JOURNAL OF BACTERIOLOGY, Feb. 2005, p. 923–929 Vol. 187, No. 3
0021-9193/05/$08.00+0 doi:10.1128/JB.187.3.923–929.2005

Growth Kinetics of Extremely Halophilic *Archaea* (Family *Halobacteriaceae*) as Revealed by Arrhenius Plots

Jessie L. Robinson,[1] Brandy Pyzyna,[1] Rachelle G. Atrasz,[1] Christine A. Henderson,[1] Kira L. Morrill,[1] Anna Mae Burd,[1] Erik DeSoucy,[1] Rex E. Fogleman III,[1] John B. Naylor,[1] Sarah M. Steele,[1] Dawn R. Elliott,[1] Kathryn J. Leyva,[2] and Richard F. Shand[1]*

Department of Biological Sciences, Northern Arizona University, Flagstaff,[1] and Department of Microbiology, Arizona College of Osteopathic Medicine, Midwestern University, Glendale,[2] Arizona

Received 22 August 2004/Accepted 31 October 2004

Members of the family *Halobacteriaceae* in the domain *Archaea* are obligate extreme halophiles. They occupy a variety of hypersaline environments, and their cellular biochemistry functions in a nearly saturated salty milieu. Despite extensive study, a detailed analysis of their growth kinetics is missing. To remedy this, Arrhenius plots for 14 type species of the family were generated. These organisms had maximum growth temperatures ranging from 49 to 58°C. Nine of the organisms exhibited a single temperature optimum, while five grew optimally at more than one temperature. Generation times at these optimal temperatures ranged from 1.5 h (*Haloterrigena turkmenica*) to 3.0 h (*Haloarcula vallismortis* and *Halorubrum saccharovorum*). All shared an inflection point at 31 ± 4°C, and the temperature characteristics for 12 of the 14 type species were nearly parallel. The other two species (*Natronomonas pharaonis* and *Natronorubrum bangense*) had significantly different temperature characteristics, suggesting that the physiology of these strains is different. In addition, these data show that the type species for the family *Halobacteriaceae* share similar growth kinetics and are capable of much faster growth at higher temperatures than those previously reported.

The family *Halobacteriaceae* in the domain *Archaea* presently is comprised of 18 genera and 49 validly described species (International Committee on Systematics of Prokaryotes [http://www.the-icsp.org]). All members are extreme halophiles, requiring at least 1.5 M NaCl for growth, but grow optimally in 2.0 to 4.5 M NaCl (6, 20). All have exceptionally high internal cation concentrations that approach 6 M in some species (e.g., *Halobacterium salinarum*) (21). In addition to exhibiting halophilicity, four genera (*Natronococcus*, *Natronomonas*, *Natronorubrum*, and *Natronobacterium*) are also alkaliphilic, growing optimally between pH 9.5 and 10.0 (20). These organisms have been isolated from a wide variety of environments, including the sediment of a cold, hypersaline lake in Antarctica (*Halorubrum lacusprofundi*) (3), the Dead Sea (*Haloferax volcanii*) (16), the Great Salt Lake (*Halorabdus utahensis*) (31), a hypersaline soda lake in Egypt (*Natronomonas pharaonis*) (27), and a salt crystal from a Permian halite deposit (*Halosimplex carlsbadense*) (30). There is also a report of haloarchaeal 16S rDNA amplified from inside a black smoker (28), but no haloarchaeons have been cultured from this source. All haloarchaea grow aerobically, and one species (*Halobacterium salinarum*) can grow phototrophically using bacteriorhodopsin as a light-driven proton pump (19).

Since ". . .growth is the core of bacterial physiology. . ." (11) and since all experiments in cellular regulation are fundamentally physiological, it is essential to understand the growth physiology of the organism one is studying. Toward this goal we generated a complete Arrhenius plot for the haloarchaeon *Haloferax mediterranei* (22, 25; J. L. Robinson and R. F. Shand, unpublished data [http://jan.ucc.nau.edu/~shand]). This growth physiology experiment revealed the following: (i) the organism is eurythermal, growing between 12 and 55°C with generation times of 752.5 and 1.67 h, respectively, (ii) it has an optimal temperature plateau (from 47 to 51°C; g_{avg} = 1.20 ± 0.01 h, where g_{avg} represents average generation time) instead of a single optimum temperature, and (iii) it has an inflection point (i.e., a deviation from the Arrhenius portion of the plot) at 33°C. We also discovered that all physiologically based experiments involving this organism have been conducted under suboptimal growth temperatures. The first (and only other) correlation between growth rate and temperature for a haloarchaeon was produced for *Halorubrum lacusprofundi* (3) by use of a temperature gradient incubator. Unfortunately, balanced growth conditions were not established and optical density readings at the higher growth temperatures were confounded by flocculent growth.

Extremely halophilic *Archaea* possess eukaryote-like features such as multisubunit RNA polymerases, homologues to eukaryotic transcription factors, TATA-box promoters (18, 29), and leaderless transcripts (4); these features make them distinct from *Bacteria* at the molecular level. Moreover, given the diversity of environments from which the haloarchaea are isolated and the lack of detailed information regarding their growth physiology, we generated Arrhenius plots for 14 type species. These data provide critical information for elucidating their physiological, metabolic, and genetic features.

* Corresponding author. Mailing address: Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640. Phone: (928) 523-9970. Fax: (928) 523-7500. E-mail: Richard.Shand@nau.edu.

TABLE 1. Strains, media, and sources

Type species	Medium	Source[a]
Haloarcula vallismortis	ATCC 1863[b,c]	ATCC 29715
Halobacterium salinarum (NRC-1)[d]	GN101(8)	S. DasSarma
Halobaculum gomorrense	ATCC 2169[b]	ATCC 700876
Halococcus morrhuae	ATCC 1863[b,c]	ATCC 43103
Haloferax volcanii (DS70)[d]	ATCC 1176[b]	C. Daniels
Halogeometricum borinquense	ATCC 213[b,e]	R. Vreeland
Halorubrum saccharovorum	ATCC 876[b,f]	R. Vreeland
Haloterrigena turkmenica	ATCC 1863[b,c]	R. Vreeland
Natrialba asiatica	ATCC 805[b,g]	C. Litchfield
Natrinema pellirubrum	ATCC 1863[b,c]	R. Vreeland
Natronobacterium gregoryi	ATCC 1590[h]	P. Jablonski
Natronococcus occultus	ATCC 1590[h]	P. Jablonski
Natronomonas pharaonis	ATCC 1590[h]	P. Jablonski
Natronorubrum bangense	ATCC 1590[h]	JCM 10635

[a] ATCC, American Type Culture Collection; JCM, Japan Collection of Microorganisms.

[b] These media were prepared by using ATCC recipes (http://www.atcc.org) with the following modifications: media were filter sterilized, and trace elements (0.2 μM $CuSO_4 \cdot 5H_2O$, 11.6 μM $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 1.8 μM $MnSO_4 \cdot H_2O$, 1.5 μM $ZnSO_4 \cdot 7H_2O$) were added. Trace elements were aliquoted, stored at −20°C, and added just prior to inoculation. Trace elements were thawed only once.

[c] $FeCl_2$ and $MnCl_2$ were excluded.

[d] NRC-1 was chosen because its genome has been sequenced (18); DS70 was chosen because it is widely used to study haloarchaeal genetics.

[e] Made as one solution. $CaCl_2 \cdot 2H_2O$ was used in place of $CaCl_2 \cdot 6H_2O$ (filter sterilized using a prefilter).

[f] Autoclaved, cooled to room temperature, and then filter sterilized using a prefilter.

[g] $FeSO_4$ was excluded.

[h] These media were prepared by using ATCC recipes with the following modifications: media were filter sterilized and contained SL-6 trace elements (3.5 μM $ZnSO_4 \cdot 7H_2O$, 1.5 μM $MnCl_2 \cdot 4H_2O$, 48.5 μM H_3BO_3, 8.4 μM $CoCl_2 \cdot 6H_2O$, 0.59 μM $CuCl_2 \cdot 2H_2O$, 0.84 μM $NiCl_2 \cdot 6H_2O$, 1.3 μM $Na_2MoO_4 \cdot H_2O$). Trace elements were aliquoted, stored at −20°C, and added just prior to inoculation. Trace elements were thawed only once. The medium was incubated at 41°C overnight, cooled to room temperature, and then filter sterilized using a prefilter.

MATERIALS AND METHODS

Haloarchaeal strains, media and growth conditions. The type species and the appropriate medium for each are listed in Table 1. At the start of this project there were 14 genera (20); since then, four new genera (*Halorhabdus*, *Halomicrobium*, *Halobiforma*, and *Halosimplex* [http://www.the-icsp.org]) have been added to the family and are not represented in this study. Cultures were grown at 200 rpm in model G76 and C76 orbital shaking water baths (New Brunswick Scientific Co. Inc., Edison, N.J.) with gabled covers (essential for reducing evaporation). Temperature was monitored with ASTM (American Society for Testing and Materials) thermometers accurate to ±0.2°C. To promote efficient aeration, 125-ml tribaffled flasks (Kimble Glass, Vineland, N.J.) with Morton-type closures containing 25 ml of medium were used. Growth was monitored spectrophotometrically using a dual-beam BioSpec 1601 (Shimadzu Scientific Instruments, Inc., Columbia, Md.). All samples with an optical density at 600 nanometers (OD_{600}) >0.10 were diluted to an OD_{600} between 0.05 and 0.10 before reading. To mitigate volume effects (especially in cultures with slow growth rates), no more than 15 ml of culture was removed from the culture flask. A survey of growth media at 41°C for *Haloarcula vallismortis*, *Halococcus morrhuae*, and *Natrinema pellirubrum* included ATCC 1863, GN101, and NRC817, in addition to the recommended medium (see Table 1 and Table 2) (8).

TABLE 2. Growth medium survey

Strain	Recommended medium (g in h)	Medium used (g in h)
Haloarcula vallismortis	ATCC 112 (8.5)	ATCC 1863 (4.1)
Halococcus morrhuae	ATCC 112[a]	ATCC 1863 (3.4)
Natrinema pellirubrum	ATCC 330[a]	ATCC 1863 (3.8)

[a] No growth after 20 h (*Natrinema pellirubrum*) or 48 h (*Halococcus morrhuae*) of incubation, but both eventually grew.

Criteria for establishing balanced growth and determining generation times. Prior to collection of Arrhenius plot data, cultures were placed into balanced growth (1): a minimum of eight generations in exponential phase (at least two subcultures). To ensure they remained in balanced growth, the cultures were grown from an OD_{600} of 0.01 to an OD_{600} of no more than 0.20 (~4.5 doublings). The starting temperature for each Arrhenius plot was 41°C. Once in balanced growth, the 41°C cultures were subcultured bidirectionally into aerated media in 2°C increments; balanced growth was then reestablished. This was repeated until both the maximum temperature and 23°C were reached. Due to the prolonged generation times at lower temperatures, it was impractical to grow these organisms below 23°C (3, 22).

Minimum requirements for growth curves from which generation times were determined were as follows: (i) samples were taken at least once every generation, (ii) each growth curve had a minimum of six time points, (iii) R^2 was ≥0.995, and (iv) the generation times of the last two growth curves differed by ≤20%. Typically, it took one or two growth curves after balanced growth had been established to meet these criteria; however, in some cases it took as many as 10. An organism was considered not able to grow when the OD_{600} remained ≤0.03 and no growth occurred for 48 h.

Statistical analysis. A Student t test was used to determine whether differences in temperature characteristics were significant (see Fig. 2). Since the correlation coefficients were remarkably high ($R^2 \geq 0.984$) (see Table 3), the within-group variance was negligible. Therefore, the slopes were compared.

RESULTS AND DISCUSSION

Methodological rationale. Cultures were shifted from one temperature to another in 2°C increments to prevent long lags in growth and a heat shock or cold shock response. Heat shock genes are essential for growing cells, and "[i]nduction of the heat shock proteins during pre-adaptation at moderate temperatures increases survival of the organism at higher temperatures" (14).

The necessity for placing cultures into balanced growth prior to collecting data has been stated by Neidhardt et al. (17): "Unless growth is monitored throughout a physiological experiment, the results may not be reproducible. . . . In fact, a physiological experiment done with a poorly characterized culture is *all but useless*." We have shown that the growth rate of *Halobacterium salinarum* NRC817 decreases from its initial growth rate as early as OD_{600} = ~0.1 (25). However, this is an extreme example, and all organisms examined in this study grew linearly to an OD_{600} of >0.2. Consequently, an OD_{600} of ≤0.2 was chosen as a conservative maximum at which to subculture in order to maintain balanced growth. This had the additional benefits of (i) preventing spectrophotometric interference due to accumulation of gas vesicles (26) and (ii) preventing physiological perturbations due to decreased oxygen availability at higher temperatures (5) and pH changes in media that were relatively unbuffered.

In context of microbial physiology, an Arrhenius plot takes the form shown in Fig. 1D. The linear portion of the plot where growth rate correlates with temperature is called the Arrhenius, physiological, or "normal" portion, and the slope of this portion is called the temperature characteristic (11). Traditionally, temperature is plotted as 1,000/T (in degrees Kelvin) (9), however, we have converted to the more familiar degrees Celsius. The log of the specific growth rate constant ($k = 0.693/g$) is plotted on the abscissa.

Growth medium survey. Although we used published medium recipes for growing the organisms, we found that three species grew poorly ($g > 8$ h) in the recommended medium. For these organisms a survey was done to determine whether some other medium would be better at supporting growth (see

TABLE 3. Summary of the growth physiology of 14 type species of haloarchaea

Strain	Maximum temp in °C (*g* in h)[a]	Optimum temp in °C (*g* in h)[b]	Reported optimum temp in °C[h]	Inflection point in °C (*g* in h)	*g* at 23°C (h)	R^{2c}	Slope[d]
Haloarcula vallismortis	55 (18.4)	43–49 (3.04 ±0.20)	40	29 (7.99)	28.4	0.995	0.029
Halobacterium salinarum	50 (1.86)	49–50 (1.86)	35–50	31 (7.23)	25.8	0.995	0.033
Halobaculum gomorrense	54 (6.62)	43–49 (2.71 ±0.14)	40	31 (6.01)	17.5	0.988	0.032
Halococcus morrhuae	57 (3.87)	51 (1.52)	30–45	33 (5.59)	18.0	0.996	0.032
Haloferax volcanii[e]	49 (3.81)	45 (1.83)	40	31 (5.30)	16.5	0.995	0.034
Halogeometricum borinquense	58 (3.15)	49–53 (1.77 ±0.06)	40	35 (4.05)	18.1	0.992	0.027
Halorubrum saccharovorum	55 (13.7)	45 (3.03)	50	31 (7.81)	22.3	0.984	0.030
Haloterrigena turkmenica	57 (2.95)	51 (1.50)	45	29 (6.56)	18.1	0.994	0.030
Natrialba asiatica	51 (5.27)	45 (2.59)	35–40	31 (6.71)	19.6	0.996	0.028
Natrinema pellirubrum	57 (3.69)	51 (1.87)	NR[f]	31 (7.49)	19.6	0.997	0.029
Natronobacterium gregoryi	52 (4.36)	47 (2.23)	37	31 (5.98)	22.2	0.989	0.031
Natrononococcus occultus	54 (4.00)	45 (2.77)	35–40	31 (7.46)	31.8	0.985	0.029
Natronomonas pharaonis	56 (7.45)	43–45 (2.13 ±0.03)	45	27 (15.31)	30.3	0.997	0.055[g]
Natronorubrum bangense	50 (3.50)	43 (2.75)	45	29 (11.75)	34.4	0.997	0.046[g]

[a] Maximum temperatures were determined to within 1°C.
[b] Optimal temperatures are ±1°C; average generation times are given for ranges.
[c] R^2 of Arrhenius portion.
[d] Slope of Arrhenius portion (i.e., the temperature characteristic).
[e] Grew for several subcultures at both 51 and 53°C but was unable to sustain growth.
[f] NR, not reported.
[g] These temperature characteristics are significantly different from the temperature characteristics of the other 12 type species (see Fig.2).
[h] Data from reference 20.

Materials and Methods). Table 2 shows that all three of these strains grew faster ($g \leq 4.1$ h) in a medium other than that usually associated with these organisms, and these media were used in this study.

Growth kinetics. Fig. 1 shows the Arrhenius plots for 14 type species of the family *Halobacteriaceae*. Nine species had higher temperature optima than those previously reported (the largest difference was for *Natronobacterium gregoryi* [10°C]), two were equal to those previously reported, two were lower (by 2 to 5°C) than those previously reported, and one had no previously reported temperature optimum (Table 3). Generation times at optimal growth temperatures were not reported for any of the original descriptions of the 14 type species, but in this study, six were <2 h and eight were ≤3 h. Like *Haloferax mediterranei*, five of the organisms (*Haloarcula vallismortis*, *Halobacterium salinarum*, *Halobaculum gomorrense*, *Halogeometricum borinquense*, and *Natronomonas pharaonis*) (Table 3) had optimal temperature plateaus spanning 2 to 7°C. This is atypical, as most organisms have a single temperature optimum (see references 2, 7, and 15 for examples). Four strains (*Halobacterium salinarum*, *Halococcus morrhuae*, *Natrialba asiatica*, and *Natrononococcus occultus*) had reported growth temperature optima spanning 5°C to (a remarkable) 15°C, but this study revealed that only one of those strains (*Halobacterium salinarum*) grew equally well at more than one temperature.

The maximum temperatures for growth were ≥49°C (Table 3). This thermotolerance reflects, in part, the high temperatures (45 to 50°C) (24) of the environments from which these organisms often are isolated. In contrast, *Halorubrum lacusprofundi* ACAM34, isolated from Deep Lake in Antarctica, is not a psychrophilic haloarchaeon, as its temperature optimum and maximum are 36 and ~42°C, respectively (3); note that this represents results obtained with a sample from a hypersaline lake whose temperature is <0°C for 8 months of the year. For 10 of the 14 species, the temperature maximum was within 4 to 7°C of the temperature optimum, with one species (*Halobacterium salinarum*) having the same temperature (50°C) for both (Fig. 1, Table 3). These two cardinal temperatures typically are not far apart due to thermal denaturation of proteins which sets the maximum temperature for growth. In contrast, three species (*Natrononococcus occultus*, *Halorubrum saccharovorum*, and *Natronomonas pharaonis*) had much greater differences between their optimum and maximum temperatures (9, 10, and 11°C, respectively) and gave results that do not conform to the typical growth-temperature profile. There is little correlation between the difference in maximum and optimum temperatures and the difference in generation times. For example, both *Haloarcula vallismortis* and *Haloterrigena turkmenica* have a 6°C difference between their maximum and optimum temperatures; however, the difference in generation times is 15.4 h for *Haloarcula vallismortis* but is only 1.45 h for *Haloterrigena turkmenica* (Table 3).

Haloarchaeal growth at the lower temperatures departs from the Arrhenius portion at a nearly uniform 31 ± 4°C and is linear between the inflection point and 23°C. This same departure is seen with psychrophiles, mesophiles, and thermophiles (2, 7, 9, 15), but the inflection point is shifted in response to the overall growth profile. For example, the inflection point is 10°C for *Vibrio psychroerythrus* (15), 21°C for *Escherichia coli* (9), about 31°C for haloarchaea (Table 3 and Fig. 2), and 53°C for *Bacillus stearothermophilus* (15). This represents a fundamental change in the physiology of the organism at these temperatures (10), and just as with *E. coli*, a new suite of cold-shock proteins presumably is synthesized (9, 10).

Uniformity of the Arrhenius portion. Fig. 2 shows the relationships of the 14 temperature characteristics. In every case, the relationship is linear ($R^2 \geq 0.984$) (Table 3) and spans as much as 22°C (for *Haloterrigena turkmenica*, 29 to 51°C) and as little as 12°C (for *Halobaculum gomorrense*, 31 to 43°C). Remarkably, the temperature characteristics of 12 of the 14 spe-

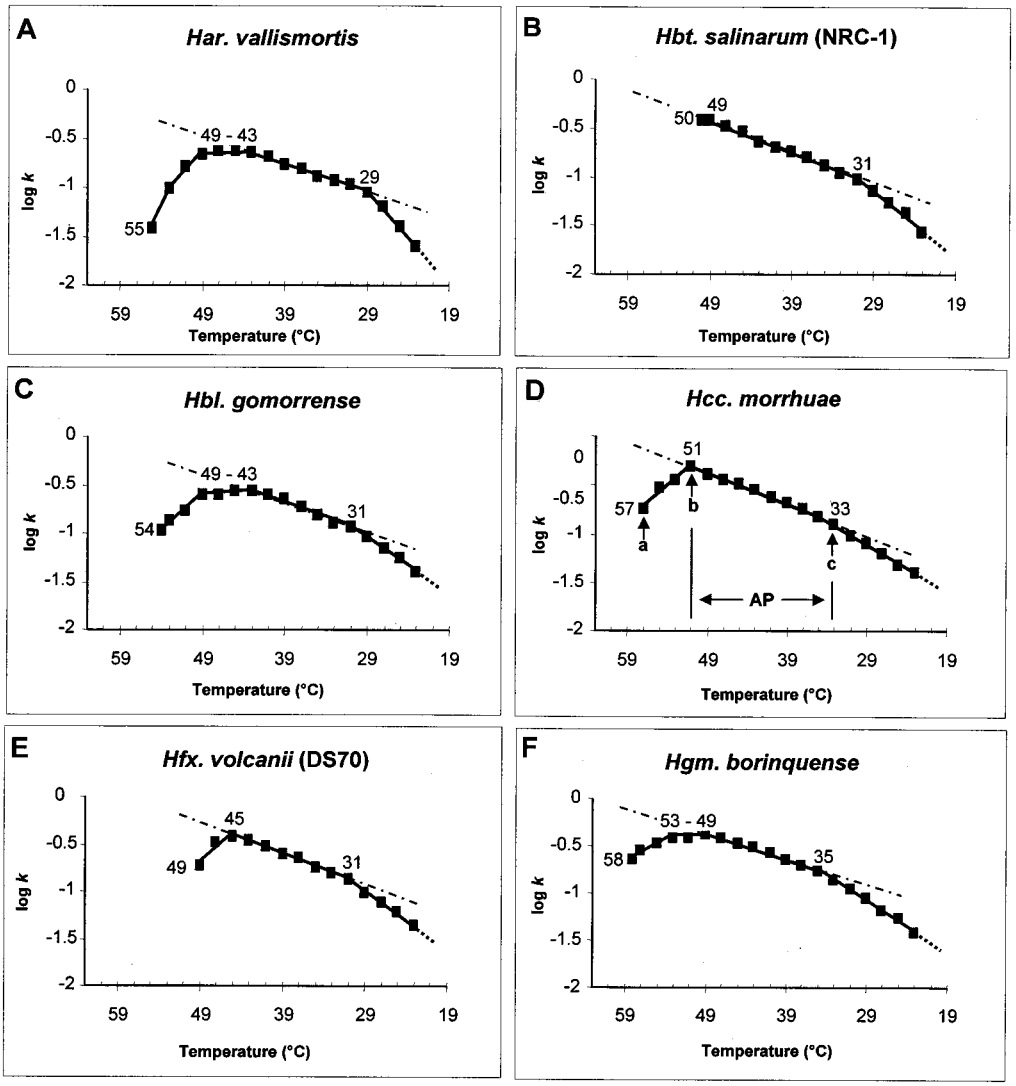

FIG. 1. Arrhenius plots for 14 type species of the family *Halobacteriaceae* ($k = 0.693/g$). — · —, regression line for the Arrhenius portion; · · · ·, extrapolation toward minimum growth temperature. (D) a, maximum temperature; b, optimum temperature; c, temperature at the inflection point; AP, Arrhenius portion.

cies are nearly parallel (Fig. 2 and Table 3), with the slope ranging between 0.027 and 0.034, a difference of only 0.007. The temperature characteristic for *Haloferax mediterranei* (slope = 0.033) also falls within this group (data not shown). The two notable exceptions are haloalkaliphiles, *Natronomonas pharaonis* and *Natronorubrum bangense* (Fig. 2 and Table 3). The difference in the temperature characteristics for these two species from the results obtained with the other 12 is statistically significant ($P \leq 0.001$) and suggests that the physiology of these two organisms is fundamentally different. While alkaliphilicity might be a contributing factor, the other two haloalkaliphiles (*Natronobacterium gregoryi* and *Natronococcus occultus*) group with the 10 neutrophiles. For comparison, we determined the temperature characteristics for *Halorubrum lacusprofundi* (slope = 0.040; $R^2 = 0.943$) and *E. coli* (slope = 0.032; $R^2 = 0.981$) from published data (3, 9). The temperature characteristic for *Halorubrum lacusprofundi* was significantly different ($P \leq 0.001$) from those of the 12 haloarchaeons, but, interestingly, that of *E. coli* was not.

Significance. The heavy lifting has been done with respect to determining optimum growth temperatures for several species in the family *Halobacteriaceae*. Optimization experiments for any other parameter can now be done in a single water bath. Although we did not conduct an extensive survey to determine whether other species would grow better in a medium different than that recommended, we suspect that for many of the organisms listed in Table 1 there are media that will yield faster generation times.

The prevailing view is that the haloarchaea ". . .have relatively long generation times (e.g., 3 to 4 h for *Haloferax volcanii*; 8 to 12 h for *Halobacterium* spp.)" (Halohandbook [http://www.microbiol.unimelb.edu.au/micro/staff/mds/HaloHandbook]; see also references 12 and 13). However, under optimal growth temperatures, these organisms grow much faster than has been as-

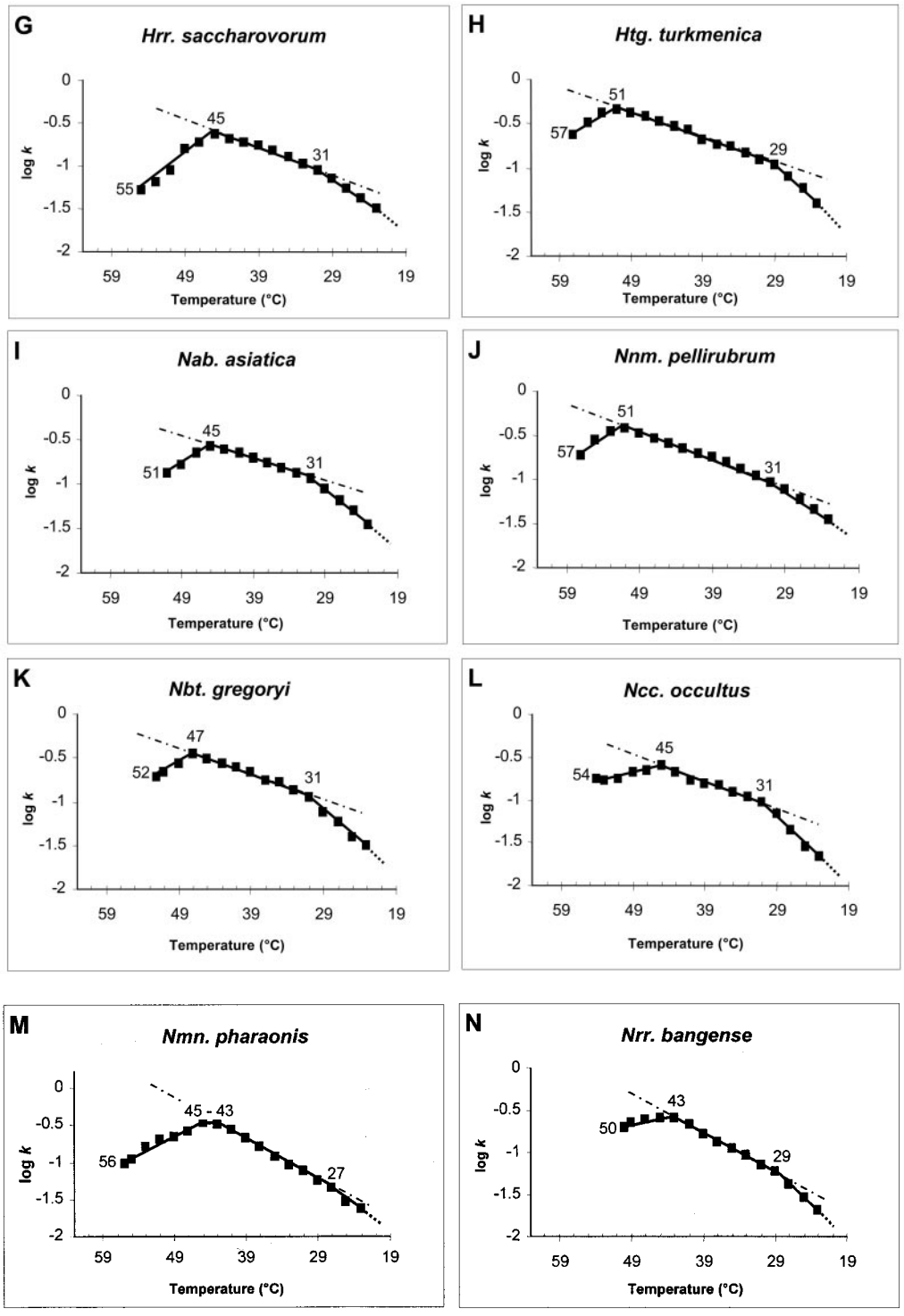

FIG. 1—*Continued*.

sumed; *Haloferax volcanii* DS70 and *Halobacterium salinarum* NRC-1 have generation times of 1.83 and 1.86 h, respectively (Table 3). Consequently, growth at optimal temperatures significantly reduces generation times.

As a group, populations of these organisms double in 1.5 to 3.0 h; however, some researchers recommend that haloarchaeons be grown below optimum temperatures (between 37 and 42°C) (23). This recommendation was made in part for convenience but

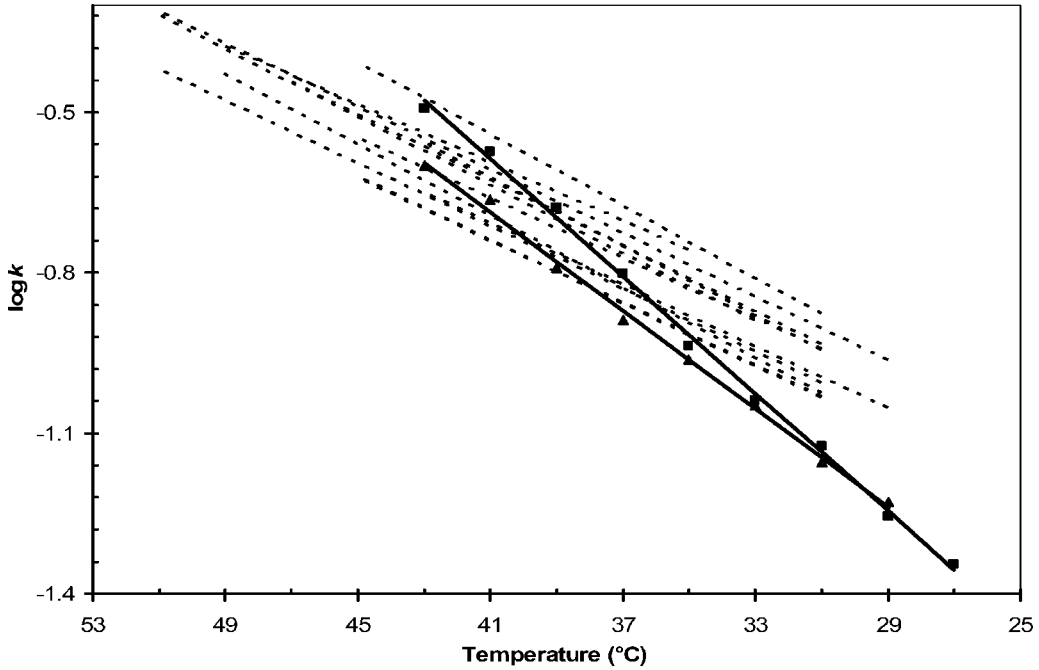

FIG. 2. Comparison of the temperature characteristics of the 14 type species of *Halobacteriaceae* listed in Table 1. ■, *Natronomonas pharaonis*; ▲, *Natronorubrum bangense*; ----, temperature characteristics of the other 12 type species (see Fig. 1 and Table 3 for details).

also for concern about oxygen solubility in high salt. While oxygen solubility might be an issue at temperatures near the maximum, practical steps for enhancing oxygen diffusion at optimal temperatures (e.g., the use of baffled culture flasks) have been described previously (25).

In addition to cardinal growth temperature and generation time results, careful growth studies can reveal much about the physiology of an organism. The temperature characteristics for *Halorubrum lacusprofundi*, *Natronomonas pharaonis*, and *Natronorubrum bangense* suggest there is something fundamentally different about their physiology that deserves further study.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM05600-02 from the National Institutes of Health and a grant from the Arizona Board of Regents' Technology and Research Initiative Funds (Proposition 301) to R.F.S. K.J.L. was supported through Intramural Grants from Midwestern University.

We thank Chuck Daniels, Shil DasSarma, Peter Jablonski, Carol Litchfield, and Russ Vreeland for supplying strains and Stephen Shuster for help with statistical analysis.

REFERENCES

1. **Campbell, A.** 1957. Synchronization of cell division. Bacteriol. Rev. **21:**263–272.
2. **Chablain, P. A., G. Philippe, A. Groboillot, N. Truffaut, and J. F. Guespin-Michel.** 1997. Isolation of a soil psychrotrophic toluene-degrading *Pseudomonas* strain: influence of temperature on the growth characteristics on different substrates. Res. Microbiol. **148:**153–161.
3. **Franzmann, P. D., E. Stackebrandt, K. Sanderson, J. K. Volkman, D. E. Cameron, P. L. Stevenson, T. A. McKeen, and H. R. Burton.** 1988. *Halobacterium lacusprofundi* sp. nov., a halophilic bacterium isolate from Deep Lake, Antarctica. Syst. Appl. Microbiol. **11:**20–27.
4. **Fuglsang, A.** 2004. Compositional nonrandomness upstream of start codons in archaebacteria. Gene **332:**89–95.
5. **Gary, K.** 1989. On-line electrochemical sensors in fermentation. Am. Biotechnol. Lab. **7:**26–33.
6. **Grant, W. D., and H. Larsen.** 1989. Family *Halobacteriaceae* Gibbons 1974, 269^{AL}, p. 2218–2232. *In* J. T. Stanley, M. P. Bryant, N. Pfennig, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 3. Williams & Wilkins, Baltimore, Md.
7. **Guillou, C., and J. F. Guespin-Michel.** 1996. Evidence for two domains of growth temperature for the psychrotrophic bacterium *Pseudomonas fluorescens* MF0. Appl. Environ. Microbiol. **62:**3319–3324.
8. **Haseltine, C., T. Hill, R. Montalvo-Rodriguez, S. K. Kemper, R. F. Shand, and P. Blum.** 2001. Secreted euryarchaeal microhalocins kill hyperthermophilic crenarchaea. J. Bacteriol. **183:**287–291.
9. **Herendeen, S. L., R. A. VanBogelen, and F. C. Neidhardt.** 1979. Levels of major proteins of *Escherichia coli* during growth at different temperatures. J. Bacteriol. **139**:185–194.
10. **Ingraham, J. L., and A. G. Marr.** 1996. Effect of temperature, pressure, pH, and osmotic stress on growth, p. 1570–1578. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella* cellular and molecular biology, 2nd ed., vol. 2. ASM Press, Washington, D.C.
11. **Ingraham, J. L., O. Maaløe, and F. C. Neidhardt.** 1983. Growth of the bacterial cell, p. ix, 227–265. Sinauer Associates, Inc., Sunderland, Mass.
12. **Juez, G.** 1988. Taxonomy of extremely halophilic archaebacteria, p. 3–24. *In* F. Rodriguez-Valera (ed.), Halophilic bacteria, vol. II. CRC Press, Boca Raton, Fla.
13. **Kushner, D. J.** 1993. Growth and nutrition of halophilic bacteria, p. 87–103. *In* R. H. Vreeland and L. I. Hochstein (ed.), The biology of halophilic bacteria. CRC Press, Boca Raton, Fla.
14. **Lengeler, J. W., G. Drews, and H. G. Schlegel (ed.).** 1999. Biology of the prokaryotes, p. 658–660. Thieme, Stuttgart, Germany.
15. **Mohr, P. W., and S. Krawiec.** 1980. Temperature characteristics and Arrhenius plots for nominal psychrophiles, mesophiles and thermophiles. J. Gen. Microbiol. **121:**311–317.
16. **Mullakhanbhai, M. F., and H. Larsen.** 1975. *Halobacterium volcanii* spec. nov., a Dead Sea halobacterium with a moderate salt requirement. Arch. Microbiol. **104:**201–214.
17. **Neidhardt, F. C., J. L. Ingraham, and M. Schaechter.** 1990. Physiology of the bacterial cell, p. 216, 227–229. Sinauer Associates, Inc., Sunderland, Mass.
18. **Ng, W. V., S. P. Kennedy, G. G. Mahairas, B. Berquist, M. Pan, H. D. Shukla, S. R. Lasky, N. S. Baliga, V. Thorsson, J. Sbrogna, S. Swartzell, D. Weir, J. Hall, T. A. Dahl, R. Welti, Y. A. Goo, B. Leithauser, K. Keller, R. Cruz, M. J. Danson, D. W. Hough, D. G. Maddocks, P. E. Jablonski, M. P. Krebs, C. M. Angevine, H. Dale, T. A. Isenbarger, R. F. Peck, M. Pohlschroder, J. L. Spudich, K. H. Jung, M. Alum, T. Freitas, S. Hou, C. J. Daniels, P. P. Dennis, A. D. Omer, H. Ebhardt, T. M. Lowe, P. Liang, M. Riley, L.**

Hood, and S. DasSarma. 2000. Genome sequence of *Halobacterium* species NRC-1. Proc. Natl. Acad. Sci. USA **97:**12176–12181.
19. **Oesterhelt, D., and G. Krippahl.** 1983. Phototrophic growth of halobacteria and its use for isolation of photosynthetically-deficient mutants. Ann. Inst. Pasteur Microbiol. **134B:**137–150.
20. **Oren. A.** 2001. The order Halobacteriales, p. 2, 6–19. *In* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt (ed.), The prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, 3rd ed. Springer-Verlag, New York, N.Y. [Online.]
21. **Oren, A.** 2002. Halophilic microorganisms and their environments, p. 211. Kluwer Academic Publishers, Dordrecht, The Netherlands.
22. **Perez, A. M.** 2000. Growth physiology of *Haloferax mediterranei* R4 and purification of halocin H4. M.S. thesis. Northern Arizona University, Flagstaff, Ariz.
23. **Rodriguez-Valera, F.** 1995. Cultivation of halophilic archaea, p. 13–16. *In* F. T. Robb, A. R. Place, K. R. Sowers, H. J. Schreier, S. DasSarma, and E. M. Fleishmann (ed.), Archaea, a laboratory manual. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
24. **Rodriguez-Valera, F.** 1993. Introduction to saline environments, p. 1–23. *In* R. H. Vreeland and L. I. Hochstein (ed.), The biology of halophilic bacteria. CRC Press, Boca Raton. Fla.
25. **Shand, R. F., and A. M. Perez.** 1999. Haloarchaeal growth kinetics, p. 411–424. *In* J. Seckbach (ed.), Enigmatic microorganisms and life in extreme environments. Kluwer Academic Publishers, Dordrecht, The Netherlands.
26. **Shand, R. F., and M. C. Betlach.** 1991. Expression of the *bop* gene cluster of *Halobacterium halobium* is induced by low oxygen tension and by light. J. Bacteriol. **173:**4692–4699.
27. **Soliman, G. S. H., and H. G. Trüper.** 1982. *Halobacterium pharaonis* sp. nov., a new, extremely haloalkaliphilic archaebacterium with low magnesium requirement. Zentbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **3:**318–329.
28. **Takai, K., T. Komatsu, F. Inagaki, and K. Horikoshi.** 2001. Distribution of archaea in a black smoker chimney structure. Appl. Env. Microbiol. **67:**3618–3629.
29. **Thompson, D. K., J. R. Palmer, and C. J. Daniels.** 1999. Expression and heat-responsive regulation of a TFIIB homologue for the archaeon *Haloferax volcanii*. Mol. Microbiol. **33:**1081–1092.
30. **Vreeland, R. H., S. Straight, J. Krammes, K. Dougherty, W. D. Rosenzweig, and M. Kamekura.** 2002. *Halosimplex carlsbadense* gen. nov., sp. nov., a unique halophilic archaeon, with three 16S rRNA genes, that grows only in defined medium with glycerol and acetate or pyruvate. Extremophiles **6:**445–452.
31. **Waino, M., B. J. Tindall, and K. Ingvosen.** 2000. *Halorhabdus utahensis* gen. nov., sp. nov., an aerobic, extremely halophilic member of the Archaea from Great Salt Lake, Utah. Int. J. Syst. Evol. Microbiol. **50:**183–190.