CONTINUITY OF PSYCHROPHILIC AND MESOPHILIC GROWTH CHARACTERISTICS IN THE GENUS *ARTHROBACTER*

NORMAN G. ROTH AND ROBERT B. WHEATON

Research Laboratories, Whirlpool Corporation, St. Joseph, Michigan

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ABSTRACT

ROTH, NORMAN G. (Whirlpool Corp., St. Joseph, Mich.) AND ROBERT B. WHEATON. Continuity of psychrophilic and mesophilic growth characteristics in the genus *Arthrobacter*. J. Bacteriol. **83**:551-555. 1962—The effect of temperature on growth of seven members of the genus *Arthrobacter* was determined at 0, 7, 20, 30, and 37 C. In general, no sharp cutoff point was observed between growth-temperature requirements of psychrophilic and mesophilic bacteria. There appeared to be a continuous gradation among members of this genus in ability to initiate and maintain growth at 0 C. The total number of generations produced was not greatly affected by incubation temperature, provided that growth occurred. However, the time required to attain maximal growth was greatest at psychrophilic temperatures.

Temperatures at which bacterial growth can occur cover a broad range extending from near the boiling point of water to below its freezing point. This range is divided into three rather artibrarily defined groups: thermophiles, mesophiles, and psychrophiles. Thermophiles appear to have temperature optima 10 to 25 degrees centigrade above those of mesophiles (Ingraham, 1958). This spread serves to distinguish these two groups. A similar degree of distinction does not appear to exist between the mesophile and psychrophile groups. Considerable disagreement has existed among the several published definitions of psychrophiles. They have been defined by Lamanna and Mallette (1953) as organisms that grow best below 20 C, and by Smith and Conant (1960) as organisms that grow in a temperature range between −5 and 30 C, with an optimum between 10 and 20 C. Mesophiles are defined by the latter authors as organisms that grow between 10 and 45 C, with an optimum between 20 and 40 C. Sulzbacher (1950) isolated strains of *Pseudomonas* capable of growing at −6 C, but showing optimal growth above 20 C. These organisms fit neither group by these definitions. Ingraham and Stokes (1959) attempted to correct this dilemma by their definition of psychrophiles as "bacteria which grow appreciably and often abundantly at 0 C within 2 weeks." This is probably the most useful definition to date. However, all of these definitions tend to indicate that mesophiles and psychrophiles occupy definite clear-cut groups, with perhaps some overlap between genera or families.

This paper reports the study of several cultures of the genus *Arthrobacter* that appear to form a continuous series between the expected characteristics of psychrophiles and mesophiles.

MATERIALS AND METHODS

Organisms. In a screening program for psychrophilic bacteria, a number of isolates were obtained from eviscerated fresh-water fish which had been stored at 5 ± 2 C for 7 days. Samples of these fish were macerated in sterile water in a Waring blender, streaked on Difco tryptone glucose extract (TGE) agar and incubated at 5 ± 2 C for 7 days. Pure cultures were obtained by isolation of single colonies and restreaking on TGE agar. Of the many isolates recovered, several produced bright lemon-yellow, circular, entire, glistening, opaque colonies on TGE agar. Microscopic examination disclosed pleomorphic, gram-variable, nonspore-forming rods that fragmented and became coccoid with age. These characteristics most closely fit the morphology of the genus *Arthrobacter* as described by Conn and Dimmick (1947). Extensive biochemical and serological examinations confirmed their identity as members of the genus *Arthrobacter*. However, they did not correspond ideally to any of the several species described in Bergey's Manual (Breed, Murray, and Smith, 1957). Their biochemical reactions appeared to occupy a position intermediate between those of *A. aurescens* and

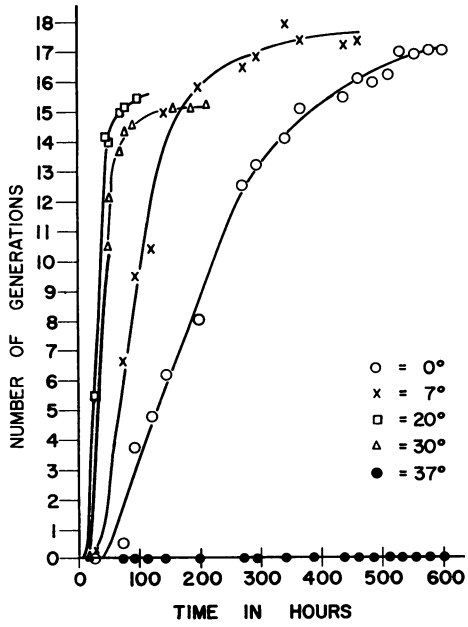

FIG. 1. *Growth curves of Arthrobacter sp. #77.*

A. citreus. In serological tests, all of our isolates were agglutinated by *A. globiformis* antiserum; only feeble or no cross-reaction was observed with cells and antisera of *A. citreus* and *A. aurescens*. The isolates used in these growth studies bore the numerical designations 62, 77, and 88.

Four known *Arthrobacter* species were obtained from the American Type Culture Collection and also used for growth studies in these tests: *A. aurescens*, ATCC 13344; *A. citreus*, ATCC 11624; *A. globiformis*, ATCC 8010; and *A. simplex*, ATCC 6946.

Growth conditions. Growth characteristics of the three isolates and the four ATCC cultures were studied at five temperatures. Cultures consisted of 50 ml of Difco nutrient broth in 250-ml flasks. These were inoculated with 0.1-ml water suspensions containing $50 \times 10^4 \pm 20 \times 10^4$ live cells per inoculum. Cells for the inoculum were obtained from TGE agar cultures grown at 20 C for 72 hr. The flask cultures were incubated without shaking at 0 ± 0.5, 7 ± 0.5, 20 ± 1, 30 ± 1, and 37 ± 1 C. Conventional incubators were used for incubation at 20, 30, and 37 C. The 0- and 7-C incubators were specially built, insulated, stainless steel, top-opening boxes with thermostatically-controlled heaters and continuous air circulation. They were placed in a chest-type freezer held at -7 ± 2 C. Temperature control was effected by bucking the freezer with the incubator heaters. This system presented two advantages for psychrophilic incubation: the nearly constant ambient temperature resulted in close control of incubator temperatures, and the incubator doors were located so that cold air from the freezer entered during sampling. Thus, periodic warm-ups were avoided; any temperature change tended toward a conservative cooling that was quickly compensated for by the incubator heaters.

Samples were aseptically removed periodically, and growth was assayed by the standard pour-plate method in TGE agar. Plates were incubated at 20 C for 72 hr. All samples were run in duplicate, and all growth curves reported are averages of two such independent replications.

To make all data comparable, results are reported as number of generations vs. incubation time. Number of generations was determined by the formula (Clifton, 1957): ($\log_{10}$ count at time t $-$ $\log_{10}$ count at time 0)/$\log_{10}$ 2.

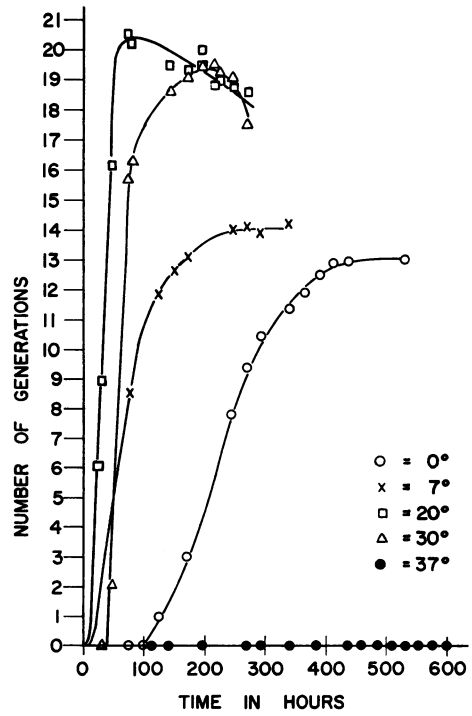

FIG. 2. *Growth curves of Arthrobacter sp. #88.*

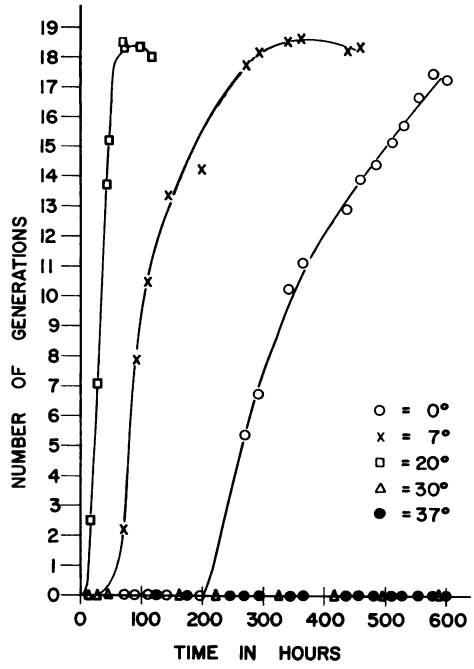

FIG. 3. *Growth curves of Arthrobacter sp. #62*

RESULTS

Figure 1 shows growth curves of isolate #77, plotted as incubation time in hours vs. number of generations. Growth at 0 C proceeded after a relatively short lag period (25 hr) and ultimately resulted in 17 generations. Growth at 7 C proceeded with a shorter lag period and a steeper slope or higher rate of growth during the logarithmic-growth period. Similar lag periods with still more rapid rates of growth were demonstrated at 20 and 30 C. No growth was observed at 37 C within the 600-hr maximal incubation time. This culture isolate was capable of rapid and efficient psychrophilic growth; however, its most rapid growth rate appeared to be attained at 20 C. Incubation temperature seemed to have little effect on the total number of generations ultimately produced.

Figure 2 shows a similar series of growth curves of isolate #88. In this case, the lag period at 0 C was increased from the 25 hr demonstrated by the previous culture to about 100 hr. Unlike the previous culture, there was some decrease in the total number of generations produced at 0 and 7 C. Again, most rapid growth was produced at 20 C, and no growth was demonstrated at 37 C.

Figure 3 shows curves of isolate #62. Good growth occurred at 0 C; however, the lag period was increased to about 200 hr. Most rapid growth was again demonstrated at 20 C, and no growth was observed at either 30 or 37 C.

These three isolates, numbers 77, 88, and 62, represent true psychrophiles by the definition of Ingraham and Stokes (1959). That is, "they grow appreciably and often abundantly at 0 C within 2 weeks" (i.e., 336 hr). More than 10 generations were produced by these three isolates within the prescribed incubation period.

Growth curves of *A. aurescens* are shown in Fig. 4. This organism grew at 0 C after a lag period of about 275 hr. Only slightly more than three generations were produced within the 336-hr time limitation imposed by definition. However, ultimate growth after 600 hr was as great at 0 C as at mesophilic temperatures. Although this is appreciable growth at 0 C, it is doubtful if this organism would be considered a psychrophile by the Ingraham and Stokes definition (appreciable or abundant growth in 2 weeks), especially if the observation were made as colony development on solid media. Tendencies toward preference for mesophilic growth by this organism were demonstrated by the somewhat retarded, but appreciable growth at 37 C, and most rapid growth at 30 C instead of 20 C.

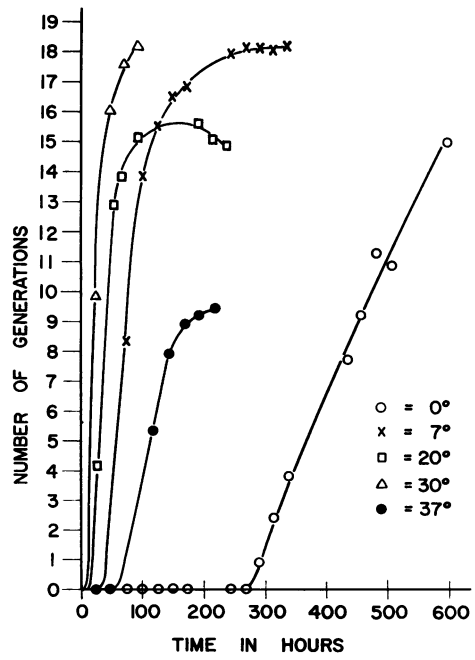

FIG. 4. *Growth curves of Arthrobacter aurescens*

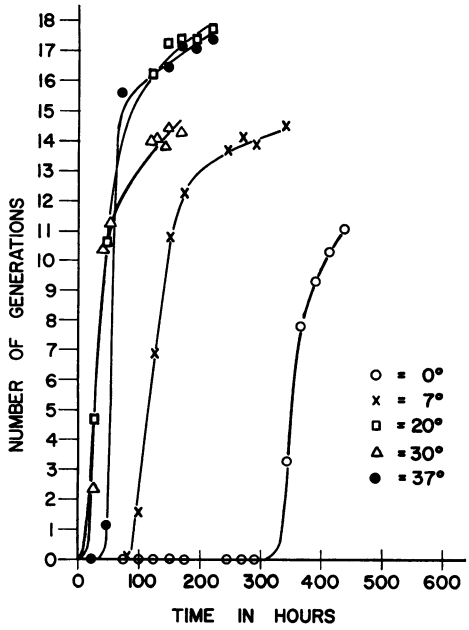

FIG. 5. *Growth curves of Arthrobacter citreus*

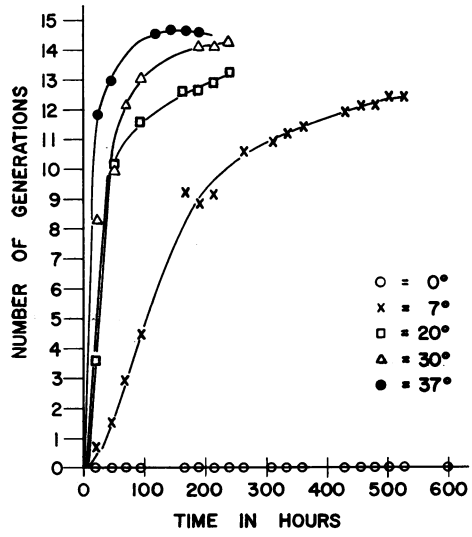

FIG. 6. *Growth curves of Arthrobacter globiformis*

Figure 5 shows growth of *A. citreus*. Growth curves of this organism are quite similar to those of *A. aurescens*. *A. citreus* showed essentially complete growth at 0 C in 600 hr, but not in 2 weeks (336 hr). However, a greater preference for mesophilic growth by *A. citreus* over that shown by *A. aurescens* seems to be exhibited by the increased (315 hr) lag period at 0 C and more rapid growth with a shorter lag period at 37 C.

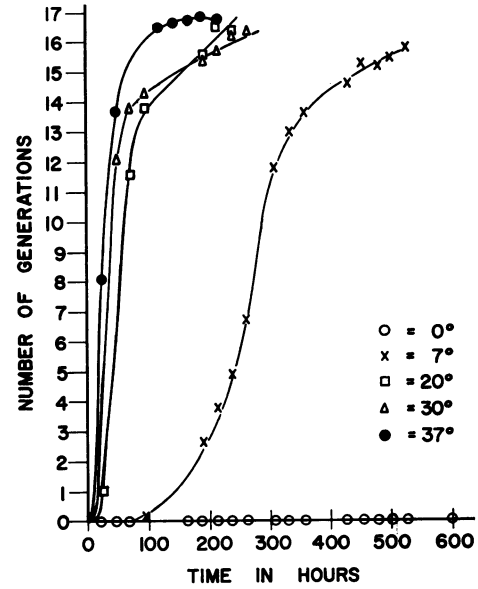

FIG. 7. *Growth curves of Arthrobacter simplex*

TABLE 1. *Lag times of Arthrobacter cultures*

Organism	Lag time	
	0 C	37 C
	hr	*hr*
#77	25	>600
#88	100	>600
#62	200	>600
A. aurescens	275	75
A. citreus	315	50
A. globiformis	>600	5
A. simplex	>600	5

Figure 6 shows growth curves of *A. globiformis*. This organism failed to grow at 0 C within 600 hr, and produced more rapid growth with increased incubation temperature up to 37 C. Growth at 7 C proceeded with a rather short lag period. There can be little doubt that this organism represents a true mesophile.

Growth curves of *A. simplex* are shown in Figure 7. Growth of this typical mesophile fits the same general pattern as *A. globiformis*, except that growth at 7 C proceeds only after a longer lag period, probably indicating a narrower temperature latitude of optimal growth.

DISCUSSION

These results graphically point up the difficulty of establishing an arbitrary definition of a psychrophile, based on incubation time at 0 C or at any other temperature. Among the seven members of the genus *Arthrobacter* described, no cut-off region between psychrophiles and mesophiles is apparent in either time or incubation temperature. Even though some of the members are definitely psychrophiles by the current definition, and some are unquestionably mesophiles, there is an apparent continuous pattern in the vigor with which these organisms can initiate and maintain growth under cold-temperature conditions.

Table 1 shows lag periods of the seven *Arthrobacter* cultures at the key incubation temperatures of 0 and 37 C. The tendency toward increasing lag time at 0 C is accompanied by a concomitant tendency toward decreasing lag time at 37 C. This apparently indicates that a decreasing tendency toward psychrophilic growth is accompanied by an increasing tendency toward mesophilic growth. This pattern displays itself as a gradual transition through this series of seven *Arthrobacter* cultures.

Growth temperature did not greatly affect the total number of generations produced. All of the psychrophilic members of this group exhibited their most rapid growth and shortest lag periods at mesophilic temperatures. As reported with a number of other psychrophiles (Ingraham and Stokes, 1959), none of these *Arthrobacter* cultures showed optimal growth at 0 or 7 C.

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