Psychrophilic bacteria grow at a relatively rapid rate at or below 45°F (7.2°C) and are capable of forming visible colonies on plates incubated for ten days at $7 \pm 0.5°C$. Since psychrophiles grow at refrigeration temperatures, they are primarily responsible for limiting the keeping quality of milk and many dairy products in which they may produce a wide variety of spoilage defects. The psychrophiles most frequently encountered are in the genus *Pseudomonas*, although many bacterial genera contain psychrophilic species. They are found in water, soil, and dirty equipment, and their elimination from milk and dairy products is largely concerned with removing these sources of contamination. The various factors affecting the survival, growth, enumeration, and biochemical activities of psychrophiles were discussed. The fundamental question of why psychrophiles are capable of growing at low temperatures is still to be answered.

Information on these and other aspects of psychrophiles was documented and interpreted with the purpose of summarizing the available information and encouraging further investigation of this important group of microorganisms.

The purpose of this review is to summarize the existing information on psychrophilic bacteria, interpret this information, and suggest some of the areas where further information is necessary or desirable. The rational of this review is the increasing and accelerating interest in this group of microorganisms. This diverse group of bacteria are linked by their ability to grow at refrigeration temperatures. Their importance to the dairy industry has been accentuated by the advancements in refrigeration facilities, increased regard for keeping quality, and longer holding times resulting from every-other-day pickup, less frequent home deliveries, and transportation over greater distances. Although of relatively recent concern to market milk processing, the psychrophiles have long received the attention of butter and cottage cheese manufacturers and much of our present knowledge of these bacteria was obtained by investigators of these dairy products. While of great practical importance to the dairy industry, the psychrophiles are also of great academic interest, since they pose the yet unexplained phenomenon of growth at low temperatures.

The subject of psychrophilic bacteria has been previously reviewed in relation to general bacteriology by Berry and Magoon (23) and Ingraham and Stokes (152), in relation to food by Ingram (153) and by Mossel and Ingram (206), in relation to marine bacteriology by Zobell (333) and Reay and Shewan (247), and in relation to dairy by Davis (64), Doetsch and Scott (71), and Thomas (294, 295). In addition to the foregoing comprehensive reviews, various aspects of dairy bacteriology closely related to psychrophilic bacteria have been separately treated: ropy defect (293), psychrophiles in raw milk (296), psychrophiles in pasteurized milk (300), butter spoilage (69, 283), and surface taint (84, 240, 249, 314, 328).

**DEFINITION**

The definition of psychrophiles has plagued bacteriologists since the term...
was first used by Schmidt-Nielsen (258) in 1902 to describe those bacteria observed by him and by previous workers (55, 91, 92, 96, 97) which grew at 0°C. The term psychrophile was an unfortunate etymological choice, since the roots implied cold loving. The year after its coinage it was criticized by Müller (207), who demonstrated that while these organisms were capable of low-temperature growth they grew more rapidly at elevated temperatures. This observation has been repeatedly confirmed, without exception, by subsequent investigators (54, 79, 137, 150, 187, 199, 239, 254, 329, 334). Kruse (168), in a 1910 textbook, suggested that these bacteria might better be called psychro-tolerant, but this suggestion or others of a similar nature (64, 79, 112, 254) did not gain acceptance and the term psychrophile has persisted.

The different motivations of the fundamental and the applied bacteriologists, and within the latter group the different motivations of those interested in dairy, soil, meat, poultry, or fish, have produced definitions of psychrophiles almost as numerous as the investigators. Hence, today psychrophiles are defined in essentially four major different ways based on (a) optimum growth temperature, (b) ability to grow at low temperature, (c) the method of enumeration, and (d) criteria which are independent of the temperature.

Each of these bases of definition is worthy of discussion, since each exemplifies certain characteristics of psychrophilic bacteria that are of interest, and indeed, each definition limits those organisms that may be considered as psychrophilic.

**Optimum growth temperature.** Unfortunately, most contemporary textbooks of general bacteriology define psychrophilic bacteria as those having an optimum growth temperature below some specified temperature, generally 20°C, but occasionally 15°C or even 10°C. The appeal of this definition is that it is specific, it conforms with the accepted nomenclature of mesophile and thermophile, and it is consistent with the etymology of the term. The optimum growth temperature, however, is difficult to accurately determine and even those investigators who prefer this definition seldom analyze for the optimum growth temperature before labelling a bacterial culture as being psychrophilic. Whether the optimum growth temperature is in reference to fastest rate of multiplication, maximum attainable population, or an undefined combination of both is a further complication. Bacteria with optimum rates of multiplication at temperatures below 20°C have been observed by only a very few investigators (170, 172, 178, 310); hence, the number of bacteria which could be called psychrophilic by this definition is unrealistically small. Optimum temperatures for producing maximum populations are usually lower than those for the optimum rate of multiplication (73, 99, 110, 137, 254, 263) and many bacteria have been observed to develop maximum crops at temperatures below 20°C. The time necessary for a culture to reach a maximum population at low temperature, however, is impractically long and the variation of maximum populations obtained at various temperatures is impractically small. Three cultures of psychrophiles studied by Greene and Jezeski (110) required a minimum of 1,347 hr.
to develop a maximum crop at 5° C., and the maximum population attained at
this temperature was not more than fivefold greater than the population ob-
served at 20° C. Further, Hess (137) observed that *Pseudomonas fluorescens*
required 29 days to produce a maximum population at 5° C. which varied only
twofold from the maximum population produced at 20° C. The use of either
criteria of optimum temperature for defining psychrophiles renders the routine
enumeration of psychrophiles impractical and makes psychrophiles of only
pedantic interest.

In addition to the optimum growth temperature, the maximum temperature
for growth is occasionally stated or implied in the definition of a psychrophile.
Most textbooks suggest that psychrophiles have a maximum growth temperature
of 30° C., but psychrophiles with a maximum growth temperature greater than
30° C. have been frequently encountered (1, 110, 112, 120, 140, 145, 159, 173,
180, 186, 318, 326, 329). Bedford (22) observed an organism which grew from
−5 to 45° C., Seleen and Stark (268) observed an organism that grew at both 5
and 42° C., and Ingraham (150) observed two organisms which grew well at
1° C. and had maximum growth temperatures of 40° C. or higher. Hucker (145)
defined psychrophiles as those bacteria which grow at 0 but not at 32° C., but
he did not encounter any such organisms in his extensive study of bacteria from
frozen vegetables. Earlier, however, Bedford (22) had isolated a number of
organisms which complied with the Hucker definition, as have other investi-
gators (110, 150, 199). Since the maximum growth temperatures of organisms
capable of low-temperature growth are so variable, the inclusion of a limiting
maximum growth temperature in the definition of a psychrophile would serve
no useful purpose.

The minimum growth temperature of psychrophiles is also the minimum
temperature of bacterial growth and it would seem unnecessary to include a
limiting minimum growth temperature in the definition of psychrophiles. Text-
books, however, set the minimum growth temperature of psychrophiles some-
where in the range of −5 to 5° C., but usually at 0° C. Hess (138) observed
bacterial growth at −6.5° C. and cites the work of a number of previous investi-
gators observing bacterial growth from −1 to −7.5° C. In addition to the
literature cited by Hess, a number of other investigators have observed bacterial
growth at temperatures below 0° C., i.e., −3° C. (120, 137), −4° C. (23, 165),
−5° C. (105, 118, 119), −7° C. (144, 288), and −8.9° C. (279). Ingram (153)
reported in his review having personally observed halophilic bacteria growing
on bacon at −10° C. Hence, to paraphrase a statement by Berry and Magoon
(23), the minimum growth temperature of psychrophiles, and therefore of
bacterial growth, is approximately −10° C., and growth below this temperature
appears to be unlikely.

*Ability to grow at low temperature.* The author prefers this basis for the
definition of psychrophiles, by which, psychrophilic bacteria are those bacteria
which grow at a relatively rapid rate at refrigeration temperatures. While
few readers would dispute this statement, most of them would challenge it as a
definition, but it adequately describes that group of bacteria which are being
considered in this review and is commensurate with the usual connotation of the word psychrophile as used in the dairy industry. The disadvantage and usefulness of this definition is in its vagueness; what is meant by relatively rapid and refrigeration temperatures? Most investigators are quite definite concerning what is meant by refrigeration temperatures and have specified every whole number temperature between 0 and 10°C or between 32 and 50°F. The choice of temperature is usually a compromise between the facts, that lower temperatures reduce the likelihood of including bacteria of predominantly mesophilic characteristics and that higher temperatures reduce the likelihood of excluding bacteria that might significantly contribute to the microflora of a sample held at the higher refrigeration temperatures (e.g., 50°F.). The more usually specified limiting temperatures in the dairy industry have been 5°C (41°F.) and 45°F. (7.2°C.).

For consistency, it is important that the definition of psychrophiles and their method of enumeration reflect each other. Since Standard Methods for the Examination of Dairy Products (285) has set the counting procedure at a maximum temperature of 7°C., the limiting temperature for psychrophile growth might logically be set at 45°F. (7.2°C.) without seriously deviating from the established dairy counting procedure or with the concept of psychrophiles held by investigators in other fields. With this choice of temperature the above definition takes a more definite form: psychrophilic bacteria are those bacteria which grow at a relatively rapid rate at or below 45°F. (7.2°C.). The relatively rapid was retained in the definition by design. The definition could be made more specific by assigning a maximum generation time—a generation time of approximately 15 hr. would result in the formation of a visible colony on a plate in ten days—but this would then require the determination of generation times, make plate-counting procedures inconclusive, be less understandable to the layman, and be of little or no practical value.

Methods of enumeration. The conditions prescribed for the isolation or the enumeration of psychrophiles either reflect the investigators’ definition of psychrophiles or serve as a definition per se. Historically, the dairy bacteriologists have contributed, and are still contributing, their share to the wide variety of media, incubation times, and incubation temperatures used to enumerate, and hence, to tacitly define psychrophiles. A table showing these incubation conditions would serve no purpose other than to show the great variety employed and such a partial table has already been presented by Thomas (295) in his review. Clearly, however, one would expect to experience different counts and be dealing with a different flora if they used any two of the following four diverse incubation schemes reported in the dairy literature: 2-4°C. for five days (201); 5-7°C. for seven to ten days (285); 10°C. for 14 days (189); or 10°C. for 20 days (304). A survey made by Alford (3), however, of investigators working with psychrophiles, showed that most dairy bacteriologists used 5°C. for seven days and standard plate count agar medium, while 28 investigators of other food products reported using 11 different media and ten different incubation conditions.
Ingraham and Stokes (152) have defined psychrophiles as those bacteria that form easily visible colonies on solid media within 14 days at 0 °C. While the bacteria so obtained are certainly psychrophiles, the temperature is too low and the incubation time too long for this method of enumeration and definition to be greeted with enthusiasm by investigators working with refrigerated food and dairy products.

The method of choice for the enumeration of psychrophiles is that given by Standard Methods for the Examination of Dairy Products (285), by which the psychrophilic bacterial count is determined by those organisms capable of forming visible colonies on plates incubated at 5–7 °C for seven to ten days. This choice seems to be an excellent compromise between the more restrictive methods proposing lower temperatures or shorter times and the less selective methods proposing higher temperatures or longer times. Further, this method of enumeration is commensurate with the previously suggested definition. Thomas et al. (297a) has recently supported this incubation scheme by strongly recommending that plates be incubated for ten days at 7 ± 0.5 °C for the determination of psychrophiles. Serious consideration should be given to this recommendation, in the interest of establishing a universally usable standard procedure for the enumeration of psychrophiles.

Criteria which are independent of the growth temperature. Most investigators are, understandably, reluctant to define psychophilic bacteria, and avoid this problem by characterizing them. Hence, in addition to their ability to grow at low temperatures one finds statements to the effect that "it is generally agreed that psychrophiles do not survive pasteurization" or "they are predominantly gram-negative rods." These statements are true and it is equally true that psychrophiles are predominantly catalase-positive, asporogenous, resistant to penicillin, nonacid-forming, motile, resistant to the toxicity of basic dyes, etc. It is unlikely that a nonmotile organism growing at low temperature would be excluded as a psychrophile, but for some unknown reason it is not as unlikely that a thermoduric organism or a gram-positive organism be so excluded. One study carefully demonstrated the growth of several thermoduric bacteria at 5 °C and concluded that they were not psychrophilic because they were thermoduric (30). In one paper studying psychrophiles in raw milk only gram-negative rods were considered for further study (regardless of their growth temperature), which resulted in the interesting observation that one of the psychrophilic isolates did not grow at low temperature (191). In a subsequent paper from the same laboratory, using milk from reasonably the same source, organisms which grew at low temperature (regardless of their gram outcome) were investigated and species of the genus Micrococcus were found to be prominently present (8). This is not meant to imply that either thermoduric or gram-positive psychrophiles are of great importance, for they are not, but simply to point out that those features which in general characterize psychrophilic bacteria are not as yet acceptable in defining them.
ESTIMATION OF PSYCHROPHILES

Most of the methods of enumerating or estimating psychrophiles have been more appropriately discussed in the sections of this review on definition and keeping quality. There are several interesting aspects of determining psychrophilic populations, however, that require further elaboration.

The present recommended method for the enumeration of psychrophiles—incubation at 5-7°C for seven to ten days (285)—has the disadvantage in quality control work of requiring too long a time before results are obtained. Based on the reasonable assumptions that the psychrophilic flora in a sample is predominantly gram-negative, and that the measurement of the gram-negative flora would in turn give an indication of the level of psychrophilic contamination, several quality control tests have been devised using higher incubation temperatures for shorter times. These tests require the presence in the growth medium of an inhibitor for gram-positive organisms. Elliker (83) suggested the use of violet red bile agar for detecting the surface spoilage organisms in cottage cheese, and Collins (50) demonstrated that this medium could be successfully used to enumerate Pseudomonas viscosa, Pseudomonas fragi, and Alcaligenes metalcaligenes in 48 hr. at 25°C. Druce et al. (75) showed that colonies of Pseudomonas, Alcaligenes, Flavobacterium, and Achromobacter all grew on violet red bile agar in 20-24 hr. at 30°C, but the colonies were usually small and often white. Several species of Bacillus also grew under these conditions. Simonart and Lambert (277) suggested the use of penicillin in agar medium for the enumeration of gram-negative organisms and Broitman et al. (35) used the surfactant Naecoral NR SF to inhibit gram-positive organisms in their reduction test for keeping quality. These latter authors also tested sodium lauryl sulfate in this respect, with less success. Gyllenberg et al. (116) added crystal violet to ammonium lactate agar to increase the selectivity of this agar for pseudomonads.

Mesophilic bacteria surviving heat-treatment have been shown to require different conditions for the resumption of growth than those required for the growth of nonheated cells (162, 214, 216) and this is also true of psychrophilic bacteria. Heat-treated psychrophiles differ from nonheated controls in being more fastidious in their nutrient requirements for initiating growth, in having a more pronounced lag phase at low temperature, and in being more sensitive to the pH of the growth medium (130, 131, 134, 171, 173). Hence, these differences need to be taken into account in enumerating heat-treated psychrophiles. On the other hand, psychrophilic bacteria surviving treatment with chlorine (173) or iodophors (132) do not appear to require a change in the conditions necessary for the resumption of growth.

The influence of the diluent used in the counting of psychrophiles may seriously affect the results. The influence of the type of diluent on the survival of Pseudomonas putrefaciens in dilution blanks was thoroughly investigated by Wagenaar and Jezeski (315). They showed that there was a wide variance in the toxic effect of distilled water on the strains tested and that for this
organism gelatin-phosphate diluent was the most reliable of the many solutions tested.

Incubation of plates at 32° C. has been shown to be the most favorable temperature for obtaining maximum bacterial counts on freshly, 24 hr. or less, pasteurized milk (21). After refrigerated storage, however, presumably due to the growth of psychrophiles, incubation of plates at lower temperatures gave maximum bacterial counts. Nelson and Baker (217) investigated the influence of time and temperature of plate incubation on the bacterial counts of milk held under refrigeration and recommended that the incubation of plates at 21° C. for four days or at 25° C. for three days be used for the detection of high bacterial counts, due to growth during refrigeration. These same incubation times and temperatures were subsequently recommended by Van der Zant and Moore (309, 310) for obtaining maximum counts in frozen desserts made from refrigerated mixes and for counting isolated cultures of psychrophilic pseudomonads. These incubation schemes were not designed to estimate psychrophiles, but only to adequately include psychrophiles in viable bacterial counts of milk samples.

MICROORGANISMS INVOLVED

The bacterial genera most commonly found to contain psychrophilic species are Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, Escherichia, and Aerobacter. The general consensus is that Pseudomonas is the most commonly encountered and this is true not only in dairy products (6, 90, 154, 172, 259, 260, 261), but also in meat (17, 18, 40, 121, 164, 289), fish (9, 104, 177), poultry (19), and eggs (93). Earlier investigators, however, found Achromobacter to be predominant (9, 88, 105, 301, 319), but this discrepancy was probably due to the nomenclatural changes in the newer editions of Bergey's Manual of Determinative Bacteriology (34), as was found to be the case in the reclassification by Brown and Weidemann (40) of the psychrophiles isolated by Empey and Scott (87). Psychrophilic bacteria, however, are not limited to the six genera listed above. Given in Table 1 are genera which have been reported to contain psychrophilic species and additional references to a few of the investigators who have isolated and observed their growth at low temperature.

It is evident from Table 1 why psychrophilic bacteria are considered to be predominantly gram-negative, nonspore-forming rods. (It is fashionable, although redundant, to include the characteristic of being nonspore-forming in this description. There are no spore-forming gram-negative rods, with the possible exception of the gram-variable and rather rare bacterium Methanobacterium omelionskii.) Only the last nine genera listed are gram-positive—Arthrobacter is gram-variable—and, with the exception of Micrococcus, have only infrequently been observed in dairy products. Many investigators are reluctant to include Lactobacillus, Streptococcus, and Micrococcus as psychrophiles, despite the fact that they have been clearly shown to grow at low temperatures, and there is a temptation to create and use definitions which exclude these organisms. This approach may allow the dairy microbiologist to incautiously conclude that only
TABLE 1
Genera containing psychrophilic bacteria and references to the investigators who have isolated them and observed their growth at low temperatures

<table>
<thead>
<tr>
<th>Genera</th>
<th>Isolated from milk and dairy products</th>
<th>Isolated from other sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em></td>
<td>26, 50, 66, 85, 139, 140, 237, 286, 305</td>
<td>137, 150, 239, 276, 284</td>
</tr>
<tr>
<td><em>Achromobacter</em></td>
<td>26, 66, 85, 139, 154, 245, 259, 260, 261, 281</td>
<td>18, 22, 104, 129, 164, 177, 289</td>
</tr>
<tr>
<td><em>Alcaligenes</em></td>
<td>1, 2, 26, 66, 139, 154, 259, 260, 261, 286</td>
<td>18, 177</td>
</tr>
<tr>
<td><em>Flavobacterium</em></td>
<td>139, 154, 172, 259, 260, 261, 286, 329</td>
<td>18, 22, 104, 105, 129, 137, 145, 159, 177</td>
</tr>
<tr>
<td><em>Escherichia</em></td>
<td>26, 90, 125, 259, 260, 261, 286, 296, 302</td>
<td>120</td>
</tr>
<tr>
<td><em>Aerobacter</em></td>
<td>8, 26, 66, 90, 110, 125, 191, 228, 248, 262, 286, 296, 302</td>
<td>129</td>
</tr>
<tr>
<td><em>Acetomonas</em></td>
<td></td>
<td>18, 40, 208</td>
</tr>
<tr>
<td><em>Serratia</em></td>
<td>139, 297</td>
<td>22</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>45, 66, 135, 164, 187, 248</td>
<td>55, 120</td>
</tr>
<tr>
<td><em>Chromobacterium</em></td>
<td>64, 202, 265</td>
<td>87, 175, 281, 299</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td></td>
<td>92, 104</td>
</tr>
<tr>
<td><em>Arthrobacter</em></td>
<td>8, 302</td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>26, 90, 248</td>
<td>5, 99, 121, 164, 218, 287</td>
</tr>
<tr>
<td><em>Microbacterium</em></td>
<td></td>
<td>18, 22, 108, 324</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>26, 90, 187</td>
<td>18, 99, 270, 299</td>
</tr>
<tr>
<td><em>Micrococcus</em></td>
<td>8, 25, 64, 135, 187, 286, 298, 302, 304</td>
<td>18, 22, 36, 38, 87, 88, 104, 105, 120, 129, 177, 199, 299</td>
</tr>
<tr>
<td><em>Corynebacterium</em></td>
<td></td>
<td>25, 104, 177</td>
</tr>
<tr>
<td><em>Bacillus</em></td>
<td>2</td>
<td>299</td>
</tr>
<tr>
<td><em>Sarcina</em></td>
<td>135</td>
<td></td>
</tr>
<tr>
<td><em>Actinomyces</em></td>
<td></td>
<td>119</td>
</tr>
<tr>
<td><em>Brevisibacterium</em></td>
<td></td>
<td>227</td>
</tr>
</tbody>
</table>

gram-negative rods are psychrophilic, the disadvantages of which have already been discussed.

References to the genera *Citrobacter* and *Klebsiella*, although clearly defined (250) and convincingly supported (58), were included in Table 1 under *Escherichia* and *Aerobacter*, respectively. Other workers have observed the low-temperature growth of coliforms, but did not distinguish between the separate genera (61, 251, 272). Schnütze and Olson (261), in a study of the distribution of psychrophiles in commercial dairy products, observed that a surprising number of their isolates (10.8%) were coliforms. A more thorough examination of these isolates showed that the majority of these coliforms most closely resembled, but did not correspond in detail with, *Aerobacter cloacae* (262).

The genus *Arthrobacter* is a recent addition to the psychrophilic genera and is included for the first time in the 1957 edition of *Bergey’s Manual of Determinative Bacteriology* (34). *Arthrobacter* was reported by Andrey and Frazier (8) to be the predominant psychrophilic organisms in bulk tank milk from
barn-fed cows and Thomas et al. (299) reported isolating an organism from raw milk which resembled *Arthrobacter*.

While the predominant psychrophilic genus is *Pseudomonas*, the predominant species of this genus are *P. putrefaciens*, *P. fragi*, and *P. fluorescens*. One or all of these species are invariably chosen by investigators as typical in their studies of the various characteristics of psychrophiles, such as lipolysis, proteolysis, heat resistance, spoilage, effect of sanitizing agents, etc. Briefly, *P. putrefaciens* is primarily associated with surface taint in butter and proteolytic defects, *P. fragi* with rancidity and fruity odor, and *P. fluorescens* with rancidity and proteolytic defects. These organisms will be discussed throughout this review, but their importance merits separate mention.

**Sources**

That the natural sources of the prominent psychrophilic bacteria are water (27, 50, 56, 70, 127, 135, 145, 161, 255) and soil (27, 91, 97, 135, 145, 150, 178, 181, 182, 205, 207, 255, 327) has been abundantly demonstrated. In older literature these organisms were known as water bacteria and they have been repeatedly and consistently found in every conceivable water source; farm water supplies (176, 203, 204, 292, 296, 300, 302, 327), dairy plant water supplies (28, 57, 126, 143, 154, 176, 179, 181, 182, 200, 202, 205, 230, 233, 240, 274, 275, 283, 292, 327), municipal water supplies (57, 264, 317), ditches, lakes, and streams (97, 181, 327), salt water (22, 91, 97, 333), and fresh water ice (129). When such water comes in direct contact with a dairy product—washing of butter and cottage cheese or residual rinse water on equipment—it may cause a serious psychrophilic contamination of the dairy product and result in its rapid spoilage.

While the initial source of psychrophiles in the dairy industry is the water supply, a secondary, and perhaps more frequently encountered source of contamination, is improperly cleaned equipment and utensils. Usually, equipment and utensils have been implicated in a general way (89, 127, 147, 181, 182, 240, 253, 296, 300, 303, 305), but occasionally specific equipment has been cited as the source of psychrophilic contamination: pipelines (126, 147, 179, 283), milk cans (204), butter churns (181, 283), holding tanks (159, 176, 179), cream pumps (283), valves (192, 147), and milking machines (111, 296).

Psychrophilic bacteria in raw milk were recently studied by Thomas et al. (299) and thoroughly reviewed by Thomas (296). Less clear are the sources of psychrophiles in pasteurized milk, except in general terms, despite the recent and lucid review of the subject by Thomas et al. (297). Psychrophilic bacteria in pasteurized milk are considered to be post-pasteurization contaminants and in freshly pasteurized milk psychrophiles are usually not demonstrable. After low-temperature storage, however, surveys have shown psychrophiles to be present in most samples (63, 156) and frequently all samples tested (95, 172, 252, 253, 318, 323). This low magnitude but high incidence of psychrophiles in pasteurized milk is one aspect of sources that may be worthy of further clarification and study.
KEEPING QUALITY

Both the growth rate and biochemical activity of bacteria are reduced by a reduction in temperature. The keeping quality of milk and dairy products is, therefore, logically increased by a reduction in storage temperature and this logic has been uniformly supported by the literature (13, 31, 32, 50, 60, 62, 63, 128, 174, 189, 201, 215, 229). At low-temperature storage, however, psychrophiles may increase markedly (16, 20, 41, 253), replace the normal flora (2, 85, 241, 252, 323), and ultimately be the cause of spoilage (26, 28, 41, 42, 50, 155, 193, 224, 270). These observations have led to the demonstration and conclusion that keeping quality and psychrophilic contamination are closely related (16, 27, 29, 31, 35, 226).

It is now, perhaps, understandable why the standard plate count has been shown to be of little value for predicting the keeping quality (15, 29, 41, 42, 252), but it is surprising to find that the psychrophilic count is also of little value in this respect (25, 29, 35, 42, 226, 252). Presumably, this is because a low level of contamination with a biochemically active, rapidly growing psychrophile may result in a poorer keeping quality than that resulting from a relatively large number of biochemically inert psychrophiles. Olson et al. (225) emphasized that the type of contaminating organism is more important than the number.

In general chemical tests, including protein stability, tyrosine and tryptophane production, pH and acidity have proved unsuccessful for use in predicting the keeping quality, although Nutting (219) reported reasonable success in correlating the lactate ion concentration to the keeping quality of raw milk, but this could not be extended to pasteurized milk. Neither methylene blue reduction (10, 102, 142, 169) nor resazurin reduction (13, 14, 47, 102, 169) as normally performed have provided good indexes for keeping quality, but these tests are more closely correlated to the standard plate count than to the psychrophilic count.

In the development of keeping quality tests most investigators have recognized the importance of psychrophilic bacteria. In their test for keeping quality, Hiscox et al. (141) ran a methylene blue reduction test at 15.5°C. to favor the detection of psychrophiles. They found that the reduction of the dye coincided with the appearance of a flavor defect. To predict the keeping quality of pasteurized milk, Broitman et al. (35) measured triphenyl tetrazolium chloride reduction in milk to which Naaccosal NR SF was added to inhibit gram-positive bacteria. A statistical treatment of their data, however, would have shown that the 95% confidence limits for predicting keeping quality varied from ± 2 days to ± 6 days, depending on the reduction time. Day and Doan (67) measured neutetrazolium reduction in evacuated tubes and concluded that a positive reduction test occurred an average of 3.75 days prior to spoilage of the sample. Statistical treatment of their data would have shown a 95% confidence limit for predicting the keeping quality of ± 3 days. Gyllenberg et al. (116) developed a keeping quality test based on the ability of ammonium lactate-crystal violet agar to detect pseudomonads in milk. Virtanen (313) suggested that the meas-
urement of catalase in butter might be used as a keeping quality test, since the psychrophilic bacteria which spoil butter produce large amounts of this enzyme. While not actually predicting keeping quality, the Moseley test described by Johns (156) is valuable for quality control and gives an indication of psychrophilic growth by comparing the increase in standard plate count at 32°C before and after storage of the sample at 45°F for five days. Johns (157, 158) also indicated that psychrophiles were largely responsible for the marked increase in counts observed in many samples preliminarily incubated for 18 hr. at 55°F. As yet, there is no test available for accurately predicting keeping quality. Such a test, however, must take into account the level of psychrophilic contamination, its rate of increase with storage, and its activity in producing deleterious effects. This is a difficult set of criteria to meet, but is certainly worth the effort of an imaginative investigator.

During the last decade a great trend toward the bulk tank storage of raw milk has occurred. This trend has instigated a number of studies on the bacteriological quality of bulk tank raw milk and on the feasibility of the accompanying every-other-day pickup schedules. These studies have shown that the bacterial counts in raw milk do increase, that this increase is due to the growth of psychrophiles, but that this increase is usually slight (12, 189, 190, 191, 241, 307). It has been strongly cautioned, however, that to maintain raw milk at high quality the temperature must be kept below 40°F. and rigorous sanitation must be employed (12, 20, 192, 234, 296).

Inglis (149) concluded that the quality of milk increased by conversion to bulk tanks, but Atherton (11, 12, 15) showed that while there was some improvement on the whole, there was no overwhelming evidence of improved quality, since many farmers delivered milk of lower quality after conversion to bulk tank. VanDemark and March (307) demonstrated that the blend temperatures did not affect the growth rate of psychrophiles, provided there was rapid cooling and provided the temperatures were not as high as 70°F. In general, every-other-day pickup did not appear to affect the storage quality of raw milk (13, 296, 307), but Smillie et al. (280) in Scotland experienced significantly higher counts in such milk. The suggestion has been made that psychrophilic counts be given consideration in the establishment of standards for bulk tank raw milk (234, 280), but Atherton (12) concluded that a standard plate count at 32°C gave a valid estimate of the bacteriological quality.

The ultimate failure of keeping quality due to spoilage by psychrophilic bacteria is manifested in a large and varied number of defects. A few of the criticisms, odors, and flavors that may develop are fruity (50), rancid (70), putrid (49, 70, 78, 84, 240, 283, 325-329), ropy (100, 101, 293, 298), discoloration (202, 321), cheesy (84, 136, 230), soapy (213), bitter (7, 196), sweaty feet odor (77), skunk-like (48), potato odor (33, 223), fishy taint (197), and aroma loss (81, 84, 232). A good discussion of these defects and the specific psychrophilic bacteria producing them can be found in some textbooks (98, 122) and the types of butter spoilage have been thoroughly discussed and reviewed by Demeter (69).
Many of the defects, perhaps commensurate with their importance to the dairy industry, have received very little attention. While the psychrophilic bacteria responsible for these defects have been identified, little information is available on the products responsible, the substrates from which they are formed, the conditions of formation, and the metabolic pathways involved. In 1902, Eichholz (80) first observed that fruity odor (then called strawberry odor) was caused by *P. fragi*, but it was not until 1958 that Pereira and Morgan (237) identified ethyl isovalerate as the responsible compound and showed its mode of formation. Similar studies are still to be done on most of the odors and flavors caused by psychrophiles.

**PUBLIC HEALTH SIGNIFICANCE**

Psychrophilic bacteria are not of public health significance insofar as they are not known to produce disease. Only two investigators, Fischer (92) in 1893 and Conradi and Vogt (55) in 1901, have claimed to have observed psychrophilic pathogens, but these reports have not been confirmed or extended in the roughly 60 yr. since their publication. That the growth of psychrophilic bacteria might produce harmful products has been suggested (54, 246, 303), but no supporting data were given. Hence, it would seem reasonable to assume that psychrophilic bacteria are not a hazard to public health. Neither are psychrophiles an index of fecal contamination, for they were not found to be present in feces (50).

In 1957, Ohye and Scott (221) observed that *Clostridium botulinum* Type E was capable of germination and growth at low temperatures. This was confirmed by Schmidt et al. (257), who observed germination, growth, and toxin production in beef stew at 43° F. in 24 days. Hence, while this organism is an obligate anaerobe and probably grows too slowly to be considered a psychophile, it remains a very unlikely, but potentially dangerous, contaminant in milk pasteurized at ultra-high temperatures or in improperly sterilized sterile milk, even though these products might be held under refrigeration. This organism has not been studied in milk.

**METHODS OF CONTROL**

**Pasteurization.** The preponderance of evidence shows that psychrophiles do not survive either laboratory pasteurization (26, 226, 252, 253, 266-268, 297, 300, 301, 317, 318) or commercial pasteurization (226, 252, 253). In fact, many of the prominent psychrophiles have been demonstrated to be extremely sensitive to heat, viz. *P. fragi* (46, 148, 231, 233), *P. viscosa* (46, 161, 231-233), *P. mephitica* (161), *P. fluorescens* (46, 173), and *P. putrefaciens* (182, 326). Davis and Babel (66) studied the heat resistance of bacteria producing slime on cottage cheese and the most heat-resistant isolate did not survive 2.5 min. at 145° F., and some isolates did not survive 0.25 min. Pasteurization has also been shown to be effective in the control of psychrophilic bacteria in plant water supplies (264). Hence, it is logical to conclude that the presence of psychrophiles in pasteurized dairy products and milk is a result of post-pasteurization
contamination and it has been proposed (318) that psychrophiles might be used as an index for post-pasteurization contamination.

The above evidence and conclusions, however, do not preclude the existence of thermoduric psychrophiles. Generally, only a few isolates in a given study have been shown to be thermoduric; four of the 722 isolates of Erdman and Thornton (89), six of the 41 isolates of Jezeski and Macy (154), and four of the 188 isolates of Stark and Scheib (286) survived pasteurization. Atherton et al. (15, 16) showed that their thermoduric isolates grew at 50° F., but not at 45° F. This supported the contention that the inclusion as psychrophiles of those bacteria growing at or slightly below 50° F. was responsible for the demonstrated thermodurics (64, 225). For example, 50° F. would allow the inclusion of the viridans and enterococci streptococci, the latter of which are thermoduric (271). The possibility of thermoduric psychrophiles, however, is not eliminated by a lower temperature, since growth of thermodurics has been experienced by several investigators at and below 45° F. (2, 30, 163, 201). Abd-el-Malek and Gibson (1) described and named Alcaligenes to lerans which was psychrophilic and capable of surviving pasteurization. Gyllenberg et al. (114) confirmed both the psychrophilic—growth at 5° C.—and the thermoduric nature of this microorganism, which has probably been overlooked in the literature, due to its inability to grow with glucose as the sole source of carbon. Contrary to being of minor interest, A. tolerans was found by these authors to be the most prominent species in pasteurized milk. Clearly, the further investigation of thermoduric psychrophiles is desirable, although they will probably be of no more than remote practical significance to the market milk industry.

Refrigeration. While there is no temperature above freezing which will completely curtail the growth of psychrophilic bacteria, these organisms grow more slowly as the temperature is reduced. At lower temperatures the decrease in growth rate is quite dramatic, particularly as the organisms approach their minimum growth temperatures. In detailed studies of the growth rates of psychrophiles, it has been shown that the temperature coefficient for growth increased with decreasing temperature in the low temperature range (110, 150, 263). Greene and Jezeski (110) reported that a decrease in temperature from 5 to 0° C. increased the keeping time of milk approximately the same number of days as did cooling from 30 to 5° C. Psychrophilic bacteria can be controlled, although not eliminated, by low-temperature storage and a few degrees difference in temperature in this range may greatly affect the growth rate.

Halogens. Hypochlorite is generally agreed to be the most effective bactericide available for the control of psychrophiles. There is considerable variation in the recommended concentrations of hypochlorite and time of action necessary to produce a satisfactory kill, but these might reasonably fall in the rather narrow range of 5 p.p.m. of hypochlorite acting for 5 sec. to 50 p.p.m. acting for 1 min. (26, 51, 66, 167, 183, 231). From 2 to 10 p.p.m. of available chlorine have been recommended for the adequate control of psychrophiles in water supplies (143, 225, 255, 283), but probably 5 p.p.m. available chlorine would be adequate except under exceptional circumstances. It has long been known
that the germicidal activity of hypochlorites is markedly reduced by an increase in pH, a reduction in temperature, or the presence of organic matter and these factors must be considered in the determination of the efficacy of hypochlorites. A concentration of chloramine-T of at least 250 p.p.m. in a solution of not greater than pH 7.0 would appear to be about as rapid in germicidal action as 50 p.p.m. of the slower (alkaline) hypochlorites (320). Commercial chloramine-T products, however, are not generally adjusted to such a low pH. Iodine compounds in the few studies made have been shown to be bactericidal to psychrophiles at approximately equivalent concentrations and times of action to those of the hypochlorites (26, 132, 316).

*Quaternary ammonium compounds.* The application of quaternary ammonium compounds to dairy sanitation was thoroughly reviewed by Elliker (82) in 1950. He expressed the then-current opinion that these compounds were more germicidally active in an alkaline pH range and were reasonably ineffective against gram-negative bacteria. This opinion suggested that quaternary ammonium compounds would not be of great value in the control of a psychrophilic flora. It has since been demonstrated that gram-negative bacteria may be effectively killed by these compounds with the proper adjustment of pH, usually to a slightly acid solution (256, 282). In a more recent study (231) of the activity of quaternary ammonium compounds against psychrophilic bacteria, it was shown that 100 p.p.m. for 5 min. at pH 7.2 was required to destroy all of the cultures studied, that the germicidal activity was increased by the addition of pyrophosphate, and that quaternary ammonium compounds were still not as effective as hypochlorites.

*Sodium chloride.* Salt at the normal concentrations encountered in dairy products is not a strong deterrent to the growth of psychrophiles. All of the psychrophilic isolates of Bonner and Harmon (26) were capable of growth in trypticase soy broth containing 5% sodium chloride. Of the 15 strains of *P. putrefaciens* examined by Long and Hammer (182), all grew in litmus milk containing 4% salt, six grew at 6% salt, one grew at 8% salt, and none grew at 10% salt. A strain of this same bacterium studied by Wolochow *et al.* (326) grew at 4%, but not at 6% salt in skim milk. The conclusion that salt offers little protection against psychrophilic spoilage bacteria in cottage cheese is certainly commensurate with the results of the above studies and the practical experience of cottage cheese spoilage. Salting tends to delay spoilage in butter (70, 182), but it can not be depended upon to prevent spoilage (49, 125). The effect of the salt concentration in butter on the black discoloration produced by *Pseudomonas nigrifaciens* is particularly fascinating, since this discoloration does not occur in unsalted butter or in butter salted to greater than 1% (321).

*Sorbic acid.* Sorbic acid has been shown to selectively inhibit catalase-positive microorganisms (86, 331), which would include most psychrophiles. For any reasonable lethal activity, however, the pH must be less than 5.0 (26, 117) and Habib (117) found a striking absence of lethal effect at 20°C and below. Sorbic acid concentrations of 0.10% or greater impart an objectionable flavor to cottage cheese (26, 103) and the formation of resistant mutants has been
observed (332). Therefore, the conflicting results concerning the efficacy of sorbic acid in controlling psychrophiles in cottage cheese is not surprising. Geminder (103) has reported extended keeping quality of cottage cheese treated with sorbic acid, while Bonner and Harmon (26) demonstrated that even impractically high concentrations of sorbic acid were not lethal to their test psychrophiles. These latter authors realized, however, that lack of cidal activity may not reflect the ability of sorbic acid to inhibit growth, which is all that is necessary to improve keeping quality. Further information is necessary to seriously evaluate the utility of sorbic acid to the dairy industry.

Antibiotics. The effect of nine different antibiotics on a number of strains of *P. fluorescens*, *P. fragi*, and *P. putrefaciens* was examined by Heather and Van der Zant (133). Their results showed that the majority of the test cultures were more sensitive to tetracycline, oxytetracycline, and chlorotetracycline than to the other antibiotics and that penicillin had no inhibitory effect on any of the test cultures. Other investigators have not tested the effect of antibiotics on psychrophiles directly, but have shown an improvement in the keeping quality of raw and pasteurized milk by the addition of antibiotics (94, 108, 269, 273). Penicillin has been only slightly effective in the improvement of keeping quality, but the tetracyclines have had a definite retarding affect on bacterial spoilage. Olson and Ball (222), however, experienced variable results in controlling bacterial spoilage of cottage cheese with 6 p.p.m. aureomycin (chlorotetracycline) and Olson et al. (226) found that the keeping quality of pasteurized milk was not extended by the addition of 0.2 μg. per milliliter of aureomycin. Additional studies are needed to establish the minimum concentrations of antibiotics necessary for consistent extension of keeping quality and to evaluate the possible utility of antibiotics in the dairy industry.

pH. Microorganisms which produce an alkaline reaction in litmus milk and are strongly proteolytic generally are reasonably sensitive to increases in acidity. Since the psychrophiles are largely this type of microorganism, it is reasonable to expect that their growth and activity should be sensitive to a reduction in pH, and indeed, this is the case. The growth rates of all the psychrophilic isolates of Bonner and Harmon (26) were materially reduced below pH 5.4. *P. putrefaciens* was commonly destroyed by pH 5.3 or below (182), *P. fragi* and *A. metalcaligenes* were effectively retarded at pH 5.0 (231, 233), *P. viscosa* developed slowly at pH 4.8 (231), and slime on cottage cheese was observed at pH 4.7 (66) and at pH 4.6 (50). Hence, it appears that the growth of psychrophiles is progressively retarded from about pH 5.4 to an estimated pH of 4.5.

Carbon dioxide. It has long been recognized that carbonation is an effective means of controlling the growth of microorganisms in carbonated beverages (72, 166, 322), extending the storage life of chilled beef (88), and delaying the slime formation by psychrophiles on cut-up chicken (220). An early study by Prucha et al. (243, 244) of the effect of carbonation on the keeping quality of dairy products concluded that carbonation did not prevent, nor in most cases did it retard to any great extent, the deterioration of dairy products. Hence, use of carbon dioxide for the preservation of dairy products has been limited
to oxygen replacement, a role which it shares with nitrogen. Revived interest in the possible use of carbon dioxide, however, may be stimulated by the recent report of Tsantilis and Kosikowski (306), who showed that cottage cheese under a carbon dioxide atmosphere consistently retained a fresh flavor over extended storage periods at refrigeration temperatures and that carbon dioxide was superior to nitrogen in this respect.

**Humidity.** A knowledge of the effect of humidity on the survival of psychrophiles is important in evaluating the possible effect of atmospheric conditions on and the magnitude of atmospheric transport of these organisms. Such a study was made by Brown (36-38), in which each of the three psychrophiles tested showed a minimal death rate in the region of 65 to 70% relative humidity. Above this region there was a positive, and below a negative, correlation between death rate and humidity. All of the bacteria tested died under airborne conditions at the test temperatures of 0 to 10 °C. This study was carefully done and conclusive, but needs to be confirmed and extended.

**Special creaming mixture.** Mather and Babel (194), in the development of a special creaming mixture for the control of the diacetyl content of cottage cheese, noticed a delay in flavor defects when their creaming mixture was employed. In a subsequent study (195), these authors showed that the special creaming mixture consisting of 1.5 parts by weight of 20% cream and one part by weight of an acidified culture of *Streptococcus citrovorum* (i.e., *Leuconostoc citrovorum*) prevented slime formation on cottage cheese inoculated with *P. fragi* and *P. putrefaciens*. In addition, coliform organisms were inhibited and loss of the diacetyl content was prevented. This inhibitory effect was shown not to be due to the volatile acids present in or the slightly lower pH of the creaming mixture. As yet, the inhibitory factors or compounds present in this special creaming mixture have not been determined.

**BIOCHEMICAL ACTIVITY**

It is within the scope of this review to briefly inspect the biochemical activities of some of the psychrophilic bacteria, but only insofar as the subject is germane to dairy considerations and only at the level of rather gross observations. Even at this level—or perhaps because it is at this level—psychrophilic bacteria appear to present conflicting characteristics. They are considered to be reasonably inactive in the reduction of dyes (109, 142), but rapidly cause the destruction of diacetyl (81, 84, 316), presumably by reduction to acetyl-methylcarbinol and possibly even to 2,3-butylene glycol (232). Some psychrophiles are active phosphatase producers, notably *P. putrefaciens* (124), but do not appear to increase the phosphatase in milk samples (15). A number of psychrophiles are oxidase-producing, but are not responsible for oxidized flavors in milk. In fact, when large inoculums of these organisms were added to milk the development of oxidized flavor was markedly or completely inhibited (53). Psychrophiles are generally characterized as being inert toward carbohydrates, yet they are in a large part responsible for the elucidation of the pathways of carbohydrate degradation (113, 330).
The fact that psychrophiles usually produce little or no reaction in litmus milk is probably responsible for their being considered biochemically inert. When psychrophiles do produce a reaction in litmus milk they usually cause an increase in alkalinity (146, 164, 253, 299a). Jones and Thomas (159) observed a psychrophilic *Flavobacterium* that produced no visible change in sterile milk in seven days at 22°C. Further, psychrophiles sometimes are slow to produce spoilage defects (14, 235, 299, 318). Broitman *et al.* (35) observed a sample of milk with a psychrophilic count of 190,000,000 per milliliter which kept for six days at 4.5°C. As though to make up for their relative inactivity in litmus milk, psychrophilic bacteria are usually strongly lipolytic, proteolytic, or both. Roughly 90% of the psychrophiles isolated by Schultze (259) were either lipolytic or proteolytic and 66% were both.

**Lipolytic activity.** Despite the obvious and long recognized importance of the lipolytic activity of bacteria in dairy products, other than the demonstration of the presence of lipolytic bacteria (32, 68, 85, 123, 154, 286) or the observation that a particular bacterium produced rancidity (6, 7, 74, 184, 202, 239, 286), very little work has been directed toward the study of lipolysis by psychrophilic bacteria. A notable exception is the detailed study of the lipase of *P. fragi* by Nashif and Nelson (209-211). These authors demonstrated that this lipase was preferentially produced at low temperatures and none was produced by these organisms when growing at 30°C or higher. This lipase, once produced, had a temperature optimum of 40°C, had a pH optimum of 7.0 to 7.2, exhibited a specificity for tricaprylin, was relatively inactive towards tributyrin and triolein, and was heat stable, requiring heating to 99°C for 20 min. to completely inactivate. Of immense practical significance, butter containing residual lipase from *P. fragi* undergoes considerable fat degradation during storage even at −10°C, but especially at 5°C and above. This low-temperature lipolysis has also been noted by other investigators (32, 236), 283).

The ability to produce lipase varies considerably between different genera (212), between different species of the same genus (106, 212, 312), and even between strains of the same species (106, 180, 210). The optimum temperature of lipase production also varies markedly with the producing organism (212). The lipases produced exhibit different specificities toward triglycerides (52, 85, 180, 186) and have different pH optima for activity (4, 185, 186, 209). Cutchins *et al.* (59) suggested that lipase might be an adaptive enzyme, but most workers have not agreed with this contention (4, 186, 210). There is general disagreement when lipases from different sources are compared with respect to heat stability, substrate specificity, optimum temperature for production and activity, adaptive characteristics, and inactivating agents. The existing knowledge of microbial lipases, however, is only fragmentary and further study may resolve many of these disagreements. One point of agreement is that lipolytic psychrophiles are frequently encountered (139, 268, 289, 292, 302, 309, 311) and that many, if not most, of the fat-splitting bacteria are psychrophilic (278).

**Proteolytic activity.** Psychrophiles which possess proteolytic activity have been frequently encountered (6, 38, 146, 154, 238, 268, 292, 309) and, indeed,
the majority of isolates of psychrophilic bacteria have been observed to be proteolytic (259, 286, 302). It was observed very early that a common failure of milk held at low temperatures was due to proteolysis (236). Holding of milk at low temperatures is selective against those bacteria which normally produce acid from lactose, and selective for the psychrophilic bacteria which preferentially attack fats and proteins. Pure culture studies of proteolytic bacteria, however, have shown that the activity of proteolytic enzymes increases with increasing temperature. Van der Zant (308), in an investigation of the extracellular proteolytic enzymes of *P. putrefaciens*, showed that this enzyme system had an optimum temperature of 40°C in attacking casein and 45°C in attacking albumin. At 5°C, there was only slight activity toward casein and no activity toward albumin. In a further study of the endocellular proteolytic enzyme system of this same organism (44), the optimum temperature for activity was again shown to be high. Peterson and Gunderson (239) have studied the proteolytic enzymes from *P. fluorescens*. They reported that the liberation of the extracellular proteolytic enzymes was greatest at 0°C and decreased with increasing temperature. The activity of the endocellular proteolytic enzymes from these bacteria grown at low temperature was greater per milligram of protein than when the cells were grown at higher temperature. After elaboration, both the extracellular and endocellular systems increased in activity with an increase in temperature. Investigation of the characteristics, production, and activity of proteolytic enzymes systems from psychrophilic bacteria has only been started and present knowledge is very sparse.

THE PHENOMENON OF LOW-TEMPERATURE GROWTH

The psychrophilic bacteria are obviously distinguished from other bacteria by their ability to grow at low temperatures. The fundamental problem is why these organisms grow at low temperatures or what special properties they possess which enable them to grow at low temperatures. From a different point of view, the question may be asked as to why nonpsychrophilic bacteria fail to grow at low temperatures or what special properties they possess which deter their growth under these conditions. It is apparent that the solution to the problem of low-temperature growth is to be found only by comparing the responses of psychrophiles and nonpsychrophiles to variations in temperature, and revealing the factors responsible for the differences in these responses.

As the temperature is lowered there are some cellular activities which have been shown to increase or to qualitatively appear. Jordan and Jacobs (160) concluded that *Escherichia coli* used nutrients more efficiently at 15°C than at 35°C for the formation of cellular material. Thiel (290, 291) showed an increase in the production of lactic acid, acetic acid, and alcohol by streptococci and an increase in the ratio of acetic acid formed to sugar utilized by lactobacilli when the temperature was reduced. Dorn and Rahn (73) showed that the efficiency of fermentation as an energy source for growth is greatest at low temperatures. Concerning pigment production, it has been observed that the optimum temperature for pigment production is lower than that for growth.
(65, 140, 300, 321), that a *Flavobacterium* produced a more brilliant yellow pigment at 5 than at 20°C (138), that the pigment formation of *P. nigrifaciens* was greater at 4 than at 15°C and absent at 25°C (321), and that two *Pseudomonas* species produced a blue-black pigment at 3°C, but not at a temperature of 15°C or higher (140). The preferential liberation of proteolytic and lipolytic enzymes at low temperatures by some bacteria has already been discussed. While these observations show some of the possible gains in activity with the lowering of temperature and are of interest, they do not bring one much closer to the understanding of low-temperature growth.

The groundwork for understanding low-temperature growth has been laid by Brown (39), Ingraham (150), and Ingraham and Bailey (151), who all compared the growth and activities of a psychrophile with a nonpsychrophile. Brown (39) concluded from his study that the systems involved in the oxidation of glucose and gluconic acid by a psychrophilic pseudomonal and *P. aeruginosa* did not differ greatly in those properties which determine their temperature relations. The oxygen uptake, with glucose by the psychrophile, however, had a lower temperature characteristic (slope of an Arrhenius plot) than that of *P. aeruginosa*, which showed that the psychrophile was less sensitive to changes in temperature. The growth rate of the psychrophile studied by Ingraham (150) was shown to have a strikingly lower temperature characteristic than that of the mesophile. In a subsequent study, Ingraham and Bailey (151) observed that the temperature coefficients (Q10) of the tested psychrophiles for glucose oxidation, acetate oxidation, and formate oxidation were lower than those for the tested mesophiles. When glucose oxidation by cell-free extracts of these organisms was measured, however, the temperature coefficient of the mesophile was lowered and corresponded with that of the psychrophile. Also, the temperature characteristics of the activities of three different dehydrogenase systems were the same for cell-free preparations from either psychrophiles or mesophiles. Summarily, the rate of growth or rate of oxidative metabolism of psychrophiles are less sensitive to changes in temperature than those of nonpsychrophiles, but this difference in the temperature response of the rate of oxidative metabolism disappears when cell-free extracts are tested. This suggests that the difference in the temperature response of psychrophiles and nonpsychrophiles is associated with the integrity of cellular organization. The work so far has shown only the plausibility of this suggestion and not its reality. The fundamental problem of why psychrophiles grow at low temperature or, conversely, why nonpsychrophiles do not grow at low temperature, is still to be solved.

REFERENCES


(23) BEERY, J. A., AND MAGOON, C. A. Growth of Microorganisms at and Below 0° C. Phytopathology, 24: 780. 1934.


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(81) ELLIKER, P. R. Effect of Various Bacteria on Diacetyl Content and Flavor of Butter. J. Dairy Sci., 29: 93. 1945.


(142) Horbs, B. C. The Part Played by Bacteria in the Reduction of Methylene Blue in Milk. J. Dairy Research, 10: 35. 1939.


(165) Kiser, J. S. Effects of Temperatures Approximating 0 ° C. upon Growth and Biochemical Activities of Bacteria Isolated from Mackerel. Food Research, 9: 257. 1944.


(204) Morris, C. S. Cryophilic Bacteria as a Cause of Milk Samples Failing in Methylene Blue Test. Dairy Ind., 7: 63. 1942.


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