Energetics of Streptococcal Growth Inhibition by Hydrostatic Pressure

PHILIP MATSUMURA[1] AND ROBERT E. MARQUIS*

Department of Microbiology, The University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Growth of *Streptococcus faecalis* in complex media with various fuel sources appeared to be limited by the rate of supply of adenosine-5'-triphosphate (ATP) at 1 atm and also under 408 atm of hydrostatic pressure. Growth under pressure was energetically inefficient, as indicated by an average cell yield for exponentially growing cultures of only 10.7 g (dry weight) per mol of ATP produced compared with a 1-atm value of 15.6. Use of ATP for pressure-volume work or for turnover of protein, peptidoglycan, or stable ribonucleic acid (RNA) did not appear to be significant causes of growth inefficiency under pressure. In addition, there did not seem to be an increased ATP requirement for ion uptake because cells growing at 408 atm had significantly lower internal K^+ levels than did those growing at 1 atm. Pressure did stimulate the membrane adenosine triphosphatase (ATPase) or *S. faecalis* at ATP concentrations greater than 0.5 mM. Intracellular ATP levels were found to vary during the culture cycle from about 2.5 μmol/ml of cytoplasmic water for lag-phase or stationary-phase cells to maxima for exponentially growing cells of about 7.5 μmol/ml at 1 atm and 5.5 μmol/ml at 408 atm. N,N'-dicyclohexylcarbodiimide at a 10 μM concentration improved growth efficiency under pressure, as did Mg^{2+} or Ca^{2+} ions at 50 mM concentration. These agents also enhanced ATP pooling, and it seemed that at least part of the growth inefficiency under pressure was due to increased ATPase activity. In all, it appeared that *S. faecalis* growing under pressure has somewhat reduced ATP supply but significantly increased demand and that the inhibitory effects of pressure can be interpreted largely in terms of ATP supply and demand.

Hydrostatic pressure plays a major role in marine ecology in that it restricts colonization of deep waters and sediments by nonbarotolerant organisms. Pressure in the ocean increases by about 1 atm for every 10 m of depth to a maximum of about 1,160 atm. The average pressure on the ocean floor is about 380 atm, and this pressure is inhibitory for growth of most bacteria, especially at the low ambient temperatures of the deep ocean. Microbial barophysiologists have been concerned for nearly a century with questions of just how pressure inhibits growth and the ways in which barotolerant organisms differ from closely related barosensitive ones. For eucaryotic microorganisms, a major determinant of barotolerance appears to be the sensitivity of microtubular structures to pressure disaggregation (18, 24). Disaggregation results in loss of motility, rounding up of cells, and inhibition of chromosome separation. For procaryotic cells, which do not contain microtu-

bules, pressure inhibition of growth is generally thought to be the result of interference with biosynthetic processes. However, it is probable that under at least some nonoptimal growth conditions, denaturation or disaggregation of polymeric structures may be important in procaryotes as well as in eucaryotes (13, 19). The procaryotic biosynthetic process that has been found to be most barosensitive is protein synthesis (12, 20), and this finding had led to the conclusion that growth inhibition by pressure is mainly a reflection of inhibition of protein synthesis (2, 21).

Our previous work (14, 17) with *Streptococcus faecalis* indicated that the barotolerance of this particular bacterium could be altered markedly simply by changing the source of fuel for growth. For example, the bacterium was extremely barosensitive when pyruvate was the fuel, and it would not grow at pressures greater than about 200 atm. With glucose as fuel, growth occurred at pressures as great as 550 atm. If 50 mM $CaCl_2$ or $MgCl_2$ was added to

[1] Present address: Biology Department, University of California at San Diego, La Jolla, CA 92037.

the glucose medium, growth was possible at pressures as high as 750 atm at 30°C or, as we have found subsequently, 900 atm at 37°C. Clearly, this is a remarkable range of barotolerance for a single organism, and it is not in accord with the notion that pressure inhibition of growth is due primarily to inhibition of protein synthesis. In this paper, we examine further the energetics of streptococcal growth under pressure and the role of adenosine-5′-triphosphate (ATP) supply in barotolerance.

MATERIALS AND METHODS

Bacterium and growth conditions. *Streptococcus faecalis* ATCC strain 9790 was grown in the tryptone-Marmite medium we have described previously (14). The organism grows very poorly in this medium unless some readily catabolized fuel source is added. For the experiments described here various sugars were added to the medium to yield a concentration of 10 g/liter. The sugars were sterilized separately and, after addition, the pH of the medium was adjusted to 7 with KOH solution. Unless indicated otherwise, the growth temperature was 30°C.

Cultures to be pressurized were placed in sterilized plastic syringes or pliable plastic vials with screw caps (Costar liquid nitrogen vials from Fisher Scientific Co., Pittsburgh, Pa.). With plastic syringes, it was necessary to introduce vent holes at the base of the plastic plunger so that air trapped in the rubber tip could escape during pressurization. Syringes and vials were filled in such a way that no air remained in contact with the culture. They were then placed in standard pressure chambers and compressed by the use of an hydraulic pump. The exact procedure has been described in detail (13). The syringes were fitted with hypodermic needles that were jabbed into rubber stoppers to make a seal. Samples were obtained by depressurizing the cultures, removing a vial or squirting out some of the culture from a syringe, and then repressurizing the cultures. The pressurization-depressurization procedure seems not to be harmful to bacteria and does not affect their growth (13).

Assay procedures. Lactic acid was assayed enzymatically by use of lactic dehydrogenase (4). Glucose was assayed by use of the Glucostat reagents of Worthington Biochemical Corp. (Freehold, N.J.). Ribose was assayed by means of the orcinol reaction (6), and the microdiffusion technique of Conway (7) was used to assay ammonia. Lactose was assayed by use of β-galactosidase in conjunction with the Glucostat reagents.

For determinations at ATP pools, 0.5-ml samples were mixed with 0.5-ml portions of ice-cold, 0.46 M formic acid solution containing 2 mM ethylenediaminetetraacetate. The samples were allowed to stand after vortex mixing for 30 min in an ice bath. Then they were centrifuged, and the supernatant fluid was assayed for ATP with the luciferin-luciferase preparation of Boehringer-Mannheim Corp. (New York, N.Y.). The luciferin-luciferase was dis-

solved to yield a 5-mg/ml solution in 0.1 M *N*-tris(hydroxymethyl)methyl-2-aminoethane-sulfonic acid buffer (pH 7.4) plus 10 mM MgSO$_4$. Formic acid extracts were diluted 1:10 with *N*-tris-(hydroxymethyl)methyl-2-aminomethane-sulfonic acid buffer plus 10 mM MgSO$_4$ solution; the mixture was placed in a test tube in an Aminco (Silver Spring, Md.) 10-222 microphotometer. A 0.1-ml sample was added with a push-button syringe through a special port in the instrument, and the peak intensity of the resultant light was recorded for use in estimating ATP concentration. The method proved to be very sensitive, and as little as 5 pmol of ATP could be assayed.

The membrane adenosine triphosphatase (ATPase) of *S. faecalis* was isolated essentially according to the procedure of Abrams (1), which involves separation of membrane fragments from osmotically lysed protoplasts. The membranes were washed twice with 1 mM MgCl$_2$ solution, suspended in MgCl$_2$ solution to give a suspension with 2 mg (dry weight) of membranes per ml, and frozen at −10°C until needed. ATPase assay mixtures consisted of one part enzyme-membrane preparation and one part ATP solution in 0.05 M citrate buffer, pH 6.5. The reaction mixtures were loaded into test tubes fitted with serum caps, and all air was removed with a syringe. The tubes were incubated at 1 atm or under pressure, and samples were removed at intervals. Sampling required less than 2 min. Reaction rates were constant for up to 60 min. Glass beads were added to make up the lost volume due to sampling. To stop the reaction, equal volumes of 10% trichloroacetic acid solution were mixed with samples. The mixtures were centrifuged, and the phosphate contents of supernatant fluids were assessed by the Fiske-SubbaRow method (8). Reaction velocities were then considered to be the amounts of phosphate released per unit of time per milliliter of the mixture.

Intracellular levels of K$^+$ were estimated by the method described previously (15), which involves acid extraction of washed cells and K$^+$ assay by atomic absorption spectrophotometry. For these assays, the cells were washed with cold water; the use of sucrose solutions for washing did not enhance K$^+$ retention.

Dry weights of cells were estimated by centrifuging culture samples, washing the cells once with deionized water, and then drying them to constant weight in a vacuum oven. Y$_{ATP}$ values were estimated with use of plots of mass increase versus time and ATP production versus time. Y$_{ATP}$ is expressed as grams (dry weight) of cells produced per mole of ATP consumed.

Assessment of polymer turnover. A 10-ml culture of *S. faecalis* was grown to stationary phase at 30°C in the defined medium described by Shockman (25) with either [^{14}C]proline (New England Nuclear Corp., Boston, Mass.) or [^{14}C]uracil (ICN Corp., Irvine, Calif.). The medium for protein labeling contained 450 μM [^{14}C]proline (0.222 μCi/μmol), and the medium for ribonucleic acid (RNA) labeling contained 268 μM [^{14}C]uracil (0.372 μCi/μmol). These initial cultures were diluted 1:2 with fresh, radioac-

tive medium and incubated for 1 to 2 h at 30°C to allow the cells to begin exponential growth. They were then aseptically centrifuged and washed once with deionized water, and the cells were suspended in 300 ml of fresh medium with 1,350 μM unlabeled proline or 804 μM unlabeled uracil. These diluted cultures were drawn up into syringes and incubated at 1 atm or under pressure. At intervals, samples were taken and centrifuged. Samples of the supernatant fluids were added to Aquasol (New England Nuclear Corp.), and their radioactivity was determined by use of a Beckman (Palo Alto, Calif.) LS-230 liquid scintillation counter.

RESULTS

The rate-limiting process in streptococcal growth. The data presented in Fig. 1 indicate that exponential growth rates of *S. faecalis* in complex media with various degradable sugars can be related directly to rates of ATP supply from catabolism. Glucose, galactose, and trehalose were degraded rapidly at 1 atm, and their catabolism supported rapid growth. Sucrose, maltose, and ribose were degraded less rapidly, and growth in media with these sugars was correspondingly slower. Lactose was degraded less rapidly, and growth was even slower in lactose medium. Here rates of ATP production were calculated from estimates of substrate utilization and product formation. *S. faecalis* is homofermentative under most growth conditions and metabolizes sugars via the Embden-Meyerhof pathway (3). Experiments with radioactively labeled substrates have indicated (3) that the fuel source is not used to any significant extent as a carbon source for growth in complex media. Therefore, ATP production rates during catabolism of most of the sugars used were simply equal to lactate production rates. Exceptions were ribose, lactose, and trehalose. Ribose was degraded to lactate and acetate with production of approximately 1.67 mol of ATP per mol of lactate (9). Lactose was degraded at a sufficiently slow rate at 1 or 408 atm so that arginine degradation reactions were activated. We estimated the extent of arginolysis from the amount of ammonia produced and then assumed that the organism produced 1 mol of ATP for every 2 mol of ammonia (9). At 408 atm, about 70% of the ATP produced by cells in lactose medium was derived from arginine breakdown, compared with a value of about 25% at 1 atm. For cells in trehalose medium, arginolysis made a significant contribution to ATP supply at 408 atm but not at 1 atm. Slow growth and catabolism can result also in reduced lactic dehydrogenase activity and activation of pyruvate clastic reactions (27) so that the bacteria are able to obtain 3 instead of 2 mol of ATP per glucose equivalent catabolized (9).
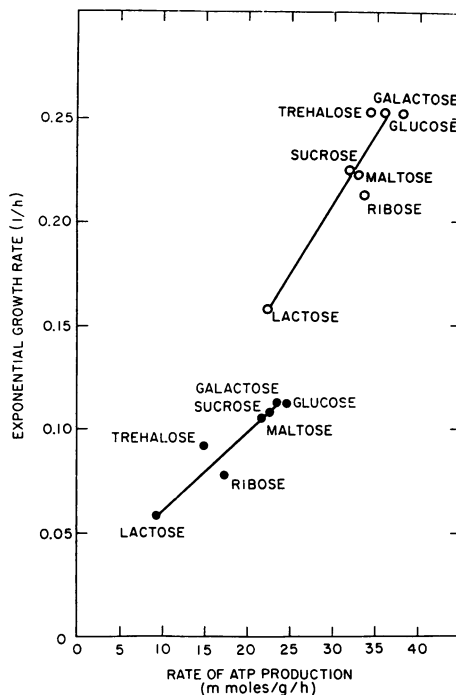

FIG. 1. *Exponential growth rates of S. faecalis 9790 at 30°C in tryptone-Marmite medium with various fuel sources plotted in relation to rates of ATP production. Exponential growth rates were calculated from the slopes of plots of* $\log_{10}$ *absorbance at 700 nm versus time. ATP production rates were estimated graphically, for example, from plots of lactate concentration versus time. Since plots of lactate concentration versus time were exponential, the rate of lactate production at any one time is equal to the initial lactate concentration* $\times k \times e^{kt}$*, where k is the exponential production constant and t is time.* (○) *Values for 1-atm cultures;* (●) *values for 408-atm cultures.*

However, even in lactose media, lactate recoveries were about 80% of those expected for homolactic fermentation; therefore, the amounts of ATP calculated as derived from pyruvate clastic reactions were relatively small components of the total.

Data presented in Fig. 1 indicate that the direct relationship between the rate of ATP production and growth rate holds at 408 atm. Both growth rate and catabolic rates were reduced at 408 atm, the former more than the latter. Thus, for example, the growth rate in glucose medium is reduced by 58%, from 0.24 to 0.11 h^{-1}, whereas the rate of ATP production is reduced only 38%, from 37 to 23 mmol/g per h. Moreover, the ATP requirements for growth under pressure appeared to be greater than those for growth at 1 atm, and the slope of the

408-atm line in Fig. 1 is only about one-half that of the 1-atm line.

Barotolerance and rate of ATP supply. The most commonly used parameter for comparing pressure sensitivities of processes is the apparent activation volume $\Delta V\ddagger$, which can be calculated by use of the following formula:

$$\Delta V\ddagger = [2.30\,RT/P - 1]\log k_1/k_p$$

where R is the gas constant, T is the Kelvin temperature, P is the pressure, and k_1 and k_p are reaction rate constants at 1 atm and under pressure, respectively. When exponential growth rate constants were used to calculate $\Delta V\ddagger$ values for growth, and when these values were plotted in relation to rates of ATP production under pressure (Fig. 2), it is apparent that there was an inverse relationship between pressure sensitivity and ATP supply. In other words, faster catabolic rates resulted in greater barotolerance. There is somewhat of a dilemma here in that growth barotolerance appeared to be determined by the rate of ATP supply, and yet, growth was more sensitive to pressure than was catabolism.

Inefficient growth under pressure. The apparent dilemma was found to be the result of inefficient growth under pressure. Even though the supply of ATP was not reduced as much as was growth, the ATP demand for growth was increased under pressure so that the bacterium

TABLE 1. Y_{ATP} values for exponentially growing S. faecalis cultures in tryptone-Marmite medium with various fuel sources

Fuel source	Y_{ATP}[a]	
	1 atm	408 atm
Glucose	15.0	10.3
Ribose	13.9	9.8
Galactose	15.7	10.5
Lactose	17.7	10.5
Sucrose	15.7	11.2
Trehalose	15.8	12.0

[a] Values were calculated with the use of plots of mass increase versus time and ATP production versus time for exponentially growing cultures.

found itself in a situation of reduced ATP supply and significantly increased demand. The degree of inefficiency under pressure can be readily appreciated from the yield data of Table 1 for exponential-phase cultures at 1 and 408 atm in media with various sugars. The average Y_{ATP} values were 15.6 and 10.7 g (dry weight) of cells per mol of ATP at 1 and 408 atm, respectively. These overall average values are close to those of 15.0 and 10.3 for cultures in medium with glucose. Thus, there appeared to be an approximately 33% reduction in Y_{ATP} at 408 atm.

The bases for growth inefficiency under pressure. Since ATP synthesis in *S. faecalis* involves only substrate-level phosphorylation, there is no possibility for catabolism to be uncoupled from ATP synthesis. Therefore, it seems that part of the ATP produced under pressure must be diverted from biosynthetic pathways. Streptococcal growth and glycolysis have been found (16) to be accompanied by an increase in volume so that some of the glycolytic energy would be diverted to pressure-volume work. However, this diversion was calculated (14) to be only about 0.54% of the energy available from glycolysis at 408 atm.

It appears from the data in Fig. 3 that enhanced turnover of protein or stable RNA under pressure does not represent much of an energetic drain. It can be seen that rates of loss of labeled proline or uracil, which had previously been incorporated into polymers by cells growing in defined medium at 1 atm, were essentially the same at 408 atm as at 1 atm. The rate of protein turnover was less than 1% per h at 1 or 408 atm, or nearly the same as that calculated by Willets (26) for growing *Escherichia coli* cells; turnover was found to be essentially independent of growth rate. Turnover rates for stable RNA were comparably low. However, since cultures at 408 atm grew more
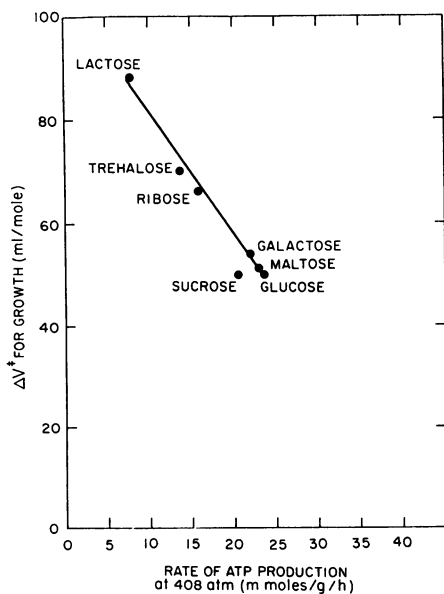

FIG. 2. *Apparent activation volumes* $\Delta V\ddagger$ *for growth of S. faecalis 9790 at 408 atm as a function of ATP production.*

slowly than those at 1 atm, the amount of turn-over per generation under pressure was greater. Still, the energy diverted to this turnover was small. For example, if 60% of the dry cell mass were assumed to be protein, then a protein turnover rate of 1% per h would mean that cells had to synthesize about 6 mg of replacement protein per g (dry weight) per h. Forrest and Walker (9) calculated that the amount of ATP required for protein synthesis is about 0.045 mol/g of protein. Therefore, the energetic drain for turnover would be 0.27 mmol of ATP per g of cells per h. Compared with ATP production rates of 37 and 23 mmol of ATP per g of cells per h for cultures in glucose medium at 1 and 408 atm, this drain is small — only about 1% of the supply. A similar calculation can be made for turnover of stable RNA.

Many bacteria have high rates of turnover of peptidoglycan. Boothby et al. (5) found that $S.$ faecalis has essentially no such turnover at 1 atm, and we could detect no turnover under pressure with cells that had been labeled with [^{14}C]glucosamine. Therefore, it seemed that polymer turnover did not represent a significant ATP drain for cells growing under pressure, unless there was an unusually rapid turnover of compounds such as messenger RNA or lipid.

It is possible that ATP could have been diverted to osmoregulatory functions. The major solute concentrated by $S.$ faecalis is K^+ (15), and ATP is thought to be required for uptake (11). The data presented in Fig. 4 show that the potassium content of $S.$ faecalis was markedly reduced during the entire growth cycle at 408 atm. In this experiment, the cells were growing in glucose medium, and the arrows indicate the times at which exponential growth was completed at 1 or 408 atm. The striking reductions in K^+ pooling under pressure did not appear to be due simply to slower growth because, when growth was slowed by cooling cultures to 15°C, there was a slight enhancement of pooling.

It appears that $S.$ faecalis growing under pressure carries out less concentrative uptake of K^+. However, at present, it is not possible to estimate whether or not this uptake was energetically inefficient at 408 atm. Cells grown at this pressure had essentially the same nucleic acid content as those grown at 1 atm; thus, one would expect that there would not be a decreased need for counter-ions to balance internal polymeric negative charges under pressure. It does seem possible that the striking reduction in K^+ content at 408 atm could adversely affect protein synthesis or other functions that depend on potassium ions (11).
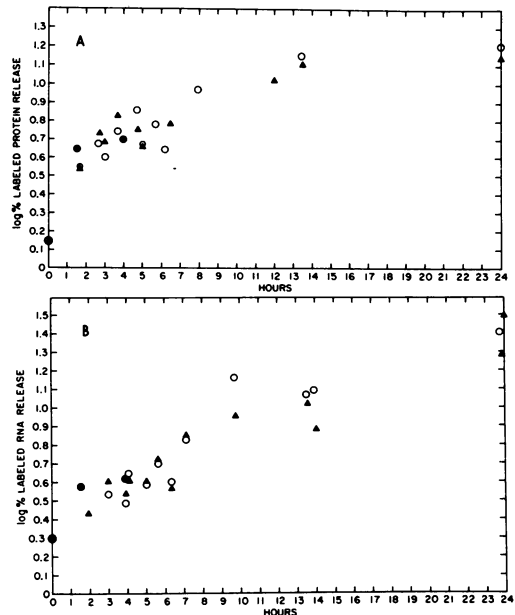

FIG. 3. *Polymer turnover in growing S. faecalis 9790 cells. (A) Release of ^{14}C from cells previously labeled with [^{14}C]proline. (○) Indicate 1-atm values; (▲) indicate 408-atm values. (B) Release of ^{14}C from cells previously labeled with [^{14}C]uracil. (○) Indicate 1-atm values; (▲) indicate 408-atm values.*

$S.$ faecalis contains a membrane ATPase that is able to catalyze hydrolysis of internal ATP (10). As the data of Fig. 5 show, this enzyme was stimulated by pressure at high ATP concentrations greater than about 0.5 mM and inhibited at lower concentrations. Here, isolated membranes were used as enzyme preparations. The response of the enzyme to pressure suggests that substrate binding is accompanied by an increase in volume, whereas activated complex formation involves a decrease in volume. This sort of behavior is exhibited by many enzymes. In this case, 408 atm of pressure caused an increase in apparent K_m from 0.93 to 2.78 mM and also a nearly threefold increase in V_{max}.

ATP pool levels for typical cultures growing at 1 and 408 atm in glucose medium are shown in Fig. 6. At 1 atm, the pool level increased from about 5 μmol/g (dry weight) at the time of inoculation with early stationary-phase cells to about 15 μmol/g shortly before the end of exponential growth at 3.5 h. The exponential growth phase in these particular experiments was relatively short because of the need to use large inocula so that there would be sufficient cells for initial ATP assays. Shortly after growth ceased, there was a sharp reduction in
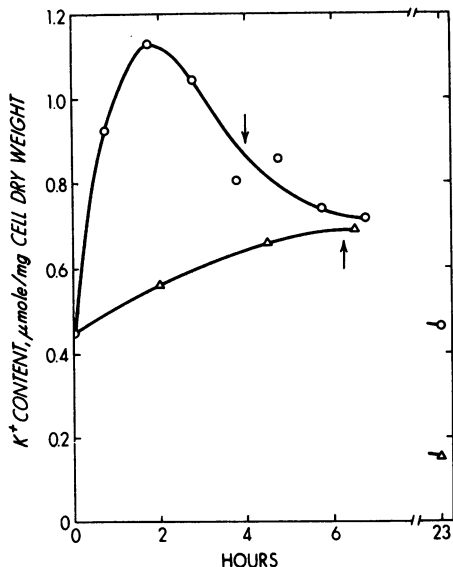
FIG. 4. K^+ contents of S. faecalis cells growing at 30°C in tryptone-glucose-Marmite medium at 1 (O) and 408 (△) atm. The arrows indicate the approximate time at which exponential growth was completed.

the pool level. For S. faecalis, the ratio of intracellular or protoplasmic water to dry weight has been found to be about 2 ml/g (15). Therefore, the maximum and minimum cytoplasmic ATP concentrations should be about 7.5 and 2.5 μmol/ml, respectively. ATP levels during exponential growth at 408 atm were significantly lower than those at 1 atm, and the maximum pool level was only about 11 μmol/g (dry weight) near the end of the exponential growth phase. In longer term experiments, the ATP pool level in stationary-phase cells at 408 atm declined to base levels of only about 5 μmol/g but did so more slowly than at 1 atm.

Although it is clearly impossible to estimate accurately the ATP concentration in the immediate vicinity of the membrane ATPase in an intact cell, it seems that cytoplasmic ATP concentrations during exponential growth must have been greater than 0.5 mM. Thus, one would expect that pressure would be stimulatory and that some of the inefficiency of growth under pressure may be due simply to hydrolytic loss of ATP catalyzed by a stimulated membrane ATPase.

Effects of barotolerance-enhancing agents. N,N'-dicyclohexylcarbodiimide (DCCD) is a potent inhibitor of the membrane ATPase of S. faecalis that can inhibit degradation of intracellular ATP (11). When 10 μM DCCD was added to S. faecalis cultures in glucose medium, growth was not significantly inhibited,

but there was a general increase in ATP pool levels with maxima of 20 and 15 μmol/g at 1 and 408 atm, respectively. DCCD also improved the efficiency of growth under pressure. Aver-
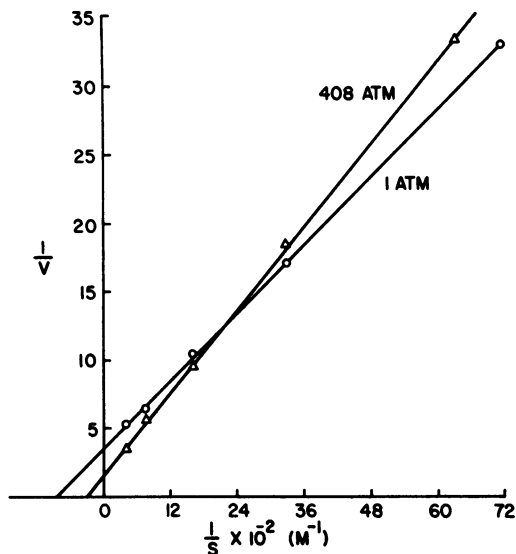

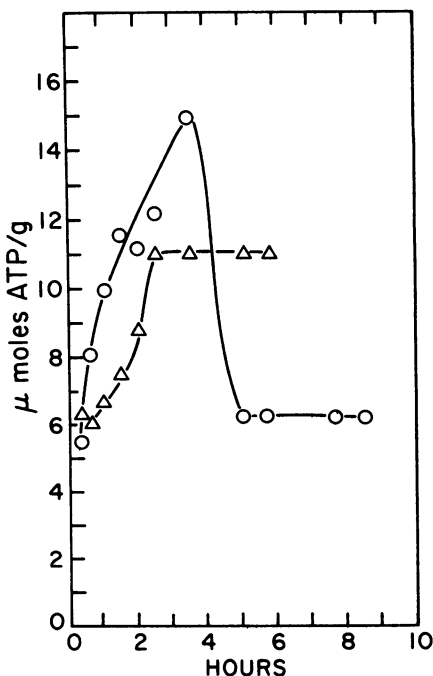FIG. 5. Double reciprocal plot of velocity versus substrate concentration for the membrane ATPase of S. faecalis 9790.

FIG. 6. ATP pool levels of S. faecalis 9790 cells growing in tryptone-glucose-Marmite medium at 30°C and 1 (O) or 408 (△) atm.

age Y_{ATP} values for cultures to which 10 μM DCCD had been added were 13.6 g/mol at 408 atm, compared with essentially the same value (13.4 g/mol) at 1 atm.

Mg^{2+} and Ca^{2+} have been found (17) to enhance barotolerance of *S. faecalis* in terms of both reduced $\Delta V \ddagger$ for growth and higher maximum growth pressures. Average Y_{ATP} values at 1 and 408 atm for cultures in glucose medium supplemented with 50 mM $MgCl_2$ were 15.6 and 15.0 g/mol. These ions also enhanced ATP pool levels to approximately the maximum attained in the presence of DCCD, and it is possible that they may have acted by inhibiting membrane ATPase, which is sensitive to the ATP-divalent cation ratio.

In this series of experiments, we were able to grow *S. faecalis* at pressures as high as 900 atm at 37°C in glucose medium supplemented with 50 mM Mg^{2+} or Ca^{2+}. The cells grown at 900 atm were greatly enlarged, thus appearing as if cell division was more severely inhibited than was cell growth. No such selective inhibition occurred at 408 atm (17). Rates and extents of growth at 900 atm were only about 10% of those at 1 atm, but still it appears that *S. faecalis* can be ranked among the most barotolerant bacteria known.

DISCUSSION

The data presented in this paper provide a basis for interpreting what had previously been a dilemma, namely, that growth of *S. faecalis* is more pressure sensitive than is catabolism, and yet it is possible to alter markedly the barosensitivity of growth simply by changing the fuel source. If we consider specifically growth in glucose medium, the exponential growth rate constant was decreased from 0.24 h^{-1} at 1 atm to about 0.10 h^{-1} at 408 atm, a 58% decrease due to pressure. The exponential rate of ATP synthesis from glycolysis was reduced by only 38% from 37 to 23 mmol of ATP per g (dry weight) per h. However, the ATP requirement was increased at 408 atm so that catabolically produced ATP was only about two-thirds as effective in promoting growth under pressure. Thus, although the rate of ATP production at 408 atm was 62% that at 1 atm, the effective supply for growth was only 0.67 × 62%, or 42%. Under pressure, the organism is in a situation of reduced ATP supply and increased demand. Presumably, the same situation can occur with other organisms in which ATP supply is limiting for growth. *S. faecalis* is a particularly useful organism for this sort of energetic study because of its limited catabolic versatility. Thus, one can predict reasonably accurately from a knowledge of end products of known catabolites just how much ATP has been synthesized.

It seems somewhat anomalous that the ATP pools increased during growth of *S. faecalis* cultures. However, pools represent only very small fractions of the total ATP that is produced, most of which is used immediately for biosynthesis and other functions. One would expect that ATP pools would be higher in cells growing under pressure since growth and biosynthesis were depressed more than were catabolic processes. The finding of lower pools in pressurized cells may simply reflect increased activity of membrane ATPase. The retention of relatively high pool levels after growth had stopped under pressure is probably a reflection of increased acid sensitivity of growth. Growth of *S. faecalis* in the media we used was limited by the build-up of metabolic acids. If these acids were neutralized, the organism could resume growth. Pressure increased markedly the acid sensitivity of growth so that cells continued to produce lactate and ATP for a relatively long period after growth stoppage. Because glycolysis is more acid resistant than is growth, it was necessary to use only exponential-phase cultures for determinations of Y_{ATP}.

Another unusual finding of this study is that slow growth under pressure does not appear to activate pyruvate clastic reactions. Thus, we found that lactate was essentially the sole product of glycolysis even when the growth rate was reduced by 408 atm of pressure from 0.24 h^{-1} to 0.11 h^{-1}. Rosenberger and Elsden (23) found with continuous cultures that a reduction in rate from 0.22 to 0.088 h^{-1} resulted in a decrease in the percent conversion of glucose to lactate from about 90 to 50%.

In all, it seems that we now have a reasonably clear view of the effects of hydrostatic pressure on growth of *S. faecalis*. At near optimal growth temperatures, pressures of 200 atm or less have little effect, or a stimulatory effect, on growth (R. E. Marquis and P. Matsumura, *In* D. J. Kushner, ed., *Microbial Life in Extreme Environments*, in press). Higher pressures slow growth and reduce yields. The slowing of growth appears to be interpretable mainly in terms of ATP supply and demand. Supply is somewhat reduced by pressure, but demand is significantly increased. The reduction in yield is due partly to inefficient growth under pressure with production of increased amounts of acid per unit of cells produced. However, it is also due in part to enhanced acid sensitivity of growth caused by pressure (19). The maximum pressure at which *S. faecalis* can grow is about 900 atm at 37°C in glucose medium supplemented with 50 mM Mg^{2+} or

Ca^{2+}. Since the efficiency of growth decreases with increasing pressure, it appears that this maximum may be set by a balance of ATP supply and demand. Certainly, the bacterium is able to carry out glycolysis at pressures in excess of 1,000 atm. Thus, ATP can be produced at high pressure, but growth does not occur. Pope and co-workers (21, 22) feel that for most bacteria the maximum growth pressure is determined by the maximum pressure at which protein synthesis occurs. They found that protein synthesis is completely stopped in a variety of organisms by a pressure of about 680 atm, even though nucleic acid synthesis can occur at this pressure. In addition, a plot of the percentage of 1-atm activity versus pressure showed (21) that catabolic processes such as glycolysis and respiration are less inhibited by pressure than is protein synthesis. The reasonable conclusion was that growth barotolerance was more likely to be governed by the barosensitivity of protein synthesis than that of catabolic reactions. However, this conclusion was reached without taking into account the increased demand for ATP under pressure, which magnifies any reduction in ATP supply. In all, we feel that growth inhibition of *S. faecalis* by pressure can best be interpreted in terms of ATP supply and demand. Perhaps for other organisms, such as *E. coli*, biosynthetic reactions may play a more major role in growth inhibition.

ACKNOWLEDGMENTS

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