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NOTES

Effect of L-Histidine on the Survival of a T-Strain of Mycoplasma

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The addition of L-histidine to the growth medium prolongs the stationary phase and the survival of a T-strain of mycoplasma. Results of an experiment performed with ^{14}C-labeled urea demonstrate that the action of L-histidine is based on the retardation of the rise of pH.

In the course of a study on the metabolism of a T-strain of mycoplasma, we observed that the addition of L-histidine to the growth medium influenced the survival of the organism. The results of our investigations are presented in this paper.

A T-strain of mycoplasma, isolated in our laboratory (4) and designated P 108, was used. The organisms were grown in the liquid medium described by Ford and MacDonald (2) supplemented with 0.05% urea, hereinafter referred to as FMD+U medium. The pH of the medium was adjusted to 6.2 with 1 N HCl. When indicated, the FMD+U medium was supplemented with either 0.016 M L-histidine or 0.05 M *N*-2-hydroxyethyl-piperazine-*N'*-2-ethane-sulfonic acid (HEPES) buffer.

The media were inoculated with a 14-h T-strain of mycoplasma culture in a ratio of 1:100. Growth was determined at various times of incubation by the colony-counting technique; samples were diluted in serial 10-fold steps in liquid media, and 0.025 ml of each dilution was inoculated onto agar medium containing 0.05 M HEPES buffer (3). The inoculated plates were incubated at 37 C with 10% CO_2 in air. Results were expressed as the number of colony-forming units (CFU) per milliliter. The pH of the media was measured with a glass electrode.

The growth curve of P 108 (Fig. 1) indicates that the addition of L-histidine to FMD+U medium does not affect logarithmic growth but prolongs the stationary phase and survival of the microorganism. The primary effect of histidine seems to be on the pH of the cultures, and this effect enhances the viability of the T-strains of mycoplasma. In fact, the effect of L-histidine is superimposable on that of HEPES buffer.

The lower pH values observed beyond the 16th hour of incubation in cultures supplemented with histidine or HEPES could either be the consequence of direct buffering effect on the medium or a reduced ammonia production due to an inhibition of the urease activity of the organism. To resolve this question, experiments were carried out to detect ^{14}C-labeled urea hydrolysis by growing mycoplasmas in FMD+U medium and FMD+U media supplemented either with histidine or HEPES. The culture apparatus consisted of a 100-ml Erlenmeyer flask attached by a ground-glass joint to a CO_2 trap containing 20% KOH. To 210 ml of each liquid medium (FMD+U; FMD+U plus histidine; FMD+U plus HEPES) 11 μCi of ^{14}C-labeled urea (specific activity, 57.5 mCi/mmol; purchased from New England Nuclear, Boston,

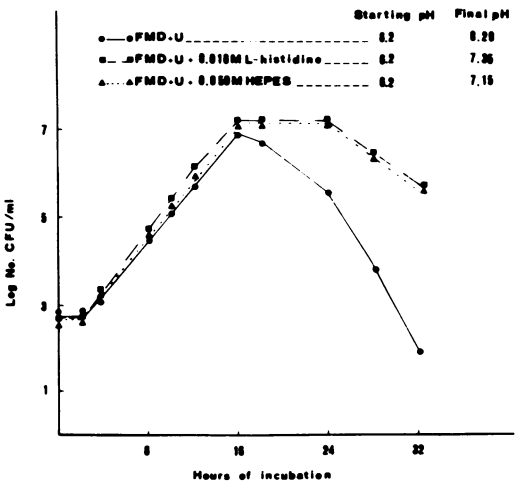

FIG. 1. *Effect of L-histidine and HEPES buffer on the growth of a T-strain of mycoplasma.*

TABLE 1. *Influence of L-histidine on ^{14}C-labeled urea hydrolysis by a growing T-strain of mycoplasma*

Time (h)	Medium								
	FMD+U			FMD+U plus 0.016 M L-histidine			FMD+U plus 0.05 M HEPES		
	Log (CFU/ml)	pH	% Urea hydrolysis[a]	Log (CFU/ml)	pH	% Urea hydrolysis[a]	Log (CFU/ml)	pH	% Urea hydrolysis[a]
0	2.30	6.20	0.00	2.30	6.20	0.00	2.30	6.20	0.00
8	3.80	6.25	0.00	4.00	6.20	0.04	3.95	6.20	0.06
16	6.30	6.50	0.40	6.50	6.25	0.68	6.40	6.20	0.60
24	7.00	7.80	63.60	7.30	6.80	71.40	7.30	6.70	72.40
32	2.50	8.05	98.00	4.50	7.20	99.30	4.45	7.00	99.00

[a] Calculated on the basis of $^{14}CO_2$ evolved by ^{14}C-labeled urea.

Mass.) was added; after a zero time sample had been removed, 0.3 ml of a 14-h T-strain of mycoplasma culture was added. The media were then distributed either in screw-capped tubes in 8-ml volumes to estimate P 108 growth and the pH of the media, or in culture apparatus in 50-ml volumes to estimate ^{14}C-labeled urea hydrolysis. All the cultures were incubated in a water bath at 37 C. After 8, 16, 24, and 32 h of incubation, the apparatus was removed from the water bath, and the growth was stopped by the addition of 2 ml of 50% trichloroacetic acid. The technique used to estimate the trend of ^{14}C-labeled urea hydrolysis was the same as that described by Ford et al. (1). The radioactivity was assayed in a Packard Tri-Carb liquid scintillation spectrometer using toluene-methanol scintillation liquor (1).

The results of this experiment (Table 1) show that the T-strain of mycoplasma logarithmically growing in FMD+U media supplemented either with histidine or with HEPES hydrolyzes more ^{14}C-labeled urea than in the unsupplemented medium, although the pH values were lower in these two supplemented media. A ^{14}C-labeled urea hydrolysis in excess of 98% was reached in all the media when the organisms were in the death phase of the growth curve (i.e., 32 h); at this time the pH of the media supplemented with histidine or HEPES was lower than that of the FMD+U medium.

In conclusion, L-histidine and HEPES do not reduce the urease activity of the T-strain of mycoplasma, and, since the maximal urease activity for these organisms was found to occur within the pH range of 5.5 to 6.5 (5), it seems likely that the action of histidine and HEPES is based on the retardation of the rise of pH.

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