PSYCHROPHILIC BACTERIA

J. L. INGRAHAM[1] AND J. L. STOKES

Western Regional Research Laboratory,[2] Albany, California, and Department of Viticulture and Enology University of California, Davis, California

CONTENTS

I. INTRODUCTION

"... our bacteria exhibit at certain temperatures a very special property which to my knowledge, at least with pure cultures, has not been previously observed.... they grow almost as well in the ice box as at the usual room temperature and even when tubes of streaked nutrient gelatin are placed in a container packed with finely crushed ice in the ice box, that is, at 0 C."
J. Forster, 1887 (19) p. 340

"The hard winter, on the one hand, and the unheated laboratories of the Institute in the winter months of 1921 and 1922, on the other hand, created favorable conditions in Odessa for the study of the effect of low temperatures on the life activity of bacteria. . . .

"During the experiments, the temperature in the laboratory was very constant. The amplitude of the temperature variations for the individual days was equal to zero; for the entire period, however, the maximum was −1.25 and the minimum, −2.5 C."
Rubentschik, 1925 (54) p. 167
(our translations)

[1] Present address: Department of Viticulture and Enology, University of California, Davis, California.

[2] Western Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

Bacteria are generally divided into thermophiles, mesophiles, and psychrophiles on the basis of the temperature ranges in which they can grow. The first two groups can be easily and accurately characterized and distinguished by their growth-temperature optima. The psychrophiles have also been defined, especially in textbooks, in terms of optimum growth temperature. But the latter has been set either so low that it fits virtually no known bacteria or so close to that of mesophiles that it fails to separate the two groups (45). There is in nature, however, an important and distinct group of bacteria which differs from thermophiles and mesophiles not so much in optimum growth temperature but rather in its ability to grow at low temperatures, at or close to 0 C. These low-temperature bacteria, commonly called psychrophiles, are widely distributed. They occur in large numbers in soil, water, foods, and other habitats where they carry out important beneficial or harmful chemical transformations at temperatures which are too low for the growth of other types of bacteria.

The information on psychrophiles has not been fully reviewed for about 25 years despite the availability of a considerable amount of new data and the obvious importance of the subject. In 1932, Hampil (28) briefly discussed psychrophiles in her general paper on the effects of temperature on bacteria. The last comprehensive review is that of Berry and Magoon in 1934 (4), although recent discussions of psychrophiles in the dairy industry have appeared (12, 52, 59). It seemed desirable, therefore, to review psychrophiles again in the light of current information about these bacteria.

II. TERMINOLOGY AND DEFINITIONS

There appears to be a common misunderstanding or lack of understanding of the growth temperatures of psychrophilic bacteria, especially of their optimum growth temperatures. This seems to be due, in part, to the misleading implications in the term, psychrophile, as discussed below. Also, the psychrophilic bacteria described in most bacteriology textbooks are essentially hypothetical organisms with growth-temperature optima that are quite different from those exhibited by psychrophilic bacteria actually isolated from natural sources.

A variety of names have been given to bacteria that can grow at low temperatures. These bacteria are called most commonly, psychrophiles, a term derived from the Greek words *psychros*, meaning cold, and *philos*, meaning loving, *i.e.*, cold-loving. They have been called, also, cryophiles and rhigophiles. Both of these names are also derived from the Greek and have essentially the same meaning as psychrophile. To the best of our knowledge, the term, psychrophile, was used first by Schmidt-Nielsen (55) in 1902 for microorganisms that can grow at 0 C, although such bacteria were described first by Forster (19) in 1887. From the very beginning, however, investigators in this field objected to the name because it implied that the organisms *preferred* low temperatures, whereas the large mass of subsequent experimental data showed, almost without exception, that these bacteria grew better at higher temperatures, generally above 20 C (3, 4, 24, 34, 35, 42, 50, 54, 64). The organisms are cold-tolerant, therefore, rather than cold-loving.

Yet most textbooks define psychrophilic bacteria as those having an optimum growth temperature below 20 C and create the erroneous impression that a relatively large group of such bacteria exist. It is difficult to understand from a study of the literature how this idea originated but, once formed, it has persisted. This does not mean that psychrophilic bacteria with an optimum growth temperature below 20 C do not exist. Indeed, there are a few isolated reports which describe such bacteria (39, 41, 42, 61) but supporting data are either lacking or appear inadequate. Also, as will be discussed later, the optimum growth temperature is difficult to determine accurately and may vary greatly depending on whether growth rate or cell yield is measured.

The recognition that low temperatures are not optimum for organisms that grow at 0 C led to the introduction of several other names as replacements for psychrophile. These included *glaciale Bakterien* (20), the species name *psychrocartericus* or cold-conquering (54), psychro-tolerant (33), eurythermic or capable of growing over a broad range of temperatures (63), and thermophobic (14). Horowitz-Wlassowa and Grinberg (33) proposed retention of the term, psychrophile, for the commonly isolated bacteria and suggested a new name, psychrobe, for the hypothetical bacteria that grow best at low temperatures. In a similar fashion, Hucker (34) considers that bacteria which can grow at 0 C and 32 C are facultative psychrophiles, whereas those which grow at 0 C but not at 32 C are obligate psychrophiles. Hucker failed to find any representatives of the latter group in an extensive survey of frozen vegetables although they have been isolated by other investigators (3, 44). It should be noted that Hucker's definitions are not based on optimum growth temperatures.

It can be claimed that truly psychrophilic bacteria exist, if maximum cell numbers, rather than the normally used maximum growth rate, are employed to establish the optimum growth temperature. Hess (31) has concluded that *Pseudomonas fluorescens* and several other bacteria isolated from fish are true psychrophiles because, in the range of 20 C to −3 C, the largest cell populations in broth cultures occurred at 5 C, although the maximum growth rate occurred at 20 C. Also, Scott (56) considered three of his low-temperature bacteria to be true psychrophiles even though they grew most rapidly at temperatures above 20 C. This conclusion was based on the observations that the mean temperature at which dk/dt (rate of change of growth with temperature) was constant was less than 15 C, that dk/dt attained its maximum value below 10 C, and that the maximum crop occurred in the range of 10 C to 15 C. There is a practical difficulty inherent in the use of cell numbers to determine optimum temperature in that months may be required to reach maximum populations at low temperatures because of the low rates of growth at these temperatures.

An additional source of difficulty in defining psychrophiles has been introduced by dairy bacteriologists. For technological reasons, psychrophiles in the dairy industry are considered to be those bacteria which can grow relatively rapidly in the range of 1.7 to 10 C—a range which occurs in commercial holding and distribution channels (Foster *et al.* (21) p. 221). The upper part of this range is too high, since it permits inclusion among psychrophiles of bacteria which are definitely mesophilic in character (27).

Despite the objections to the term, psychrophile, we have continued to use it in the present review because it is so deeply entrenched in the literature and is so widely used. The unique property of psychrophilic bacteria is the ability to grow well at 0 C. This was recognized from the very beginning of the study of psychrophiles by Forster (19), Schmidt-Nielsen (55), and other early investigators. The essentially erroneous concept that psychrophiles are distinguished by their ability to grow most rapidly below 20 C did not arise until later. If one eliminates from consideration the presently irrelevant matter of optimum growth temperature, it seems possible to arrive at a definition of psychrophiles which fits the known facts. According to our definition, *psychrophiles are bacteria that grow well at 0 C within 2 weeks*. It should be noted that this definition does not include any of the cardinal temperatures —minimum, optimum, or maximum. It can be stated, perhaps, in other ways: psychrophiles are bacteria that form colonies on solid media, easily visible to the naked eye within 2 weeks at 0 C (figure 1); or more quantitatively, psychrophiles are bacteria that have a generation time of 48 hr or less at 0 C (35). The above definition eliminates mesophiles, which usually do not grow below about 8 C, although data on this point are somewhat limited (9 (p. 95); 27), and also border-line bacteria which produce some growth, usually scant, between 0 and 8 C if incubated for many weeks or months.

There may be some merit in naming the above defined bacteria *facultative psychrophiles* and reserving the term *obligate psychrophiles* for those bacteria that not only grow rapidly at 0 C but also grow most rapidly at temperatures below 20 C, *i.e.*, the textbook type of psychrophile. The latter may have been isolated occasionally in the past and may be encountered in future investigations. This subdivision differs from Hucker's (34) in that he defines the obligate psychrophiles in terms of maximum growth temperature, *i.e.*, inability to grow at 32 C rather than on the basis of an optimum growth temperature below 20 C.

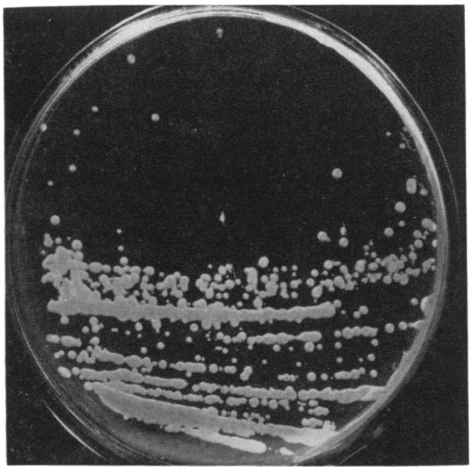

Figure 1. Colonies of *Pseudomonas fragi* on trypticase soy agar (BBL) after incubation for 10 days at 0 C (10-cm petri dish).

III. ECOLOGY AND TYPES

As previously mentioned, Forster in 1887 (4) was the first to demonstrate the ability of pure cultures of bacteria to grow at 0 C. Forster's cultures were luminous bacteria isolated from marine fish. Within a year, Forster's results were confirmed by Fischer (18) with 2 strains of luminous bacteria in his culture collection and also with 14 additional strains of freshly isolated marine bacteria. In a subsequent paper in 1892 (20), Forster showed that psychrophilic bacteria are widely distributed in nature. He found them in fresh and salt water, on the surface and in the intestines of fresh and salt water fish, in milk and meat, in garden soil and street dirt, and in canal and meadow ditch water. The concentration of psychrophiles in these places was fairly high: 1000 per ml of commercial milk, 2000 per ml of canal water, and 140,000 per g of garden soil. The psychrophiles were isolated from these habitats during the warm summer months as well as in winter. Later, Schmidt-Nielsen (55) isolated 15 different types of psychrophilic bacteria from soil and vegetables, and Müller (50) obtained 36 different types in pure culture from meat, fish, milk, vegetables, flour, soil, and air.

Numerous subsequent investigations have amply confirmed the ubiquitous nature of psychrophilic bacteria. Thus, in more recent years, psychrophiles have been isolated from dairy products including raw and pasteurized milk (6, 17, 60), cream (17), and butter (32, 38), from chilled poultry (2), fresh and frozen fish (22, 30), chilled and frozen meat (8, 16, 25, 58), sea water and mud (3, 63), frozen vegetables and fruits (34, 57), and soil (42).

It is difficult to list with certainty the genera and species of psychrophilic bacteria which have been isolated. The earliest investigators made no attempt to identify their psychrophiles except to report, for example, that they were luminescent. Subsequent investigators frequently described only the morphology, gram-staining reaction, and motility of their strains, whereas others identified their strains only as to genus. Also, in some instances, organisms were considered to be psychrophiles even though growth was determined at temperatures above 0 C and many weeks or months were required for visible growth. We shall consider as psychrophiles, however, only those bacteria that have been shown to grow well within a week or two at or close to 0 C.

Psychrophilic bacteria are preponderantly gram-negative nonsporeforming rods. Such rods, both motile and nonmotile, were dominant among the cultures isolated by Müller (50) from a variety of foods and also from soil by Lochhead (42). Many of Bedford's (3) marine bacteria belonged to the genera *Serratia*, *Flavobacterium*, and *Achromobacter*. Of a variety of common bacteria examined by Haines (27), which included aerobic sporeformers, coliform bacteria, staphylococci, streptococci, and others, only strains of *Pseudomonas* and *Achromobacter* grew well at 0 C within 2 weeks. Likewise, *Pseudomonas* strains are the predominant members of the psychrophilic population of frozen lamb and pork (58), chilled beef (8), and chilled poultry (2). Psychrophilic micrococci have also been encountered on chilled beef (15, 44). Mossel and Ingram (49) found that food spoilage at temperatures below 10 C is due primarily to psychrophilic strains of *Pseudomonas*, *Achromobacter*, and *Flavobacterium*. According to Hucker (34), the predominant psychrophilic organism in frozen vegetables is a gram-negative rod resembling *Flavobacterium esteroaromaticum*.

The psychrophilic population in dairy products is also composed mainly of gram-negative rod-shaped bacteria. These are strains of *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and occasionally *Alcaligenes* (17, 38, 41, 52, 60); strains of *Lactobacillus* and *Streptococcus* are relatively rarely reported (17). Strains of *Pseudomonas* are the most troublesome psychrophiles in the dairy industry because of their pronounced ability to produce undesirable flavors, odors, and pigments (17, 32, 47, 48, 52).

Ingraham (35) examined 54 pure cultures for their ability to grow well at 1 C. These included 32 strains of *Salmonella*, 6 strains of *Proteus*, 3 strains each of *Aerobacter* and *Pseudomonas*, 2 strains each of *Bacillus*, *Lactobacillus*, and *Streptococcus*, and 1 strain each of *Paracolobactrum*, *Escherichia*, and *Micrococcus*. Of this group of bacteria, only 2 strains, both *Pseudomonas fluorescens*, were psychrophilic. Occasionally, psychrophilic strains of *Proteus* have been isolated (10).

In a recent extensive survey of the types of psychrophilic bacteria on chilled beef, Brown and Weidemann (8) found that 182 of 189 cultures were gram-negative rods and that 93 per cent of the latter were strains of *Pseudomonas*. Included in the 189 cultures were 129 strains originally isolated by Empey and Scott and identified by them as being, in about equal numbers, strains of *Pseudomonas* and *Achromobacter*. Brown and Weidemann, however, on the basis of flagellation, pigment formation, sensitivity to penicillin, and oxidative metabolism, believe that many of the strains identified as *Achromobacter* by Empey and Scott are actually strains of *Pseudomonas*. Ayres *et al.* (2) also have pointed out that many strains previously labeled *Achromobacter* would now be classified as strains of *Pseudomonas*.

There are probably psychrophilic bacteria in the Arctic and Antarctic regions, although this has not yet been established. McLean (43) reported the presence of gram-positive cocci and gram-positive and gram-negative sporeforming and nonsporeforming rods in the snow and in association with frozen algae and seaweed at Adelie Land near the South Pole. Darling and Siple (11) isolated 178 bacterial cultures from the ice, snow, water, plant debris, and soil of Antarctica. Of these, 117 cultures were aerobic sporeforming rods, 45 nonsporeforming rods, and 16 cocci. Unfortunately, the ability of these cultures to grow at low temperatures was not determined. Since aerobic sporeforming bacteria are not usually psychrophilic, it seems likely that the sporeformers from Antarctica are also nonpsychrophilic. Some of the nonsporeforming rods and cocci, however, may be able to grow at 0 C. During the summer months in Antarctica, the temperatures may hover around 0 C or higher for several months (11, 43) and thus permit considerable growth and activity of psychrophilic bacteria.

Although most psychrophilic bacteria are gram-negative nonsporeforming rods, other morphological types and also gram-positive psychrophiles have been isolated occasionally. Thus, among Bedford's 71 psychrophilic cultures from sea water were a number of strains of *Micrococcus* and *Rhodococcus*. McLean *et al.* (44) isolated an unusually large micrococcus, *Micrococcus cryophilus*, from pork sausage. It was gram-variable, strictly aerobic, and did not ferment sugars. It grew at −4 C, maximally between 23 and 24 C, and not at all above 25 C. Seven of the 189 cultures examined by Brown and Weidemann (8) were gram-positive. Four of these were probably strains of *Corynebacterium*. The remaining three included a yellow rod with a polar flagellum, a nonpigmented rod, and a coccus.

It can be concluded that the ability to grow well at 0 C is possessed by a rather limited group of bacteria, mainly gram-negative nonsporeforming rods. Even within this restricted group, most strains belong to the genus *Pseudomonas* and the remainder to the genera *Achromobacter*, *Flavobacterium*, *Micrococcus*, perhaps *Alcaligenes*, and a few others. No anaerobic psychrophiles have been reported, although such organisms may exist.

Although we have limited this review to the psychrophilic bacteria, it might be mentioned briefly that there are some strains of actinomycetes which can grow at 0 C (26). Also, there is an extensive literature on psychrophilic yeasts and molds (4). Recently several strains of *Candida* have been described (40) which appear to be strict psychrophiles with optimum growth at approximately 11 C and no growth at 21 C or above.

IV. PHYSIOLOGY AND BIOCHEMISTRY

A. Temperature-Growth Relations

1. Minimum growth temperature. The distinguishing characteristic of psychrophilic bacteria is their ability to grow at low temperatures. The lowest temperatures at which growth is possible have been established in several investigations. Ten of Bedford's (3) marine bacteria grew at −7.5 C on agar after 48 to 98 days of incubation. No growth occurred at −10 C in 37 days, after which time the plates were discarded because the medium had crystallized. According to ZoBell (63), the majority of marine bacteria isolated in his laboratories grew at −4 C. Strains of *Pseudomonas* and *Achromobacter* will grow at −3 C (27, 46). Smart (57) reported slight but definite growth of a number of microorganisms including several bacterial cultures at −8.89 C after 5 to 7 months of incubation. Sulzbacher (58) isolated from frozen lamb 8 cultures, mostly strains of *Pseudomonas* which grew abundantly in 1 week at −6 C and slightly in 3 to 4 weeks at −8 C in veal infusion medium.

It appears, therefore, that the lowest temperature for bacterial growth is in the vicinity of −10 C. In this temperature region, multiplication is usually very slow and months may be required for visible growth. Below about −10 C, growth is prevented, probably by the increasing desiccation and high salt concentration of the media due to the progressive removal of water by freezing.

2. Optimum growth temperature. Because psychrophiles have been defined most frequently on the basis of their optimum growth temperature, the latter has assumed a special importance in this field. It is usually defined as the temperature at which the *rate* of growth is most rapid, and is usually determined roughly by visual assessment of the rate of appearance and increase of turbidity in liquid cultures or of the rate of appearance and enlargement of colonies or masses of growth on solid media.

Most of the determinations of the optimum growth temperatures of psychrophiles have been made by such visual observations and are, therefore, only rough approximations. Within this limitation, the data in the literature, as previously mentioned, indicate that the optimum temperature for the growth of virtually all psychrophiles is near or, more usually, above 20 C. For example, the optimum for Bedford's psychrophiles (3) was 20 to 25 C or higher; for those of Horowitz-Wlassowa and Grinberg (33), 20 to 37 C; for those of ZoBell and Conn (64), 18 to 37 C. Lochhead's (42) soil cultures grew better at 20 C than at 3 C, and also Hess's cultures (31) grew better at 20 C than at lower temperatures. Although *Micrococcus cryophilus* grew well at 0 C, it grew best at 23 to 24 C (44). The *Pseudomonas* strains of Sulzbacher (58), which grew abundantly at −6 C, developed best at temperatures above 20 C. The numerous psychrophiles isolated by Ingraham (35) had optimum temperatures above 20 C, some close to 40 C.

The optimum growth temperature of psychrophiles can be established more accurately by determining the generation time during the expo-

TABLE 1

Growth of Pseudomonas fluorescens in buffered nutrient broth at various temperatures (Hess (31))

Growth	Temperature			
	20 C	5 C	0 C	−3 C
Lag phase,* duration in days	1	3	4	6
Exponential phase				
Generation time in minutes	85	401	1813	3408
Duration in days	¼	3	19	40
Maximum cell count per ml $\times 10^{-6}$	995	1850	1590	1200†
Days required to reach maximum	3	29	46	60

* Includes stationary or latent phase.

† Count at termination of experiment might have gone higher with longer incubation.

nential growth phase of the culture. The temperature at which the generation time is shortest is then the optimum growth temperature. Such determinations were made as early as 1903 by Müller (50). The generation times for bacterium A, a gram-negative, rod-shaped psychrophile, at 30, 25, 12, 6, and 0 C were, respectively, 47, 51, 116, 456, and 1197 min. Thus, about ¾ hr was required for the doubling of the cell population at 30 C and as much as 20 hr at 0 C. Also, for this psychrophile, as in the case of others, growth was most rapid at temperatures above 20 C, *i.e.*, at 25 to 30 C. Müller obtained similar results with several other psychrophiles. Later, Hess (31) made a careful and detailed investigation of the generation time, duration of the lag period, the maximum cell yield, and other aspects of the growth curves of three psychrophilic bacteria at 20, 5, 0, and −3 C. The data obtained with *Pseudomonas fluorescens* are reproduced in table 1. The cell population doubled in about 1½ hr at 20 C, 30 hr at 0 C, and 57 hr at −3 C. Growth proceeded most rapidly, therefore, at 20 C. The remainder of the data in the table will be discussed subsequently.

Other determinations were made later of generation times over the entire temperature range in which growth of psychrophiles can occur (7, 23, 35, 51, 56). The usefulness of some of these data is limited by the fact that growth on solid surfaces was followed (51, 56), mixed populations were used (51), or the liquid cultures were not shaken (23) so that the oxygen supply may soon have become limiting. Recently, Ingraham (35) determined the generation times of several representative psychrophilic and mesophilic bacteria in agitated, shallow-layer, broth cultures at 2 C intervals throughout the entire temperature range of growth. The results for a typical psychrophile, a species of *Pseudomonas*, and a mesophile, *Escherichia coli*, are shown in figure 2, in which the generation times in minutes are plotted against temperature. The generation time of the mesophile is 20 hr at 10 C, 42 hr at 8 C, and so long at lower temperatures as to be indeterminable, whereas the psychrophile had a generation time of about only 3 hr at 10 C, 4 hr at 8 C, and 20 hr at 0 C. An additional difference between psychrophile and mesophile becomes apparent if an Arrhenius plot of the data is made, as shown in figure 3. For both organisms, the slope of the curve (temperature characteristic) is negative at high temperatures, *i.e.*, the growth rate decreases as the temperature increases. With decreasing temperature, the slope becomes zero (optimum temperature) and then positive and relatively constant before it again increases markedly near

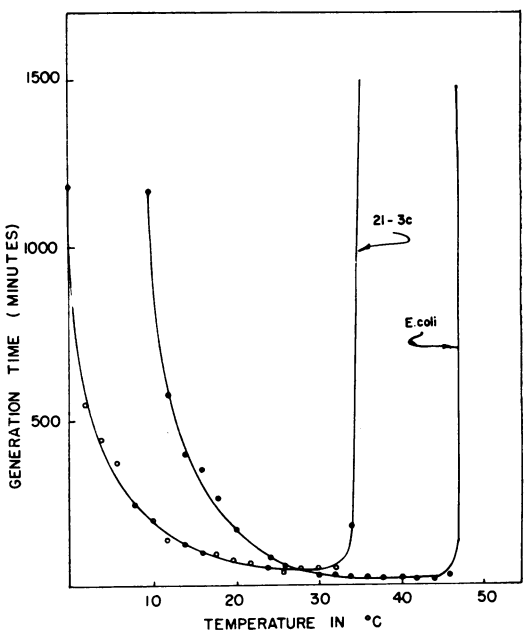

Figure 2. Effect of temperature on the generation time of a psychrophile, *Pseudomonas* sp. (21-3c) and a mesophile, *Escherichia coli* strain K-12 (Ingraham (35)).

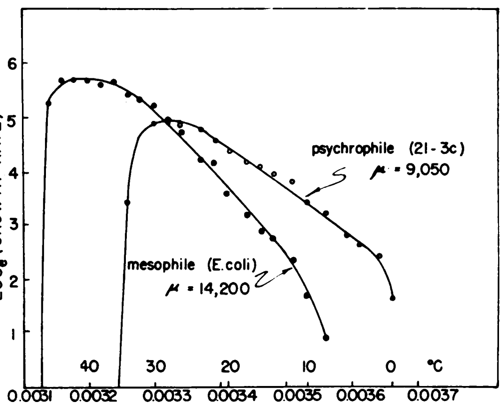

Figure 3. Arrhenius plot of the relationship between growth rate and temperature for a psychrophile, *Pseudomonas* sp. (21-3c) and a mesophile, *Escherichia coli* strain K-12 (Ingraham (35)).

the minimum growth temperature. The interesting difference between psychrophile and mesophile is that the slope of the curve in the temperature range where it is relatively constant is less for the psychrophile than for the mesophiles. In other words, the growth rate of the psychrophile is considerably less affected by decreasing temperature than the growth rate of the mesophile. Somewhat similar results were obtained by Brown (7).

Although most investigators have defined optimum growth temperature in terms of maximum growth rate, some have suggested that it be based on maximum cell crop. In general, the optimum temperature for the latter is considerably lower than that at which growth is most rapid (9, 53). It can be seen from Hess's data in table 1 that although *Pseudomonas fluorescens* grows most rapidly at 20 C, the maximum cell count occurs at 5 C. There is considerable merit in defining the optimum growth temperature as that temperature which supports the largest cell yield. But, in practice, this is difficult to determine because months of incubation may be required at low temperatures to reach maximum cell numbers, as is evident from the data of Hess. The interesting data of Dorn and Rahn (13) on lactic acid bacteria, which are reproduced in table 2, indicate how the optimum temperature for growth rate, cell yield, and fermentation may differ. For example, the maximum growth rate for *Streptococcus lactis* occurs at 34 C, the largest cell crop at 25 to 30 C, and the maximum rate of fermentation at 40 C.

Also, the optimum growth temperature may vary with the composition of the growth medium and other cultural conditions (9). This applies, also, to the minimum and maximum growth temperatures. The available data in this area are few and should be increased.

3. Maximum growth temperature. This varies over a relatively wide range for psychrophilic bacteria and is, perhaps, higher than one might expect for organisms that grow well at 0 C. The following data of Bedford (3) on psychrophilic marine bacteria is illustrative:

Maximum growth temperature	*No. of strains*
25 C	5
30 C	32
37 C	13
40 C	5
42.5 C	2
45 C	3

Most strains had a maximum of 30 C, but there was a considerable number with maxima at 37 to 45 C.

Among 36 psychrophilic bacteria isolated from spoiled chicken and from soil (35), none had a maximum growth temperature lower than 30 C. The maximum temperature for 20 strains was 30 C; for 14 strains, 35.5 to 37 C; and for 2 strains, 40 C or higher. Twenty-three of 180 psychrophilic strains of bacteria from meat (8), or 13 per cent, grew at 37 C. The maximum growth temperatures for these and the rest of the strains were not determined.

It appears, therefore, that most psychrophilic bacteria have a maximum growth temperature of about 30 C. A considerable number of strains can

TABLE 2

Optimum temperature for various metabolic functions of Streptococcus lactis and Streptococcus thermophilus in skim milk cultures (Dorn and Rahn (13))

Function	*Streptococcus lactis*	*Streptococcus thermophilus*
Maximum growth rate	34 C	37 C
Largest number of cells	25–30 C	37 C
Most rapid rate of fermentation	40 C	47 C
Largest amount of acid	30 C	37 C

also grow at 37 C and a few at temperatures as high as 45 C. Frequently, the maximum growth temperature is only a few degrees higher than the optimum growth temperature (3, 9).

4. Lag phase. The duration of this growth phase is markedly influenced by temperature. It lengthens as the incubation temperature is lowered, so that it may last for several days at low temperatures (Müller (50)). Also, Hess (31) has shown that the phase of adjustment for *Pseudomonas fluorescens*, which includes the period when there is no multiplication and the period of slow growth prior to the exponential phase, increases from 1 day at 20 C to 3 days at 5 C, to 4 days at 0 C, and to 6 days at −3 C (table 2). Similar results were obtained later by Greene and Jezeski (23) with several psychrophilic bacteria. Brown (7) compared the duration of the lag phase for a psychrophilic *Pseudomonas* species and a mesophile, *Pseudomonas aeruginosa*, over the range of 0 to 40 C. The minimum lag time of the psychrophile occurred at a lower temperature than that of the mesophile. Also, the lag time of the psychrophile was less than that of the mesophile at all but the highest temperatures.

Hess (31) has also shown with psychrophilic bacteria that the duration of the exponential phase increases and that the bacteria die more slowly as the incubation temperature is lowered.

5. Adaptation to lower temperatures. The evidence for adaptation of bacteria to growth at lower temperatures is conflicting. According to Müller (50), the ability of bacteria to grow at 0 C is a specific property of certain bacteria and cannot be acquired by subculture of mesophiles at progressively lower temperatures. Some adaptation was obtained by Horowitz-Wlassowa and Grinberg (33) by serial passage or storage of bacteria at low temperatures. For example, 5 strains of bacteria required 29 days to produce visible growth at −3 C but only 10 to 19 days after one previous subculture at 0 C. The latter, however, may have merely shortened the lag period at −3 C and may have had no effect on the exponential growth rate. Hess (30) subcultured psychrophilic strains of *Pseudomonas* and *Achromobacter* at temperatures ranging from 20 C to −3 C for approximately 4 months. Cultivation at 0 C and −3 C did not produce strains that grew better at these temperatures than the parent strains. The strains that were grown at 5 C, however, grew better at 0 C and −3 C than the parent strains. In an extensive investigation, Jennison (37) serially subcultured *Escherichia coli*, *Aerobacter aerogenes*, *Serratia marcescens*, and *Chromobacterium violaceum* for several weeks at 22 C and then compared generation times at 22, 27, 32, 37, and 42 C with those obtained when the bacteria were subcultured at the above four additional temperatures. The generation times were not affected by the previous incubation temperature except for a shortening of the generation time of *S. marcescens* at 37 C by 11 min after 4 months of previous cultivation at 37 C.

Although the available data are limited in amount, it may be concluded that bacteria do not adapt readily to growth at lower temperatures. The changes in reference to temperature which have been reported are not striking.

B. Motility

Rubentschik (54) noted that his urea-decomposing psychrophilic bacteria were motile at 20 to 24 C, but not at −1.25 to −2.5 C. In contrast, Hess (30) found that agar-slant cultures of *Pseudomonas fluorescens* grown at temperatures ranging from 20 to −6.5 C were motile at all temperatures and that motility actually lasted longer with the low-temperature cells than with those from the 20 C cultures.

C. Chromogenesis

Many psychrophilic bacteria produce diffusible and nondiffusible pigments even when grown at low temperatures. Marine psychrophiles may form yellow, orange, pink, and red pigments (3, 29). The fluorescent, greenish-yellow, water-soluble pigment complex of *Pseudomonas fluorescens* is frequently observed. Hess (30) reported that *Pseudomonas fluorescens* grown on fish-extract agar slants produces a greenish fluorescence in 1 day at 20 C, in 6 days at 5 C, and in 12 days at 0 C and −3 C. Also, *Flavobacterium deciduosum* produced a yellow pigment on agar slants at all temperatures at which growth occurred in the range of 20 C to −6.5 C, although it appeared more slowly as the temperature decreased. Ayres *et al.* (2) noted that chromogenic bacteria were predominant on chilled poultry during initial storage but were later replaced by nonchromogenic bacteria. Pigment formation by psychrophilic bacteria occurs only rarely in milk and cream stored at low temperatures (38) but more commonly in butter (32, 38, 47, 62). In general, although pigment formation by psychrophiles is often observed and is an important factor in the spoilage of fish, dairy products, and other foods, it is not a common characteristic of psychrophilic bacteria. The rate of pigment production is correlated with the rate of growth of the bacteria and as the latter decreases when the temperature is lowered, the rate of pigment formation also decreases.

D. Other Biochemical Activities

Bacteria which are growing at low temperatures must be carrying out a great many biochemical transformations involved in the synthesis of new cells and in the maintenance of the old ones. External signs of biochemical activity such as the fermentation of carbohydrates, the liquefaction of gelatin, and the production of indole also occur. At low temperatures, however, incubation for weeks or months may be necessary before these reactions are detectable due to a combination of slow growth and slow enzyme activity at these low temperatures. Even in the absence of growth, at temperatures below −10 C, biochemical changes of substrates due to psychrophiles may be detectable if long enough periods of incubation are used. The temperature at which all enzymatic activity ceases is not known.

Production of pigments bys pychrophiles has already been discussed. Luminescence of marine bacteria at 0 C was noted by Forster (19) and Fischer (18). Fermentation of glucose and other sugars by psychrophiles at 0 C and lower temperatures has been reported frequently (7, 30, 33, 50, 63). Sometimes only acid is formed rather than the acid and gas normally produced (30, 33). At 0 C and below, psychrophiles can decompose urea (54, 63), hydrolyze starch (63), form indole from tryptophan, and reduce nitrate to nitrite (30). Also, proteins can be decomposed, as indicated by the liquefaction of gelatin or the production of NH_3, CO_2, and H_2S from peptones (30, 50, 63).

These reactions may not be detectable for weeks or months depending upon the incubation temperature. Hess (30) first detected fermentation of glucose, sucrose, and maltose by strains of *Pseudomonas*, *Flavobacterium*, and *Achromobacter* at 0 C in 22 days and at −3 C in 65 to 120 days. Similarly, 41 days were required for indole formation at −3 C, 22 days for gelatin liquefaction at 0 C (46 days at −3 C), and 15 days for nitrate reduction at 0 C. Psychrophiles generally carry out the same biochemical reactions at both low and high temperatures. The reactions proceed, however, much more slowly at the low temperatures.

The ability of psychrophiles to grow at low temperatures may be due to some unique property of their enzymes. Brown (7) and also Ingraham and Bailey (36) have shown that the temperature coefficient of oxidation of several substrates is less for psychrophiles than for mesophiles. This indicates that the enzymatic activities of the psychrophiles are less affected by a decrease in temperature than those of mesophiles. However, Brown compared the oxidation of glucose and gluconic acid by a psychrophilic species of *Pseudomonas* and a mesophilic strain of *Pseudomonas aeruginosa* and concluded that these enzyme systems in the two types of bacteria may not be greatly different with respect to physical properties which determine temperature relations. Likewise, Ingraham and Bailey could not find any difference in the effect of temperature on the cell-free dehydrogenase systems for malic acid, citric acid, and glucose-6-phosphate obtained from a psychrophilic strain of *Pseudomonas* and a mesophilic *E. coli* strain.

These results may merely mean that the right enzyme systems have not yet been chosen for investigation. In any event, the mechanism which enables psychrophiles to grow at low temperatures is still unknown.

V. APPLIED ASPECTS

It is not our intention to review, in detail, the extensive literature on the occurrence, spoilage, and other aspects of psychrophilic bacteria in meats, poultry, fish, dairy products, vegetables, fruits, and other foods. This information can be found in the reviews of Berry and Magoon (4), Doetsch and Scott (12), Ayres (1), Borgström (5), and Thomas (59).

The greatly increased use of frozen and especially chilled foods in recent years and the increasingly longer periods of time between their production and consumption have greatly enlarged the importance of psychrophilic bacteria in the food industries. Although psychrophiles grow slowly at low temperatures, very large cell populations can arise within a week at 0 C and sooner at the ordinary household refrigeration temperature of 4 to 5 C. Frozen foods, which normally are processed and stored at temperatures too low for bacterial growth, frequently undergo spoilage by psychrophiles because temperatures are allowed to rise in wholesale distribution channels, in stores, and in the home. Large losses of meat, fish, poultry, dairy products, and other foods, due to undesirable odors, flavors, and odors produced by psychrophiles, are not uncommon.

There is increasing use of antibiotics to suppress the growth of psychrophiles and other bacteria on poultry and fish. The tendency, however, for such treatments to give rise to large populations of antibiotic-resistant strains of bacteria has reduced their effectiveness. Food spoilage by psychrophiles can be greatly lessened by proper sanitation during production, by maintenance of low temperatures, usually below −10 C, during distribution, and by shortening the interval between production and consumption.

VI. SUMMARY

Psychrophiles have been defined as bacteria which grow appreciably and often abundantly at 0 C within 2 weeks. They can develop below 0 C and the minimum temperature is close to −10 C. Their optimum growth temperatures lie in the range of 20 C to 40 C, and the maximum may be as high as 45 C. Psychrophilic bacteria with an optimum temperature below 20 C, frequently described in textbooks, have rarely, if ever, been found.

Psychrophilic bacteria are ubiquitous and can be isolated, sometimes in large numbers, from soil, air, fresh and salt water, common foods, and other habitats. Most psychrophiles appear to belong to the genus *Pseudomonas* and, to a much lesser extent, to the genera *Achromobacter*, *Flavobacterium*, *Alcaligenes*, *Micrococcus*, and possibly others.

At 0 C and lower, psychrophiles can luminesce, be motile, synthesize green, yellow, orange, red, and other pigments, ferment carbohydrates, decompose proteins, produce indole, reduce nitrate, and, in general, exhibit all of the biochemical activities evident at higher temperatures, but at a considerably reduced rate. The mechanism which enables psychrophiles to grow at low temperatures is not known.

Psychrophiles are important spoilage organisms in meats, poultry, fish, dairy products, and other foods stored at temperatures close to 0 C.

VII. REFERENCES

1. Ayres, J. C. 1955 Microbiological implications in the handling, slaughtering, and dressing of meat animals. Advances in Food Research, **6**, 109–161.
2. Ayres, J. C., Oglivy, W. S., and Stewart, G. F. 1950 Post-mortem changes in stored meats. I. Microorganisms associated with the development of slime on eviscerated cut-up poultry. Food Technol., **4**, 199–205.
3. Bedford, H. 1933 Marine bacteria of the northern Pacific Ocean. The temperature range for growth. Contribs. Can. Biol. and Fisheries, **7**, 431–438.
4. Berry, J. A. and Magoon, A. 1934 Growth of microorganisms at and below 0°C. Phytopathology, **24**, 780–796.
5. Borgström, G. 1955 Microbiological problems of frozen food products. Advances in Food Research, **6**, 163–230.
6. Boyd, J. C., Smith, C. K., and Trout, G. M. 1953. The role of psychrophilic bacteria in the keeping quality of commercially pasteurized milk. J. Dairy Sci., **36**, 571.
7. Brown, A. D. 1957 Some general properties of a psychrophilic pseudomonad: the effects of temperature on some of these properties and the utilization of glucose by this organism and *Pseudomonas aeruginosa*. J. Gen. Microbiol., **17**, 640–648.
8. Brown, A. D. and Weidemann, J. F. 1958 The taxonomy of the psychrophilic meat-spoilage bacteria: a reassessment. J. Appl. Bacteriol., **21**, 11–17.
9. Buchanan, R. E. and Fulmer, E. I. 1930 Effects of temperature upon microorganisms, Ch. VII. In *Physiology and biochemistry of bacteria*. The Williams & Wilkins Co., Baltimore.
10. Campbell, J. J. R. and Reed, G. B. 1947 Characteristics of some psychrophilic bacteria. Can. J. Research (Section F), **25**, 280–283.
11. Darling, C. A. and Siple, P. A. 1941 Bacteria of Antarctica. J. Bacteriol., **42**, 83–98.
12. Doetsch, R. N. and Scott, W. M. 1951 Psychrophilic bacteria in milk and cream. Milk Plant Monthly, **40**, 30–32.
13. Dorn, F. L. and Rahn, O. 1939 Definition versus measurement of optimal temperature. Arch. Mikrobiol., **10**, 6–12.
14. Edsall, G. and Wetterlow, L. H. 1947 Missed contaminations in biologic products: the role of cryophile bacteria. J. Bacteriol., **54**, 31–32.
15. Empey, W. A. and Vickery, J. R. 1933 The use of carbon dioxide in the storage of chilled beef. J. Council Sci. Ind. Research, **6**, 233–243.
16. Empey, W. A. and Scott, W. J. 1939 Investigations on chilled beef. I. Microbial con-

tamination acquired in the meatworks. Bull. Council Sci. Ind. Research, Australia, No. **126**.

17. Erdman, I. E. 1951 Psychrophilic bacteria in Edmonton milk and cream. II. Kinds. Can. J. Technol., **29**, 238–242.
18. Fischer, B. 1888. Bakterienwachsthum bei 0°C sowie über das Photographiren von Kulturen leuchtender Bakterien in ihrem eigenem Lichte. Centr. Bakteriol. Parasitenk. Abt. I, **4**, 89–92.
19. Forster, J. 1887 Ueber einige Eigenschaften leuchtender Bakterien. Centr. Bakteriol. Parasitenk., **2**, 337–340.
20. Forster, J. 1892 Ueber die Entwickelung von Bakterien bei niederen Temperaturen. Centr. Bakteriol. Parasitenk., **12**, 431–436.
21. Foster, E. M., Nelson, F. E., Speck, M. L., Doetsch, R. N., and Olson, J. C. 1957 *Dairy microbiology*. Prentice-Hall, Inc., New York.
22. Gibbons, N. E. 1934 A bacteriological study of "ice fillets." Contribs. Can. Biol. and Fisheries, Ser. C, **8**, 301–310.
23. Greene, V. W. and Jezeski, J. J. 1954 The influence of temperature on the development of several psychrophilic bacteria of dairy origin. Appl. Microbiol., **2**, 110–117.
24. Gubitz, H. 1928 Kältebakterien, mit besonderer Berücksichtigung ihrer Bedeutung für die Milchwirtschaft. Milchwirtsch. Forsch., **5**, 407–456.
25. Haines, R. B. 1931 The growth of microorganisms on chilled and frozen meat. J. Soc. Chem. Ind. (London), **50**, 223–227.
26. Haines, R. B. 1931 The influence of temperature on the rate of growth of saprophytic *Actinomyces*. J. Exptl. Biol., **9**, 45–60.
27. Haines, R. B. 1934 The minimum temperature of growth of some bacteria. J. Hyg., **34**, 277–282.
28. Hampil, B. 1932 The influence of temperature on the life processes and death of bacteria. Quart. Rev. Biol., **7**, 172–196.
29. Harrison, F. C. 1929 The discoloration of halibut. Can. J. Research, **1**, 214–219.
30. Hess, E. 1934 Cultural characteristics of marine bacteria in relation to low temperatures and freezing. Contribs. Can. Biol. and Fisheries, Ser. C, **8**, 461–474.
31. Hess, E. 1934 Effects of low temperatures on the growth of marine bacteria. Contribs. Can. Biol. and Fisheries, Ser. C, **8**, 491–505.
32. Hiscox, E. R. 1936 A pigment-producing organism (*Pseudomonas* sp.) isolated from discoloured butter. J. Dairy Research, **7**, 238–243.
33. Horowitz-Wlassowa, L. M. and Grinberg, L. D. 1933 Zur Frage über psychrophile Mikroben. Zentr. Bakteriol. Parasitenk., Abt. II, **89**, 54–62.
34. Hucker, G. J. 1954 Low temperature organisms in frozen vegetables. Food Technol., **8**, 79–82.
35. Ingraham, J. L. 1958 Growth of psychrophilic bacteria. J. Bacteriol., **76**, 75–80.
36. Ingraham, J. L. and Bailey, G. F. 1959 A comparative study of the effect of temperature on the metabolism of psychrophilic and mesophilic bacteria. J. Bacteriol., **77**, 609–613.
37. Jennison, M. W. 1935 Some quantitative relationships in bacterial population cycles. J. Bacteriol., **30**, 603–623.
38. Jezeski, J. J. and Macy, H. 1946 Cryophilic organisms in water and butter. J. Dairy Sci., **29**, 439–452.
39. Kennedy, L. and Weiser, H. 1950 Some observations on bacteria isolated from milk that grow within a psychrophilic temperature range. J. Milk and Food Technol., **13**, 353–357.
40. Lawrence, N. L., Wilson, D. C., and Pederson, C. S. 1959 The growth of yeasts in grape juice stored at low temperatures. II. The types of yeast and their growth in pure culture. Appl. Microbiol., **7**, 7–11.
41. Lawton, W. C. and Nelson, F. E. 1954 The effect of storage temperature on the growth of psychrophilic organisms in sterile and laboratory pasteurized skimmilks. J. Dairy Sci., **37**, 1164–1172.
42. Lochhead, A. G. 1926 The bacterial types occurring in frozen soil. Soil Sci., **21**, 225–231.
43. McLean, A. L. 1918 Bacteria of ice and snow in Antarctica. Nature, **102**, 35–39.
44. McLean, R. A., Sulzbacher, W. L., and Mudd, S. 1951 *Micrococcus cryophilus*, spec. nov.; a large coccus especially suitable for cytologic study. J. Bacteriol., **62**, 723–728.
45. Mitchell, P. 1951 Physical factors affecting growth and death, Ch. V. In *Bacterial physiology*. Edited by C. H. Werkman and P. W. Wilson. Academic Press, Inc., New York.
46. Moran, T. 1935 Post-mortem and refrigeration changes in meat. J. Soc. Chem. Ind. (London), **54**, 149–151.
47. Morgan, G. F. V. 1930 Some colour-producing organisms in butter washing water. New Zealand J. Agr., **41**, 315–318.
48. Morrison, H. B. and Hammer, B. W. 1941

Distribution of *Pseudomonas fragi*. J. Dairy Sci., **24**, 9–18.

49. Mossel, D. A. A. and Ingram, M. 1955 The physiology of the microbial spoilage of food. J. Appl. Bacteriol., **18**, 232–268.
50. Müller, M. 1903 Über das Wachstum und die Lebenstätigkeit von Bakterien, sowie den Ablauf fermentativer Prozesse bei niederer Temperatur unter spezieller Berücksichtigung des Fleisches als Nahrungsmittel. Arch. Hyg., **47**, 127–193.
51. Oglivy, W. S. and Ayres, J. C. Post-mortem changes in stored meats. II. The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. Food Technol., **5**, 97–102.
52. Parker, R. B., Coldwell, A. L., and Elliker, P. R. 1953 Psychrophilic bacteria—a sanitation problem. J. Milk and Food Technol., **16**, 136–139, 152.
53. Rahn, O. 1932 Growth. In *Physiology of Bacteria*. Blakiston's Son & Co., Inc., Philadelphia.
54. Rubentschik, L. 1925 Über die Lebenstätigkeit der Urobakterien bie einer Temperatur unter 0°. Centr. Bakteriol. Parasitenk., Abt. II, **64**, 166–174.
55. Schmidt-Nielsen, S. 1902 Ueber einige psychorphile Mikroorganismen und ihr Vorkommen. Centr. Bakteriol. Parasitenk., Abt. II, **9**, 145–147.
56. Scott, W. J. 1937 The growth of microorganisms on ox muscle. II. The influence of temperature. J. Council Sci. Ind. Research, **10**, 338–350.
57. Smart, H. F. 1935 Growth and survival of microorganisms at subfreezing temperatures. Science, **82**, 525.
58. Sulzbacher, W. L. 1950 Survival of microorganisms in frozen meat. Food Technol., **4**, 386–390.
59. Thomas, S. B. 1958 Psychrophilic microorganisms in milk and dairy products. Dairy Sci. Abstr., **20**, 447–468.
60. Thomas, S. B. and Chandra Sekhar, C. V. 1946 Psychrophilic bacteria in raw and commercially pasteurized milk. Proc. Soc. Appl. Bacteriol., No. 1, 47–50.
61. Van Der Zant, W. C. and Moore, A. V. 1955 The influence of certain factors on the bacterial counts and proteolytic activities of several psychrophilic organisms. J. Dairy Sci., **38**, 743–750.
62. White, A. H. 1940 A bacterial discoloration of print butter. Sci. Agr., **20**, 638–645.
63. ZoBell, C. E. 1934 Microbiological activities at low temperatures with particular reference to marine bacteria. Quart. Rev. Biol., **9**, 460–466.
64. ZoBell, C. E. and Conn, J. E. 1940 Studies on the thermal sensitivity of marine bacteria. J. Bacteriol., **40**, 223–238.