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Arginine Hydrolysis by *Mycoplasma fermentans* is Regulated by Glucose

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Abstract: Arginine metabolism by mycoplasmas has been reported to be correlated with pathogenicity. Since a clinically important mycoplasma; *M. fermentans* hydrolyses arginine as an energy source, the arginine metabolism amongst *M. fermentans* strains belonging to different isolation sites was investigated. Arginine hydrolysis was detected by the increase in NH_4^+ concentration, measured using an NH_4^+ –sensitive electrode. The method proved simple to use and gave reproducible results. In broth medium, arginine and glucose were both shown to act as energy sources for growth. It was also shown that, in contrast to previous work, glucose inhibited arginine hydrolysis during growth. There did not appear to be a tight coupling between arginine hydrolysis and growth, since there was substantial hydrolysis after growth had ceased.

Key Words: *M. fermentans,* arginine hydrolysis of mycoplasma, ammonium ion–selective electrode.

Introduction

Mycoplasma fermentans is occasionally isolated from clinical material but its natural habitat in man is unknown. It is also frequent contaminant of cell lines. It causes serious kidney infections in AIDS patients and there has been speculation that it may be a factor in the progression of HIV infection to AIDS (1). However, it has also been shown to cause fatal systemic infections in HIV–negative individuals and more recently was detected by PCR in the synovial fluid of approximately 10% of patients with inflammatory arthritis (2) and by culture in children with a diagnosis of community acquired pneumonia (3). Thus this poorly understood organism is of increasing interest.

In growth media, *M. fermentans* produces acid from glucose and also hydrolyses arginine, both of which reactions may be important in energy generation. Preliminary evidence shows *M. fermentans* is of biochemically diverse and the rate of arginine metabolism relative to glucose varies amongst isolates (4). This variation is also of potential importance in relation to pathogenicity, since arginine metabolism will lead to both depletion of arginine from host cells (5) and NH_3 production (6).

The purpose of this study is to determine the quantity of arginine hydrolysed during growth. The approach adopted was to use an ammonium ion–selective electrode to measure an end product of hydrolysis. These electrodes are widely used in analytical chemistry and have also been used to study the kinetics of protease activity in *Bacillus subtilis* (7) and ammonium permease activity in *Rhizobium leguminosarum* (8). In a study of NH_4^+ levels in renal tubules, sensitivity of NH_4^+ detection was 100–fold greater than for classical, pH–change methods (9). The detection of mycoplasma arginine metabolism using NH_4^+ –selective electrodes has not previously been reported.

Materials and Methods

M. fermentans strains and culture conditions: Table 1 lists the *M. fermentans* strains used in this study and their original sources. The strains were collected by the Central Public Health Laboratory, Colindale, UK. They were obtained as freeze–dried ampoules and upon receipt were subcultured in broth, dispensed in ampoules and stored in –70°C. Cells were grown in modified SP4 broth medium (10). Where specifically stated, arginine (10%

Table 1. Origins of *M. fermentans* strains.

Reference number	Isolation site	Original source
M200/90 (incognitus)	Kaposi's Sarcoma tissue	S.–C. Lo, Armed forces Institute, Washington, DC.
M642/95	Respiratory tract	R. Dular, Cancer Research Facility,
M671/95	Respiratory tract	Frederick, MD.
M641/95	Urethra	C. Bébéar, University of Bordeaux.
M670/95	Joint fluid	
PG18	Genital ulcer	National Collection of Type Culters (NCTC 19989)

w/v) was included in media. Cultures were incubated anaerobically at 37°C for at least 48 hours. When growth was first evident as indicated by a change in the colour of the pH indicator (phenol red) from red to yellow, the change in pH was determined using pH electrode (Russell, PHM–10–070N) and optical density was measured at 550 nm in a Gallenkamp Visi–spectrophotometer.

Determination of ammonium production: The electrode potential is a function of the logarithm of the activity of the ammonium ion [NH_4^+]. The relationship is given by the Nerst equation (11); slope of the plot of E (measured potential on a milivoltmeter) against log [NH_4^+] which at 25°C should lie in the range 54–59 mV per $\log_{10}$ reduction in ammonium activity.

Measurement of [NH_4^+]: The tip of an NH_4^+–selective electrode (BDH, 309/0297/05) was immersed in the test solution which was magnetically stirred. A double junction reference electrode (BDH, 309/1030/04) was also immersed in the same solution. Both electrodes were connected to a milivolt/pH meter (Fisher Scientific, UK, Delta 350) and potential (mV) was recorded when it became steady. The ammonium concentration in test solutions was determined using a standard curve of potential (mV) against ammonium chloride (NH_4Cl) concentration (10^{-4} to 10^{-1} M). In almost all experiments, the electrode gave a Nerstian response of 58–59 mV/$\log^{10}$ [NH_4^+] over the range 10^{-1} to 10^{-4} M NH_4Cl. The correlation coefficient of potential against $\log_{10}$ [NH_4^+], for the standard curve, was ≥0.989 in all cases.

Results And Discussion

M. fermentans strains were grown in glucose–SP4 broth medium with or without added arginine (2 g L^{-1}). At various times, culture pH, NH_4^+ concentration and cell growth, determined as optical density were measured. [NH_4^+] increased from approximately 3 mM (uninocultated SP4 medium) to a maximum of 35mM following growth in media with added arginine. The latter data indicates that unsupplemented SP4 contained minimum of approximately 6 mM arginine, since arginine hydrolysis *via* arginine dihydrolase pathway gives 2 mol NH_3 per mol.

The comparison of data for strains M200/90, M641/95 and M670/95 at different incubation times, shows that there was substantial arginine hydrolysis (NH_4^+ production) after growth had stopped (Table 2).

Table 2. The growth of various *M. fermentans* strains in SP4 media plus arginine (2 g L^{-1}) with or without added glucose. Cultures were sampled at 24, 48 and 72 h depending on the growth characteristics of the strains.

		Media					
		Glucose (–)			Glucose (+)		
Strain	incubation time (h)	OD	pH	[NH_4^+] mM	OD	pH	[NH_4^+] mM
M641/95	24	0.232	7.076	15	0.399	5.870	10
	48	0.157	7.046	30	0.402	5.366	23
M200/90	24	0.210	7.270	14	0.425	6.260	9
	48	0.220	7.295	30	0.399	5.411	15
M670/95	24	0.473	7.137	21	0.905	5.848	21
	48	0.424	7.102	34	0.650	5.730	35
M671/95	48	0.399	7.259	32	0.659	6.716	26
	72	0.367	7.257	26	0.571	6.218	26
M642/95	48	0.349	7.284	35	0.573	6.461	36
	72	0.378	7.242	26	0.455	5.703	27
PG18	48	0.267	7.333	30	0.311	6.757	28
	72	0.282	7.322	26	0.300	6.154	26

Table 3. Effect of glucose (5 g L^{-1}) and arginine (2 g L^{-1}) on ammonium production by strains M200/90 and M671/95 in SP4 medium.

Strain	incubation time (h)	$[NH_4^+]$ (mM) Arginine (+) Glucose (–)	Arginine (+) Glucose (+)	Arginine (–) Glucose (–)	Arginine (–) Glucose (+)
M200/90	24	12	9	13	10
	48	32	17	14	16
M671/95	24	25	15	13	14
	72	32	20	12	14

Table 4. Effect of glucose (5 g L^{-1}) and arginine (2 g L^{-1}) on culture optical density (OD) and pH during growth of strains M200/90 and M671/95 grown in SP4 medium.

		Arginine (+)				Arginine (–)			
	Incubation	Glucose (–)		Glucose (+)		Glucose (–)		Glucose (+)	
Strains	time (h)	OD	pH	OD	pH	OD	pH	OD	pH
M200/90	24	0.260	7.026	0.350	6.700	0.239	7.017	0.510	6.546
	48	0.345	7.090	0.522	5.500	0.282	6.874	0.624	5.320
M671/95	48	0.488	7.370	0.500	6.897	0.482	7.323	0.560	6.927
	72	0.395	7.834	0.396	6.827	0.190	7.432	0.557	6.500

Thus, there does not appear to be a tight coupling between energy generation and growth. In the presence of added glucose, growth of the test strains increased by 50 to 100%, except for strain PG18 (10%). The significance of glucose metabolism was also indicated by large falls in pH, to between 5.4 and 6.4. Glucose also appeared to reduce the rate of arginine metabolism, particularly in strains M200/90 and M641/95; in these strains, the ratio of NH_4^+ produced to culture optical density at 24 h, fell by more than 60% (from approximately 65 to 25 mmol L^{-1} per OD unit). However, arginine utilisation in the remaining strains did not appear to be affected by the presence of glucose.

Further experiments were carried out for strains M200/90 and M671/95. In strain M200/90 (incognitus), the data of Table 3 suggested that arginine utilisation was clearly repressed by glucose, whereas repression was less apparent with strain M671/95, which gave a relatively high ratio of arginine to glucose metabolism in washed cells (4). Comparison of the data in media without added glucose, shows that added arginine (2 g L^{-1}) stimulated growth of both strains (Table 4). However, the stimulation was less than observed for glucose (5 g L^{-1}) in medium without added arginine. The effect of higher concentrations of arginine were not tested, since arginine is reported to be inhibitory above 2 g L^{-1} (12). The presence of arginine, in glucose supplemented medium, had little effect on cell growth and on the final pH. This suggests that, under the experimental conditions used, arginine did not significantly inhibit glucose metabolism. It was reported that in growing *M fermentans* strain PG18, arginine hydrolysis and glucose fermentation were independently regulated, i.e. the presence of arginine did not affect the rate of glucose fermentation and *vice versa* (13). Such findings are difficult to explain, if the purpose of both arginine hydrolysis and glucose fermentation is to generate ATP. However, in the present study, the presence of glucose reduced arginine hydrolysis. This finding suggests that glucose may be preferred energy source to arginine.

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