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Variations in rRNA Content of Marine *Vibrio* spp. during Starvation-Survival and Recovery†

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The degree and temporal context of variations in ribosome content during nutrient starvation of two copiotrophic marine bacteria, *Vibrio alginolyticus* and *Vibrio furnissii*, have been examined. The organisms were starved either by nutritional shift-down or by consumption of limiting nutrients resulting from growth into stationary phase. Measurements of the amount of hybridization to 16S rRNA-specific probes revealed that the cells retained between 10 and 26% of their original rRNA content after 15 days of starvation. In *V. alginolyticus*, losses in stationary-phase cells occurred rapidly (1 to 2 days), whereas cells shifted into starvation remained larger and retained considerably more rRNA. The ability of *V. alginolyticus* to recover from starvation was assessed after cells were maintained for 2, 8, and 15 days in nutrient-depleted medium. The pattern of recovery at the level of rRNA accumulation depended upon the duration of nutrient deprivation and the manner in which it was imposed. Stationary-phase cells starved for 2 days had only slight relative increases in rRNA levels after excess nutrients were added. As the duration of starvation lengthened to 8 and 15 days, increasingly greater amounts of rRNA (30 and 70 times preenrichment values, respectively) were transcribed after nutrient enrichment. Shift-down cells recovered from 2 and 8 days of starvation without extensive rRNA production. After 15 days, nutrient enrichment caused 16S rRNA levels to increase 30-fold. The results indicate that the mechanisms controlling starvation-survival in these marine bacterial species are linked to the physiological state at the onset of starvation and that the subsequent pattern of recovery will depend upon how starvation was initiated.

Over the past decade, renewed attention has been focussed on understanding the series of distinct subcellular events which occur as nondifferentiating bacteria deplete essential nutrients and deviate from steady-state balanced growth. Physiological variability during different stages of growth on a limiting substrate is well documented (28, 42), as are many of the phenomena that occur when nutrient deprivation leads to starvation (34). Morphological, physiological, and molecular biological variations have all been studied over various time scales after the onset of starvation in different organisms (21, 29, 31). Elucidating these responses, as well as how cells recover from starvation, is thought to be particularly important to interpreting how bacterial growth occurs in natural aquatic environments such as the oceans, where nutrients are available transiently or in very low concentrations (21, 30, 31, 38).

Because of their prevalence in the marine environment, as well as their causative role in a number of diseases (4, 7), *Vibrio* spp. have been used extensively as models of growth and starvation responses in bacteria. Details of distinct morphological changes leading to the production of extremely small cells (5), alterations in basal macromolecular pools (14, 17, 37), and changes in key metabolic capabilities (12, 25, 41) have been noted, as has the production of specific, starvation-related families of proteins (18, 34). Aspects of the recovery of starved cells have been reported as well (1–3).

One of the many features of the "starvation response" exhibited by some species is the metabolism of macromolecular constituents. For example, degradation of ribosomes during the course of starvation of enteric bacteria has been known for many years (26, 27). Kaplan and Apirion (19, 20) described the pattern of ribosome dissociation and subsequent rRNA degradation to nucleotides that occurred after *Escherichia coli* was starved; the process was initiated within minutes after the cells were washed and resuspended in medium lacking essential nutrients. More recently, Davis et al. (9) examined ribosome degradation and cell mortality in phosphate-limited *E. coli* during stationary phase. They found that loss of viability followed the degradation of ribosomes, and this led to the hypothesis that the adaptive advantage gained from metabolizing unused ribosomal material is balanced against unrecoverable losses in protein synthetic capacity. Somewhat different patterns have been noted in studies of *Vibrio* spp. (2, 11). Washed cells resuspended in starvation medium retained viability over long time frames (e.g., 10 days) despite decreases in the level of total RNA. In addition, cells retained a residual ribosome population that was seemingly important in the recovery process.

The relationship between ribosomes and the growth of bacterial cells has historically been among the most well-characterized physiological systems, as reported in previous studies (6, 24, 33). Those studies and others emphasize the status of the ribosome fraction as a sensitive molecular biological indicator of physiological state (23). Although conducted predominately with enteric bacteria, such studies provide an essential foundation for investigations of various aspects of growth regulation in heterotrophic marine bacteria. One important aspect of these investigations is to elucidate how bacterial cells common in the marine environment respond to periods of nutrient deprivation and subsequent resupply—specifically, to discern how the magnitude and timing of physiological variations occur as cells deviate

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from steady-state conditions. Changes in the ribosome fraction should serve as a primary marker for these physiological variations. Herein, we report the results of investigations of the degree of ribosome degradation during starvation-survival, as well as ribosome synthesis during subsequent recovery from starvation.

Two phylogenetically related, copiotrophic strains, *Vibrio alginolyticus* and *Vibrio furnissii* (43), were studied during periods in which they were deprived of nutrients and after enrichment following starvation. The diverse and predominately nonculturable mixed communities known to be present in the sea (4, 13, 40) predict that a similarly diverse set of physiological adaptations exists (16). Although studies of culturable strains are inherently limited in their ability to reflect this heterogeneity, they can provide essential clues to the potential mechanisms underlying physiological diversity and to the time frames within which variations occur.

MATERIALS AND METHODS

Growth conditions. Stock cultures of *V. alginolyticus* (ATCC 17749) and *V. furnissii* (ATCC 35016) were maintained on Marine Agar 2216 (Difco, Detroit, Mich.). Prior to the start of nutrient depletion studies, the strains were grown overnight in a minimal artificial seawater medium (44) which had been amended with 0.1% (wt/vol) Bacto Peptone and 0.1% (wt/vol) yeast extract (termed ASWPY). These cultures were inoculated into fresh ASWPY and subsequently diluted in a semicontinuous fashion to extend exponential growth through 4 to 5 mass doublings, as assayed by changes in culture optical density at 600 nm (OD_{600}). The final late-exponential-phase cultures were harvested by centrifugation (4,500 × *g*, 10 min). The cell pellets were washed three times with minimal seawater medium lacking Bacto Peptone and yeast extract (termed ASW) and then resuspended in 50 ml of ASW. This cell suspension was then used to inoculate two 1,400-ml cultures. One, established as a long-term depletion culture, consisted of ASWPY inoculated to an initial OD_{600} of 0.05. It was allowed to grow into stationary phase. The second consisted of ASW inoculated to a starting OD_{600} of 0.20. This culture maintained the "shift-down" state achieved by washing the cells. Both cultures were subsequently maintained for a period of 15 days at 25°C with agitation (100 rpm).

Cell enumeration. Aliquots of cultures were collected aseptically, preserved with filtered (0.2-μm pore size) formalin (0.2% [vol/vol] final concentration), and stored at 4°C. Appropriate dilutions were made in particle-free 2.5% NaCl before staining with acridine orange (15). Clumped cells present in starved cultures of *V. furnissii* required an additional disaggregation procedure. The original samples were diluted in cell-free 2.5% NaCl to which was added 0.05% Tween 20 and 0.2 mg of Pronase (Sigma Chemical Co., St. Louis, Mo.) per ml. The dilutions were incubated at 37°C for 10 min, after which they were passed repeatedly through a 26-gauge needle. The final dilutions of both strains were filtered onto 0.2-μm-pore-size polycarbonate membrane filters previously stained with irgalan black (Nuclepore Corp., Pleasanton, Calif.). Cell counts were performed at a magnification of ×1,250 under epifluorescent illumination.

Shift-up experiments. The physiological response to nutrient addition was examined in shift-up experiments performed after the cultures were incubated for 2, 8, and 15 days. One-hundred-milliliter aliquots were transferred to sterile containers and amended with sterile glucose and Casamino Acids (Difco) to final concentrations of 0.1% (wt/vol). Subsamples were collected after selected incubation periods during the following 12 h in order to assess physiological responses.

RNA preparation. Samples for RNA purifications were obtained by harvesting cells contained in 2- to 8-ml aliquots by centrifugation (14,000 × *g*, 5 min). Pellets were washed once (0.4 M NaCl, 0.05 M EDTA [pH 8.0]), resuspended in extraction buffer (0.1 M LiCl, 0.05 M Tris [pH 7.5], 0.03 M EDTA, 2% sodium dodecyl sulfate [SDS]), and rapidly frozen to −80°C for storage. Subsequently, frozen cell pellets were thawed at 65°C and then heated for an additional 5 min to fully lyse the cells. The lysate was extracted once at 65°C (5 min) and once at room temperature (5 min) with acidic phenol (equilibrated against 0.05 M sodium acetate [NaOAc; pH 5.6]). This procedure was followed by one extraction with chloroform-isoamyl alcohol (24:1). In all cases, phase separation was accelerated by centrifugation (14,000 × *g*, 4 min). The final aqueous phase was ethanol precipitated. The pellets obtained after centrifugation were dried in vacuo and resuspended in distilled water to which 10× DNase buffer (1.0 M NaOAc [pH 5.6], 0.1 M $MgCl_2$) and 35 U of RNase-free DNase (Boehringer Mannheim, Indianapolis, Ind.) were added. The samples were then incubated at 37°C for 30 min and extracted once each with phenol and chloroform (both at room temperature) before a final ethanol precipitation. The resulting pellets were resuspended in distilled water and stored at −80°C. RNA concentrations were determined spectrophotometrically by measuring the absorbance in a 100-μl microcuvette at 260 nm. Standard precautions against RNase contamination were observed throughout the purification protocol (39).

Polymerase chain reaction (PCR) amplification of 16S rDNA and probe preparation. To generate a probe for Northern (RNA) hybridizations, a 1.5-kb fragment encoding the 16S rRNA subunit was amplified from DNA purified from each species. The oligonucleotide primers utilized were identical to those designated "A" and "H*" as described by Edwards et al. (10). The 20-base oligonucleotides were synthesized on a MilliGen 7500 DNA synthesizer (MilliGen Corp., Bedford, Mass.). *Taq* DNA polymerase and reaction buffer were obtained from Promega Corp. (Madison, Wis.). Approximately 100 ng of genomic DNA was added to each 50-μl reaction volume, which was then overlaid with mineral oil. Amplifications were carried out in a temperature cycler (Ericomp, Inc., San Diego, Calif.) programmed for 40 cycles consisting of denaturation at 94°C for 2 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min. The reaction products were separated from primers electrophoretically. The appropriately sized bands were excised from the agarose gel, and the DNA fragments were purified with a Geneclean kit (Bio 101, La Jolla, Calif.). The fragments were ^{32}P labelled with a random-primed DNA labelling kit following the manufacturer's instructions (Boehringer Mannheim). Unincorporated nucleotides were removed by passing the reaction mixtures through Sephadex G75 columns. In preliminary experiments (data not shown), the probes were hybridized to total RNA separated on morpholinepropanesulfonic acid (MOPS)-formaldehyde-agarose gels and blotted to nitrocellulose. After high-stringency washes (0.1× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–1% SDS, 65°C, 18 h), autoradiography revealed a single band corresponding to the 16S rRNA subunit.

Dot blot preparation. Samples of purified RNA were diluted with distilled water and then combined with 3 volumes of denaturation buffer (66% deionized formamide, 21% formaldehyde, 13% 10× MOPS buffer [0.2 M MOPS, 0.05 M

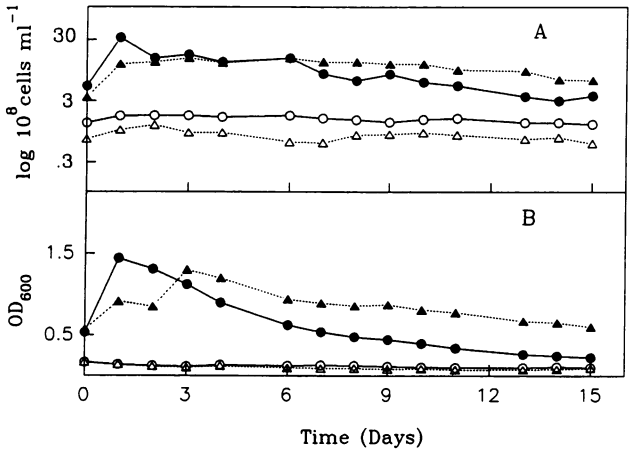

FIG. 1. Variations in culture characteristics of marine *Vibrio* spp. during long-term starvation. (A) Log cell numbers (total cell count); (B) culture OD_{600}. Symbols denote stationary-phase (●) and shift-down (○) *V. alginolyticus* and stationary-phase (▲) and shift-down (△) *V. furnissii*.

NaOAc, 0.01 M disodium EDTA, pH 7.0]), and incubated at 65°C for 5 min. They were immediately diluted with 1 volume of ice-cold 20× SSC (3.0 M NaCl, 0.3 M sodium citrate) and chilled on ice for 5 min before blotting to pure nitrocellulose membranes (Hybond-C; Amersham, Arlington Heights, Ill.). Blots were made and washed (repeated 200-μl aliquots of ice-cold 10× SSC followed by 5× SSC) by using a filtration manifold. The membranes were air dried and baked under a vacuum at 80°C for 2 h.

Northern hybridization and quantitation. Baked filters were sealed in individual bags with 10 ml of hybridization solution (6× SSPE [1.1 M NaCl, 0.06 M NaH_2PO_4, 6.0 mM EDTA], 5× Denhardt's solution [0.25 g of Ficoll per liter, 0.25 g of polyvinylpyrrolidone per liter, 0.25 g of bovine serum albumin per liter], 0.1% SDS, 0.1 mg of boiled, sonicated salmon sperm DNA per ml) and prehybridized for 18 h at 65°C before fresh hybridization buffer and the probe were added. Hybridizations were carried out at 65°C for 12 to 18 h, after which the filters were washed at high stringencies (0.5× SSC, 65°C, 12 to 18 h). Autoradiograms were made of the washed filters to confirm probe excess as well as the absence of nonspecific binding to the nitrocellulose. Afterwards, a triplicate series of 20-ng blots from each time point was cut from the filters and the radioactivity in each blot in 4 ml of Bio-Safe II scintillation cocktail (RPI Inc., Mt. Prospect, Ill.) was assayed. All blotting, hybridizations, and quantitations were performed twice with separately amplified and labelled probes. The range of counts of the triplicate blots for each sample was approximately 9% of the mean values.

RESULTS

Long-term depletion cultures. The physiological response to nutrient deprivation exhibited by cells during the course of this study was species dependent and was influenced by the manner in which starvation was imposed.

At the cellular level, greater variations occurred in stationary-phase cultures than with the shift-down treatment (Fig. 1). Both species exhibited reductions in cell mass as indicated by decreased culture absorbance (OD_{600}). There was also a decrease in the population size of stationary-phase *V. alginolyticus* (Fig. 1A). Shift-down cultures varied little over the time course; population density and OD_{600} remained constant. Of note were marked reductions in cell size (data not shown) for the shift-down and stationary-phase treatments *V. furnissii*. In addition, the cells exhibited a tendency to aggregate. "Miniaturization" (31) was also observed for stationary-phase *V. alginolyticus*; however, when shifted down into starvation medium, the organism remained approximately the same size for most of the time course. It was only in the latter portion of the experiment (after day 12) that miniaturization became evident. Aggregation was not apparent in either case.

Decreases in cellular RNA content occurred rapidly with the stationary-phase treatment of *V. alginolyticus* (Fig. 2A). Within 3 days, the RNA content of the cells had dropped to approximately 14% of the initial value. By the conclusion of

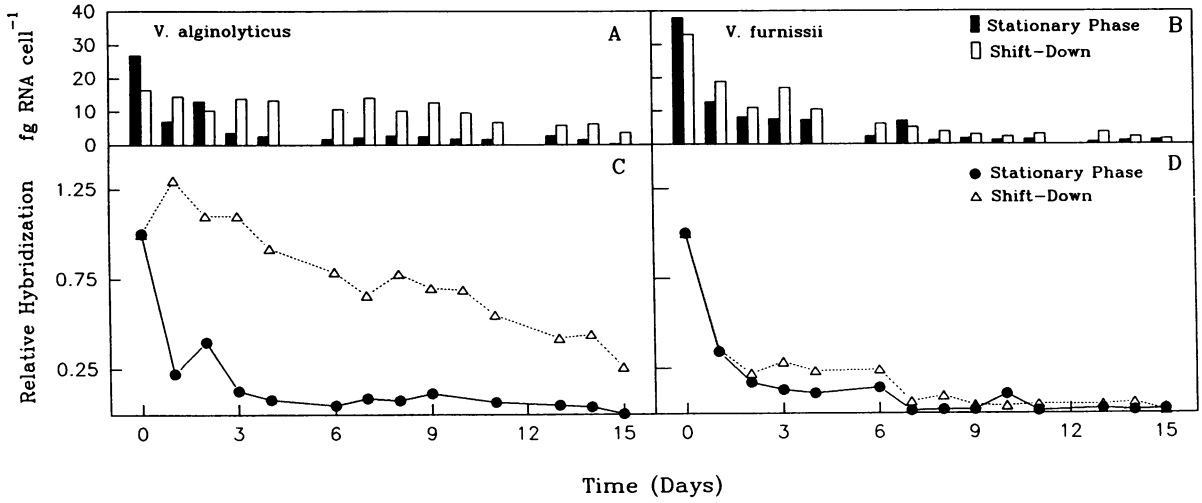

FIG. 2. Variations in subcellular characteristics of marine *Vibrio* spp. during long-term starvation. Total cellular RNA content of *V. alginolyticus* (A) and *V. furnissii* (B) and relative hybridization of 16S rRNA probe normalized to cell number and the initial time point (4 h) for *V. alginolyticus* (C) and *V. furnissii* (D) are shown.

the experiment, the RNA content of the cells had decreased approximately 99% (from 27.0 to 0.3 fg cell^{-1}). However, this was not the case with the shift-down treatment (Fig. 2A). After 7 days of starvation, those cells retained approximately 50% of their original RNA content. During the second half of the time course, RNA content decreased to 3.7 fg cell^{-1} (14% of the original content), a value 10-fold greater than that of the parallel stationary-phase cells. These changes at the subcellular level in *V. alginolyticus* and *V. furnissii* cells were much more pronounced than the apparent population responses as determined by cell counts and culture optical density (Fig. 1).

Both treatments of *V. furnissii* resulted in similar responses, as the cellular content of RNA decreased from approximately 25 to 1.5 fg cell^{-1} during the 15-day incubation period (Fig. 2B). This reflects an approximate 95% decrease in cellular RNA content.

In order to determine how rRNA content varied after the cells were exposed to starvation conditions, all cultures were sampled daily, starting 4 h after the washed cells were inoculated into the flasks containing the respective experimental treatments. This time corresponded to early stationary phase for cells inoculated into ASWPY. Samples were taken over a period of 15 days. RNA purified from these samples was processed for quantitative Northern dot blot analysis. These blots were subsequently hybridized to the PCR-generated probes to determine 16S rRNA content. Because increasingly larger numbers of cells were needed to provide the equal 20-ng loadings to nitrocellulose over the course of the experiment (a result of decreased cellular RNA content during starvation), hybridization data were normalized to cell number. These values were then expressed relative to the initial value. The stationary-phase culture of *V. alginolyticus* and both *V. furnissii* treatments exhibited similar patterns (Fig. 2C and D, respectively). 16S rRNA content decreased rapidly over the first 3 days. By the sixth day of the study, cells had less than 10% of their initial content of rRNA. At the conclusion of the experiment, the cells retained approximately 2% of their original 16S rRNA content. Conversely, *V. alginolyticus* cells shifted into starvation retained more ribosomal material (Fig. 2C). A detectable decrease occurred in three phases, however. There was little change over the first 4 days, a 20% loss over the next 6 days, and finally a more rapid decline over the last 5 days of the experiment. After incubation for 15 days, the cells retained 26% of their initial 16S rRNA content (Fig. 2C).

Shift-up responses. *V. alginolyticus* and *V. furnissii* were tested for their abilities to respond to nutrient enrichment as a function of length of time under starvation conditions. The primary focus of these experiments was to characterize the timing and magnitude of the accumulation of rRNA during the recovery of cells exposed to starvation by shift-down or growth into stationary phase. In doing so, our intent was to determine whether the manner of exposure to nutrient deprivation was of consequence to the pattern of recovery at the level of rRNA as the cells returned to rapid growth. Because the cells aggregated, considerable effort was required to obtain accurate cell counts of cultures of *V. furnissii*. Thus, the large number of samples required to characterize the shift-up responses prevented a complete set of cell counts from being made for this strain. Accordingly, only data for *V. alginolyticus* are presented herein.

V. alginolyticus recovered from nutrient depletion throughout the time course of the study. The final population density and culture mass attained after nutrient enrichment were similar for both treatments (Fig. 3 and 4). The lag prior

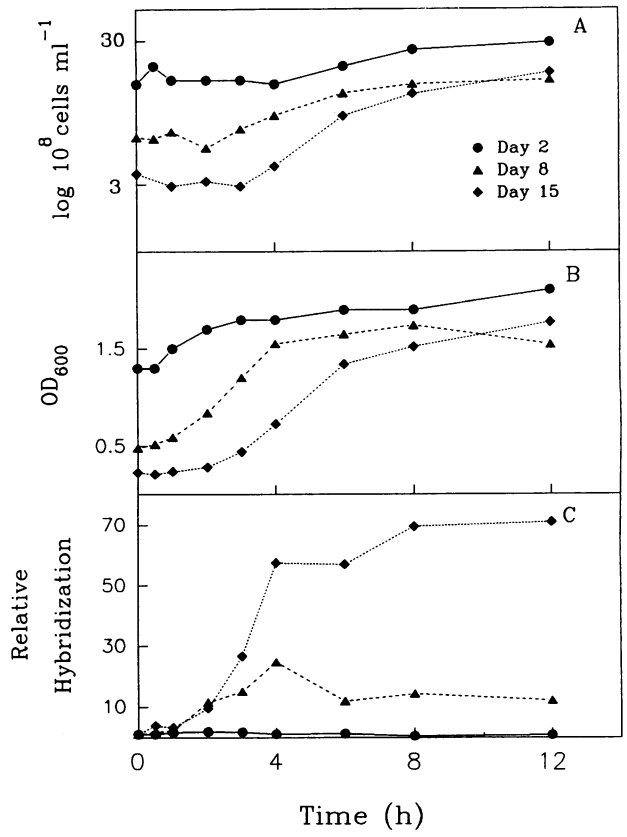

FIG. 3. Shift-up response of *V. alginolyticus* after selected periods of starvation resulting from growth of the culture into stationary phase. (A) Log cell numbers; (B) OD_{600}; (C) 16S rRNA content as indicated by relative hybridization (values normalized to cell number and to the preshift time point (0 h). Duration of starvation: ●, 2 days; ▲, 8 days; ◆, 15 days.

to the onset of cell division was somewhat longer in stationary-phase cultures, however; it ranged from 2 to 4 h (Fig. 3A). In shift-down cultures, the lag time increased from 1 h after 2 days of starvation to 2 h after 8 and 15 days in nutrient-free medium (Fig. 4A).

The pattern of recovery at the subcellular level varied with the manner in which the cells were exposed to nutrient depletion and the duration of starvation (Fig. 3 and 4). For cells in stationary phase, the magnitude of the recovery response increased with increasing starvation time and decreasing rRNA content (Fig. 3). Cells starved for 2 days increased 16S rRNA content approximately twofold. However, after 8 days, there was a much larger (25-fold) increase during the first 4 h after nutrient addition. Cells starved for 15 days showed an even more dramatic rise in 16S rRNA hybridization, with a 60-fold increase occurring over 4 h. By 12 h after nutrient addition, this value increased to more than 75-fold (Fig. 3C). The lag times prior to the rise in accumulation of rRNA increased from 1 to 2 h between days 8 and 15. Total RNA content paralleled the variations in 16S rRNA content (data not shown). During the initial shift-up, there was very little change in total RNA content. By day 8, RNA content increased to a peak of 40 fg cell^{-1} during exponential growth before dropping to 21 fg cell^{-1} as cells reentered stationary phase. The response was similar for cells starved for 15 days; however, RNA content increased over 8 h to

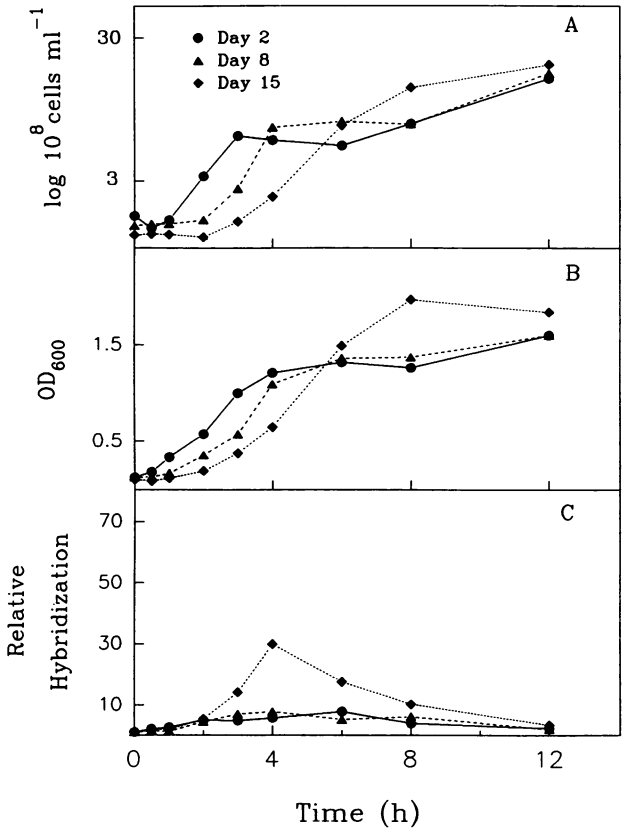

FIG. 4. Shift-up response of *V. alginolyticus* after selected periods of starvation caused by shift-down into a nutrient-free medium. (A) Log cell numbers; (B) OD_{600}; (C) 16S rRNA content as indicated by relative hybridization (values normalized to cell number and to the preshift time point (0 h). Duration of starvation: ●, 2 days; ▲, 8 days; ◆, 15 days.

approximately 50 fg cell^{-1}. Once the cells were in stationary phase, the RNA content declined to 20 fg cell^{-1}.

Cells shifted down into starvation responded differently to nutrient enrichment (Fig. 4). The ribosome content of the cells changed little after nutrient enrichment during the first 8 days of starvation. There was only an approximately sevenfold increase in hybridization over preshift values in both cases. After starvation for 15 days, a much larger response was noted. During the first 4 h after nutrient addition, the 16S rRNA content increased to 30 times the value detected before enrichment (Fig. 4C). Unlike the case with stationary-phase cells, variations in total RNA content were observed with all three experiments with the shift-down treatments. The peak values attained during exponential growth following nutrient addition (58, 64, and 86 fg cell^{-1} for cells starved for 2, 8, and 15 days, respectively) were higher than those observed for stationary-phase cultures. However, once stationary phase was reached, cellular RNA content was quite similar in all cases (15 to 25 fg cell^{-1}).

DISCUSSION

Although both *Vibrio* spp. included in this study are considered copiotrophs, they responded differently to nutrient depletion. Regardless of how starvation was initiated, *V. furnissii* responded in a manner that was similar to that of other bacterial species previously used as models (5, 31, 34). While cell counts remained stable, cell mass and rRNA decreased rapidly in response to nutrient depletion. Miniaturization of cells observed with these cultures was also typical for other starved *Vibrio* spp. (5, 31).

The distinct differences in how *V. alginolyticus* responded to nutrient deprivation that were detected indicate that this species possesses a separate mechanism to control survival during the initial phase of long-term starvation. The cells maintained in stationary-phase culture exhibited both miniaturization and decreases in population density over the course of the 15-day experiment. The apparent loss of some cells may be accounted for by their adherence to the container walls. It is also possible that some cells lysed and provided some minimal requirements needed to maintain those remaining. However, there was no evidence of cryptic growth (28). The population of exponential-phase cells shifted into starvation was more stable. There was little change in cell counts, and direct microscopic examination revealed that cell size remained constant. In addition, cell mass and rRNA content were also retained throughout most of the experiment.

The type strain of *V. alginolyticus* employed in these studies characteristically spreads on solid agar surfaces. Therefore, we were unable to compare culturable cell counts with direct microscopic enumeration (38). However, previous studies with *Vibrio cholerae* (5), as well as with a colonial *V. alginolyticus* strain, 138-2 (41), indicated that populations shifted into starvation had decreased viability (i.e., decreases in plate counts of 1 to 2 orders of magnitude) over 20-day starvation periods. The size of those cells also decreased. More recent studies in which the psychrophilic marine *Vibrio* sp. strain ANT-300 (32) was grown in chemostat and batch cultures prior to shift-down into starvation medium demonstrated three distinct stages in the starvation process; the first (0 to 14 days) was characterized by only moderate (albeit variable) losses in culture viability.

Many studies with enteric bacteria and some other marine *Vibrio* spp. have detailed how rapid shifts into starvation yield global physiological changes (18, 29, 35, 37). These changes include stringent-like responses with concurrent production of ppGpp (35–37) and suites of new starvation-specific proteins (1, 2, 18, 29). Recently, enhanced mRNA stability has been noted with starved *Vibrio* sp. strain S14 (2). The delay in rRNA degradation detected with *V. alginolyticus* during starvation may be part of such a global response. It is unknown at this time whether this delay is due to specific stabilization of ribosomes themselves or to inhibition of the degradative pathways for rRNA subunits (9, 19, 20).

Both stationary-phase and shift-down cultures of *V. alginolyticus* recovered rapidly when nutrients were resupplied to the starvation menstruum. Increased rRNA transcription and cell mass occurred prior to the onset of cell division, reflecting a typical shift-up pattern (8). However, the lag times were shorter than those previously observed for recovery of starved cells of at least one *Vibrio* sp. (3).

Over the course of the 15-day incubation period, stationary-phase cells depleted progressively more ribosomal material. The relatively small decreases in 16S rRNA content after 2 days of starvation were accompanied by a similar degree of rRNA synthesis when nutrients were added. However, as the period of nutrient depletion lengthened, the response increased in turn, mirroring the ongoing losses of ribosomes (Fig. 2C and 3C). Shift-down cells retained higher

rRNA content throughout the study, and the consequences detected during shift-up were proportionately reduced. The responses in cells starved for 2 and 8 days were similar. Extensive changes in 16S rRNA content were not detected until day 15, when the effects of starvation on ribosomes were more pronounced (Fig. 2C and 4C). Although the overall patterns in ribosome content resulting from the two modes of starvation initiation were different, they both emphasize and reflect the well-documented, pivotal roles that ribosomes play in cellular growth regulation (6, 8, 22, 24, 33).

The responses noted during the early portion of starvation of both treatments indicate that *V. alginolyticus* retained sufficient protein synthetic capacity to achieve a faster growth rate without extensive production of ribosomes. However, as the effects of starvation deepened, progressively more essential ribosomal material was lost. Stationary-phase cells deteriorated more quickly than shift-down cells, although after 15 days of starvation considerably more transcription of rRNA was required for recovery in the shift-down culture as well. Maintenance of excess ribosome capacity during slow growth as well as starvation has been proposed by others (2, 11, 22). *Vibrio* sp. strain S14 starved for as long as 200 h synthesized protein despite the presence of rifampin during nutritional shift-up, implying that new ribosomes were not required (2, 11). The results reported herein indicate that in *V. alginolyticus*, a rapid shift-down from exponential growth triggered a response which delayed the onset of extreme ribosome degradation. Davis et al. (9) hypothesized that starved *E. coli* must balance the net gain from metabolizing unused ribosomal material against irretrievable losses. Stationary-phase cells of *V. alginolyticus* continuously lost ribosomal material as starvation progressed. A rapid shift into nutrient-depleted medium, however, initiated a separate response that delayed rRNA degradation. This condition was not permanent, and the pressure of prolonged starvation ultimately overcame the physiological protection offered by this mechanism. Whereas cells undergoing both treatments were able to recover and achieve rapid growth rates, the pattern of recovery at the level of rRNA accumulation reflected the degree and manner of starvation. Transient increases in rRNA transcription and accumulation reflect the dynamics of variable physiological states in batch growth after shift-up and the inevitable return to stationary phase.

Morita (31) noted that there are a variety of possible responses to nutrient starvation. The results reported herein indicate that more than one mechanism may exist among members of a genus and even the same species, depending upon how starvation was induced. The results of studies of *V. alginolyticus* and *V. furnissii* stress that the timing of variations in the ribosome fraction during starvation and recovery is quite rapid and linked to both the existing physiological state as well as the physiological history of the organism. Assessments of changes in rRNA provide a direct and sensitive measure of this process.

Interpreting how other heterotrophic bacteria respond to nutrient flux in the natural environment will require a better understanding of the degree and timing of these responses as well as the molecular biological mechanisms underlying them. A net ecological interpretation will be possible only when additional information regarding these complex phenomena becomes available from other copiotrophic as well as oligotrophic bacteria.

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