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Arginine Deiminase System and Acid Adaptation of Oral Streptococci

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Streptococcus rattus **FA-1 and** ***Streptococcus sanguis*** **NCTC 10904 underwent phenotypic acid adaptation in batch cultures toward the end of sugar-fueled growth after the culture pH had dropped to triggering values. The bacteria could be derepressed or induced for arginine deiminase independently of acid adaptation, and arginolysis afforded protection against acid killing over and above that of acid adaptation.**

Lactic acid bacteria in dental plaque have to endure frequent cycles of acidification to pH values as low as 4.0 and alkalinization to pH values somewhat above 7. There is a hierarchy of acid tolerance among the organisms, and in general, the mutans streptococci are inherently more tolerant than organisms such as *Streptococcus sanguis*, *Streptococcus gordonii*, or *Streptococcus oralis*. This high level of inherent tolerance appears to be important for the cariogenicity of the mutans streptococci. Higher levels of inherent tolerance of oral lactic acid bacteria to acidification have been related (7) to higher levels of proton-translocating F-ATPase activity and to lower pH optima for activity of the enzymes.

Recently, it has been shown (1, 5) that *Streptococcus mutans* can adapt phenotypically to acid conditions during growth in continuous culture to become more acid tolerant, in part because of enhanced F-ATPase activity. Thus, the organism not only has a high level of inherent acid tolerance but can become phenotypically even more tolerant during growth in acidified media.

Many of the less-acid-tolerant bacteria in plaque are able to catabolize arginine by means of the arginine deiminase system (ADS). A major function of this system appears to be to protect the organisms against acid damage (4). The system is highly acid tolerant and can operate to produce NH_3 at pH values well below the minima for growth or glycolysis. NH_3 produced within the cell combines with protons to yield NH_4^+, and this reaction raises the cytoplasmic pH value to protect against acid damage to sensitive cytoplasmic structures (6).

In previous experiments (3), we found that the ADS of *Enterococcus hirae* was catabolite repressed during growth in complex medium with added glucose and arginine but was derepressed after cultures had been in the stationary phase for a time, even though residual glucose was still present. Thus, derepression of the ADS occurred at a time when the cells could be acquiring adaptive acid tolerance because of acidification of the culture. We have now undertaken to determine if the acid-adaptive response can occur in batch cultures and the relationship between derepression of the ADS and acid adaptation.

Initially, we used *Streptococcus rattus* FA-1 from our laboratory culture collection. It is a mutans streptococcus found mainly in rodent plaque but also in human plaque in certain parts of the world. *S. rattus* is the only ADS-positive species of the mutans group. The ADS in *S. rattus* is much less subject to glucose catabolite repression than is the system in, for example, *S. sanguis* NCTC 10904 (2). Fig. 1 presents data obtained with cultures of *S. rattus* FA-1 grown statically in tryptone-yeast extract medium (1) with 1% (wt/vol) glucose and 0.5% (wt/vol) arginine at 37°C. This level of arginine was sufficient to induce the ADS, while the level of glucose was not repressive. Early in the culture cycle, the bacteria had a high level of the enzyme arginine deiminase (Fig. 1), assayed by measuring production of citrulline from arginine by cells permeabilized as described previously (4). During the initial phase of growth (Fig. 1), the organisms degraded both glucose and arginine. Then, by about 5 h after inoculation, arginolysis was complete and a period of somewhat slower growth ensued, in which the bacteria continued to use glucose (assayed with the use of glucose oxidase) until the culture pH had fallen to about 5 before growth ceased. Then, the bacteria continued to produce acid until the culture pH value had dropped to about 4.5. This pattern of growth and metabolism was obtained repeatedly.

Also shown in Fig. 1 are D values for killing of the organism harvested from different phases of the culture cycle and resuspended in 1% Difco peptone solution at a pH value of 3.5 and 25°C to yield a suspension with approximately 10^9 CFU/ml. This treatment resulted in rapid killing of the cells with little or no lag, as indicated by colony counts of spread plates on tryptic-soy agar (Difco, Detroit, Mich.) of suspensions diluted in 1% Difco peptone broth. The D values indicated are the times required for killing of 90% of the population and were estimated from plots of log percentage of survivors versus time showing exponential killing. Phenotypic acid adaptation by cells in batch culture was reflected by increases in the D value for cells titrated to pH 3.5 ($D_{pH\ =\ 3.5}$). Tolerance was lowest during the initial culture phase, when the bacteria were catabolizing arginine and the culture pH was relatively high. However, by the time the culture pH had fallen to about 5.3, the bacteria had developed increased acid tolerance, as indicated by an increase in the $D_{pH\ =\ 3.5}$ to some 17 min. The cells maintained this state of enhanced tolerance until late into the stationary phase, but after about 26 h, there was a decline in tolerance. With other cultures maintained for times beyond 30 h, the tolerance fell to the level of cells harvested early in the growth phase.

These results demonstrate that phenotypic acid tolerance can develop in batch cultures. Our previous studies (1) with *S. mutans* cells from continuous culture indicated that adaptation occurred rapidly, in less than a generation, after the culture pH value was allowed to fall from 7 to 5. Presumably, in the final stages of batch culture with excess glucose, the pH value falls sufficiently low to trigger the adaptive response while the cells

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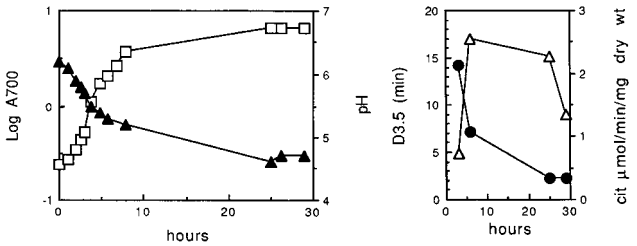

FIG. 1. Growth of *S. rattus* FA-1 in tryptone-yeast extract medium with 1% (wt/vol) glucose and 0.5% arginine in static culture at 37°C. Data are shown for A_{700} (□), culture pH (▲), D value (time for 90% killing) for cells titrated to a pH value of 3.5 (D3.5) (△), and relative activity of the arginine deiminase enzyme assayed as described previously (4) with permeabilized cells and expressed in terms of micromoles of citrulline produced per milligram (dry weight) of cells per minute (●).

are still able to grow. In these experiments, triggering pH values appeared to be between about 5.0 and 5.5. The results show also that the ADS and the adaptive response to acidification occur independently. Therefore, derepression of the ADS does not appear to be part of the acid-adaptive response.

For the second part of this study, we used an ADS-positive oral streptococcus with lower acid tolerance, *S. sanguis* NCTC 10904. As shown by the data in Fig. 2A, when the organism was grown in a medium with 0.5% arginine and 0.05% glucose plus 0.05% galactose (a nonrepressing sugar), there were two growth phases, with a prolonged interval between them. During the initial 6 h of culture, the organism's growth was fueled by the sugars and the culture pH dropped to about 5.7. This drop in pH was sufficient to trigger an acid-adaptive response, as shown by the increase in $D_{pH\ =\ 4.0}$ from less than a minute to nearly 29 min. Since this organism is less acid tolerant than

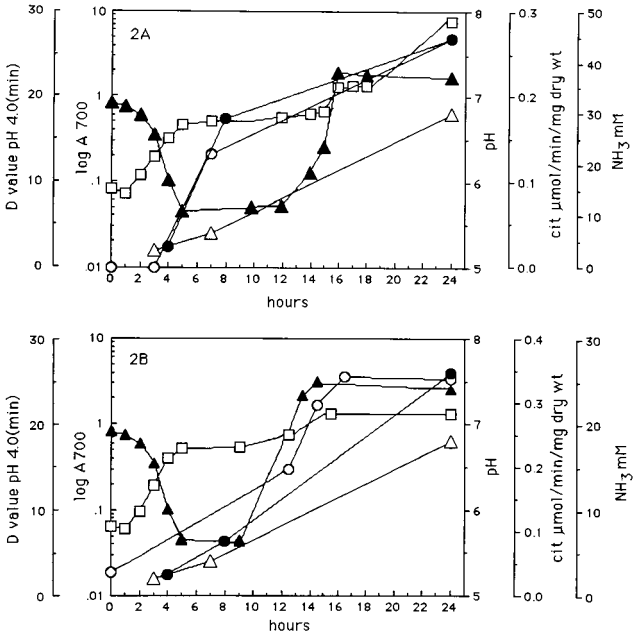

FIG. 2. Growth of *S. sanguis* NCTC 10904 in tryptone-yeast extract medium in static culture at 37°C with a mixture of 0.05% (wt/vol) galactose, 0.05% glucose plus 0.5% arginine (A) or 0.1% glucose plus 0.5% arginine (B). Data are shown for A_{700} (□), culture pH (▲), D value for cells titrated to a pH value of 4.0 (△), relative activity of the arginine deiminase enzyme in permeabilized cells (●), and level of ammonia in the culture assayed with an ammonium electrode (○).

TABLE 1. Protection by arginine of acid-adapted and unadapted cells of *S. rattus* FA-1 against acid killing at a pH value of 3.5

Cell type	10 mM arginine	$D_{pH\ =\ 3.5}$ (min)[a]
Acid adapted	No	15.2 ± 1.6
	Yes	44.1 ± 3.1
Unadapted	No	8.7 ± 2.0
	Yes	15.1 ± 2.9

[a] Data are averaged from three experiments, and standard deviations are shown. Unadapted cells were exponential-phase cells from cultures in which the pH had not dropped below 6; adapted cells were from late-exponential-phase cultures in which the pH had fallen below 5 (Fig. 1).

S. rattus, the pH value for assessing acid killing had to be increased from 3.5 to 4.0. Also, soon after completion of the first phase of sugar-fueled growth, the ADS was derepressed, as shown by increased levels of citrulline production in the assays for arginine deiminase enzyme, and after about 8 h, ammonia was present in the medium at detectable levels. The ammonia level increased sharply as the organisms degraded arginine. This degradation resulted in a second phase of growth, now arginine fueled, and an increase in the culture pH value to somewhat above 7.

As shown by the data presented in Fig. 2B, this organism showed two growth phases also when grown in tryptone-yeast extract medium with 0.5% arginine and 0.1% glucose but no galactose. The first phase was associated with catabolism of the added glucose, and the bacteria had only very low levels of arginine deiminase activity. However, after about 5 h growth stopped, but then it resumed after about 8 h. This second phase of growth was associated with utilization of arginine and with a rise in culture pH due to ammonia production. During this phase, the cells expressed arginine deiminase activity. In the interval between the two phases of growth, the bacteria developed enhanced acid tolerance, as indicated by an increase in $D_{pH\ =\ 4.0}$ from about 2 min to some 18 min.

It is clear once again that acid adaptation can occur in batch cultures in which the pH drops to a triggering value, here a value of about 5.5. It also is clear that even an organism with relatively low inherent acid tolerance can undergo an adaptive response, but this response is triggered at higher pH values. Finally, although in these cultures the ADS and the acid-adaptive response were induced at about the same time, they were independent, as indicated by the finding that acid adaptation occurred in media without added arginine (data not shown) and, therefore, without induction of the ADS.

The data in Table 1 show that arginine added to acidified suspensions of *S. rattus* FA-1 served to protect both acid-adapted and unadapted cells against acid damage, assessed here in terms of protection against acid killing of cells subjected to acidification to a pH of 3.5. The range of sensitivity is reflected in an average $D_{pH\ =\ 3.5}$ value of 8.7 min for unadapted cells without arginine to a $D_{pH\ =\ 3.5}$ value of over 40 min for adapted cells supplemented with arginine. Arginine is protective at concentrations as low as 1 mM (4), but a higher concentration of 20 mM was used in these experiments to provide an excess of the protectant.

The results presented here indicate that even strains of plaque streptococci with low tolerance for acidification can undergo phenotypic adaptation to acid conditions. This type of response is likely to be common among plaque bacteria and is likely to play important roles in plaque ecology. Thus, desirable organisms with low inherent acid tolerance can adapt phenotypically to acidification, and this adaptation would enhance

their capacity to survive in plaque acidified to potentially lethal degrees by undesirable, acid-tolerant organisms such as *S. mutans*. The acid-adaptive response does not appear to involve the ADS in ADS-positive organisms, and protection against acid damage afforded by arginolysis is in addition to that afforded by the acid-adaptive response.

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