to be unable to utilize adonitol, dulcitol, inositol, inulin, lactose, maltose, raffinose, salicin, sorbitol and sucrose. Additionally, Liu found P. aeruginosa able to ferment arabinose and rhamnose, but unable to use them as a sole carbon source. Elrod and Braun (1942) disagree in respect to arabinose, and Salvin and Lewis (1946) agree with respect to rhamnose with P. aeruginosa.

The mesophiles in the Genus Chromobacterium are perhaps related to Pseudomonas aeruginosa. Similar studies with other gram negative bacilli would be highly valuable for comparative purposes in this respect. If significant differences were to be found between other gram negative organisms and both the violet mesophiles and P. aeruginosa, the mesophiles are likely related to P. aeruginosa. Such a statement to this effect at present, however, would be premature.

The violet pigmented psychrophiles, on the other hand, are as much different from P. aeruginosa as are the violet pigmented mesophiles.

A lack of sufficient comparative studies makes it impossible to liken these organisms to any other species or genus at this time.

The occasional fermentation of raffinose and inulin by the psychrophilic cultures is of particular interest. Adams, Richtmyer and Hudson (1943) showed that the ease of enzymatic hydrolysis of sucrose, raffinose, stachyose and inulin by bakers' yeast and brewers' yeast invertase are in the proportion 100 : 23: 6.8 : 0.036 and 100 : 12.5 : 3.1 : 0.006 respectively. Accordingly, one might deduce that all cultures utilizing raffinose are capable of utilizing sucrose. Such was the case. In addition, stachyose would have been utilized by some number between 11 and 18 of the cultures studied here. Significant, however, is the fact that utilization of these carbohydrates is apparently more a matter of degree than of possession or lack of a particular enzyme by the organism. Since the enzyme would appear to be present in all psychrophiles as "sucrase", its function in the cases of inulin and raffinose is relatively unimportant.

Unfortunately no similar correlation can be found between either adonitol or rhamnose and the other carbohydrates utilized by the mesophiles.

The use of these carbohydrates in peptone-free media for separating the two groups of violet chromogens are summarized in Table VIII.

Occasional discrepancies in carbohydrate utilization shown in Tables IV and VI may be due to the use of chiefly old laboratory stock cultures for representatives of the mesophilic group. Attempts by the author to isolate mesophiles by both Corpe's Rice Grain Method and by direct agar plating of both water and soil samples were

TABLE VIII

Utilization of 11 differentially useful carbohydrates by mesophilic and psychrophilic Chromobacterium spp. in peptone free media

	Arabinose	Rhamnose	Mannose	Lactose	Sucrose	Maltose	Trehalose	Sorbitol	Adonitol	Inositol	Salicin
Mesophiles	-	-	-	-	- (+)	-	+ (-)	-	-	-	-
Psychrophiles	+	+ -	+	+	+ (-)	+	-	+	+ -	+	+

+ = utilization; (+) = utilization uncommon;
- = no utilization; (-) = no utilization uncommon

unsuccessful. These discrepancies are of minor importance only in the cases of sucrose and trehalose, however; and those for trehalose are corrected in the peptone containing media.

Linardos and Cleverdon (1955) reported that Chromobacterium spp. require organic nitrogen and carbon (as amino acids) for growth. Growth, *per se*, in the peptone free media employed here indicates the error of this statement.

Hydrogen Cyanide Production

Hydrogen cyanide production by mesophilic cultures, with two exceptions (strains Frazer's Hill and Univ. Michigan), is useful for distinguishing the two groups present. These two exceptions might possibly be eliminated by alteration of the cultural conditions, but such was not attempted.

Gelatin Destruction

Two modes of differentiation by gelatinase activity are possible. While practically all (86 per cent) the strains showed the presence of the enzyme, the relative activities are of differential value. This is summarized as follows:

TABLE IX

Gelatin destruction by Chromobacterium spp. at 72 hours determined in Nutrient Gelatin stab and on Frazier Plates

	Nutrient Gelatin	Frazier Plate
Mesophiles	+	+
Psychrophiles	- (+)	-

+ = destruction; (+) = destruction rare; - = no destruction

Pigmentation

All mesophiles produced the pigment, violacein, but the psychrophiles were variable in this respect. This is of value in that achromogenic or only partially pigmented strains can be recognized as members of the psychrophilic group at once.

Temperature

One of the best criteria for the differentiation of the two groups proved to be their temperature ranges. One group, the mesophiles, was found to grow in the range 4 to 48°C, with optima between 28 and 36°C. The other group, the psychrophiles, grew from 0 to 36°C with an optimum at 24°C.

The two groups are best distinguished by incubation at 8, 12, 28, 32, 36, 40 and 44°C (Figure 1). Only the exceptional mesophile grew above 36°C, however, Accordingly, the upper limit for diagnostic work should be confined to that level. These temperatures would be of value in comparative studies in which members of both groups were present, and in which comparable inocula of all strains were employed. Then, those cultures growing best at 8 and 12°C and poorest at 28, 32 and 36°C in 48 hours or less would be the psychrophiles. Those growing best at 28, 32 and 36°C and poorest at 8 and 12°C would be the mesophiles. High turbidity caused by a minimum of 250 million cells per cc. after 48 hours incubation at the intermediate temperatures of 16, 20 and 24°C (i.e. room temperature and slightly below) make these temperatures valueless in differential work unless a photolometer or similar

instrument is used in a standardized determination. Turbidities in this range are difficult to distinguish visually. In addition, the turbidities caused by the two different groups at these temperatures would be greatly influenced, and even inverted in value, by appreciably unequal inocula.

For the occasional determination, therefore, where only one or a few specimens are being identified, 12 and 36°C are the temperatures of choice. The differential value of these two incubation temperatures is shown as follows:

TABLE X

Differentiation of mesophilic and psychrophilic
<u>Chromobacterium</u> spp. by temperature of incubation

	48 hours incubation at: 12°C	36°C
Mesophiles	-	+
Psychrophiles	+	-

where + represents "good growth" (viable cell count in excess of 103 million/cc) and - represents "poor growth" (viable cell count less than 32 million/cc).

SUMMARY

The biochemical characteristics found useful in this study are summarized in tabular form in Table XI.

Inadequate descriptions of early named species renders impossible the attachment of any one name to either the mesophilic or the psychrophilic group with certainty. Accordingly, Sneath (1955-56) proposes that the name Chromobacterium violaceum (Schröter) Bergonzini be conserved for the type species by virtue of its wide acceptance and that this name be applied to the mesophilic strains. He has deposited strain Mentekab in the National Collection of Type Cultures in England and in the American Type Culture Collection in Washington, D. C., to serve as a type for this species.

In addition, Sneath proposed the name Chromobacterium lividum (Voges) Holland for the psychrophilic group, saying that it is the "first permissible." While this author agrees with the first proposal, it is felt that the name Bacillus membranaceus amethystinum Eisenberg, now Chromobacterium amethystinum (Chester) Holland should also be conserved and be applied to the psychrophilic strains.

However, until such names are officially accepted, it is strongly suggested that cultures be referred to only as being members of either the psychrophilic or mesophilic group.

TABLE XI

Summa-rized biochemical reactions of differential value for the psychrophilic and mesophilic *Chromobacterium* spp.

	Peptone Media: Glucose	Peptone Media: Fructose	Peptone Media: Mannose	Peptone Media: Trehalose	Peptone free Media: Arabinose	Peptone free Media: Rhamnose	Peptone free Media: Mannose	Peptone free Media: Lactose	Peptone free Media: Sucrose	Peptone free Media: Maltose	Peptone free Media: Trehalose	Peptone free Media: Sorbitol	Peptone free Media: Mannitol	Peptone free Media: Inositol	Peptone free Media: Salicin	HCN Production	Pigment on Nutrient Agar	Growth in 48 hr. broth culture: 12°C	Growth in 48 hr. broth culture: 36°C	Nutrient Gelatin Stab Tube	Frazier Plate
Mesophiles	+	+	+ (-)	+	-	-	-	-	- (+)	-	+ (-)	-	-	-	-	+	+	+	-	+	+
Psychrophiles	-	-	-	-	+	±	+	+	± (-)	+	-	+	±	+	+	-	±	-	+	- (+)	-

+ = positive reaction; - = negative reaction; ± = variable reaction;
(+)= rarely positive; (-)= rarely negative.

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APPENDIX

Addresses of Cooperating Investigators

Abrams, Dr. Arthur, Immunology Division, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.

Bortels, Dr. H., Institut für Bakteriologie, Konigin-Luise Strasse 15-16, Berlin-Dahlem, Germany

Burman, N. P., Metropolitan Water Board, London, England

Corpe, Dr. William A., Department of Biology, Western Kentucky State College, Bowling Green, Kentucky

Haynes, William C., Fermentation Section, Northern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois

Kluyver, Prof. A. J., Laboratorium voor Microbiologie, Technische Hoogeschool, Nieuwe Laan 5, Delft, Holland

McClung, Dr. L. S., Department of Bacteriology, Indiana University, Bloomington, Indiana

Marchal, Prof. J. G., Laboratoire de Microbiologie, Faculte de Pharmacie de Nancy, Nancy, France

Morton, Dr. Harry E., Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania

van Niel, Dr. C. B., Hopkins Marine Station, Pacific Grove, California

Sippel, Dr. W. L., Department of Animal Diseases, Georgia Coastal Plain Experiment Station, University of Georgia College of Agriculture Experiment Stations, Tifton, Georgia

Sneath, Dr. P. H. A., National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W. 7, England

[redacted]