

## Estimation of *Campylobacter* spp. in Broth Culture by Bioluminescence Assay of ATP

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The luciferin-luciferase bioluminescence reaction was used to estimate cell numbers of *Campylobacter jejuni* and *Campylobacter coli* in broth cultures based on a linear relationship between cell numbers (in excess of  $10^4$  to  $10^5$ ) and ATP levels. The sensitivity was lower than that obtained with *Escherichia coli*. The calculated amount of intracellular ATP per cell of *C. jejuni* and *C. coli* ranged from 1.7 to 2.1 fg.

ATP has been used to estimate microbial load based on the facts that bacterial cells contain a fairly constant amount of ATP (6) and that ATP can be used in the luciferin-luciferase bioluminescence reaction to measure the amount of ATP. Bacterial ATP must be released in its free soluble state (1, 5, 8) so that it can be reacted with luciferin in the presence of luciferase, magnesium, and oxygen to produce light. The amount of light emitted is proportional to the concentration of ATP in the sample, provided that the other reagents are present in excess. This reaction should be suitable for use with *Campylobacter* cells. The purpose of this study was to determine the sensitivity of the luciferin-luciferase assay in estimating cell numbers of *Campylobacter jejuni* and *Campylobacter coli* in broth cultures. *Escherichia coli* ATCC 11229 was used as a reference strain.

*C. jejuni* 81116, Penner serotype 6 (9) (from D. G. Newell, Public Health Laboratory, Southampton General Hospital, Southampton, England), and *C. coli* UA100, Penner serotype 5 (12) (from J. H. Bryner, National Animal Disease Center, Ames, Iowa), were grown in brucella medium (BB) (Difco Laboratories, Detroit, Mich.) and modified K broth (KB) (11) at 37°C for 24 h in a modified atmosphere containing 7% CO<sub>2</sub>. *E. coli* ATCC 11229 was grown in nutrient broth (NB) (Difco) at 37°C for 24 h. The *Campylobacter* strains would not grow in NB. Cell numbers were determined by serially diluting 24-h cultures in a sterile 0.85% saline solution and surface plating appropriate dilutions of the cultures onto brucella agar (BBL Microbiology Systems, Cockeysville, Md.). Plates were incubated at 37°C in 7% CO<sub>2</sub>.

ATP was extracted from the cells with 100 µl of culture suspension and 100 µl of nucleotide-releasing agent for bacteria (Lumac Systems Inc., St. Paul, Minn.). This mixture was reacted with 100 µl of a 1:1 dilution of luciferin-luciferase reagent (Lumit; Lumac), and the integrated light emitted in 10 s expressed in relative light units (RLU) was determined in a Biocounter M2010 (Lumac). RLU readings were converted to units of ATP by using standard curves for ATP in appropriate media and internal standardization (adding known quantities of ATP to each sample and measuring the increase in RLU). Lower readings for ATP in broth media than in distilled water were attributed to a quenching effect. This was illustrated by calculating the ratio of RLU in broth medium to RLU in distilled water. The quenching of BB, KB, and NB was 0.15, 0.18, and 0.33, respectively.

RLU emitted are directly proportional to the number of cells present in a sample (3). The linear relationship between RLU and *Campylobacter* cell numbers is shown in Fig. 1. The sensitivity of the luciferin-luciferase reaction for *Campylobacter* cells was determined with serial dilutions of the cultures in their appropriate growth media. For *Campylobacter* cells in BB or KB the sensitivity was  $10^4$  to  $10^5$  CFU/ml. The sensitivity for *E. coli* cells was  $10^3$  to  $10^4$  CFU/ml. This was comparable to the limit of detection for *E. coli* reported by Beckers and Lang (2). The lower sensitivity for *Campylobacter* cells may have been due to the quenching effect of the growth media or to lower levels of intracellular ATP per cell.

The sensitivity of the luciferin-luciferase reaction could be increased by concentrating the cells, either by centrifugation or by filtration (Table 1). Centrifugation at  $9,000 \times g$  for

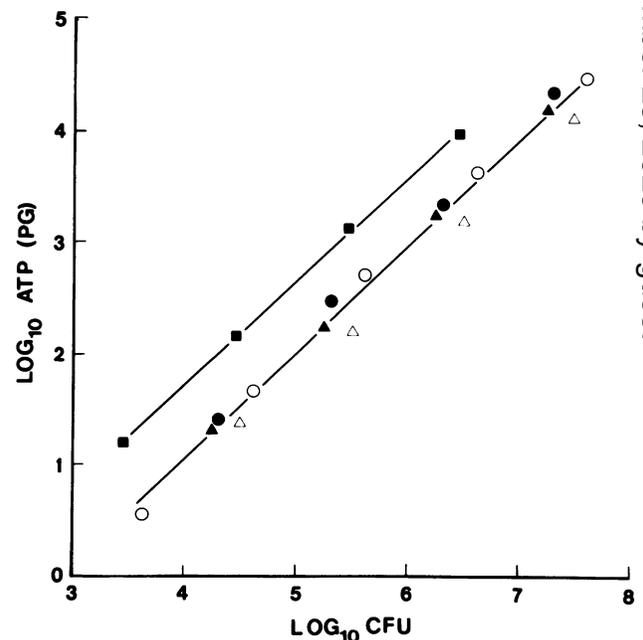


FIG. 1. Linear relationship between cell numbers and ATP levels in broth cultures of *E. coli* and *Campylobacter* spp. Symbols: ●, *C. coli* in KB; ▲, *C. coli* in BB; ○, *C. jejuni* in KB; △, *C. jejuni* in BB; and ■, *E. coli* in NB.

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TABLE 1. Effect of centrifugation or filtration on ATP estimates in *C. coli* grown in KB

Sample	Centrifugation		Filtration	
	RLU/100 $\mu$ l	CFU/100 $\mu$ l	RLU/100 $\mu$ l	CFU/100 $\mu$ l
Untreated	45,400	$4.3 \times 10^7$	53,000	$3.6 \times 10^7$
Supernatant or filtrate	8,400	$1.3 \times 10^6$	10,100	<10
Resuspended culture <sup>a</sup>	39,200	$1.1 \times 10^7$	46,000	

<sup>a</sup> From pellet or filter membrane.

min left ca. 10% of the cells in the supernatant so that some ATP was lost by this method. A greater loss of ATP after centrifugation (27,000  $\times g$  for 10 min) has been reported for several bacteria (8). Filtration with a 0.22- $\mu$ m-pore-diameter hydrophilic membrane (GVWP 01300; Millipore Corp., Bedford, Mass.) yielded results similar to those with centrifugation. The filtrate contained <10 CFU/ml; however, it still contained some ATP. This could have been due to cell lysis releasing ATP into the filtrate.

The amount of ATP per cell was calculated by using internal standardization and the least-squares best fit of the data, using the calculated intercept of the line on the ATP axis to estimate the amount of ATP per cell. The amounts of ATP per cell in *C. jejuni* and *C. coli* are shown in Table 2. As calculated from the regression line (Fig. 1), the *Campylobacter* cells contained ca. 1.8 fg of ATP per cell. This is within the range for most bacteria reported in the literature (3, 4, 7, 10). For *E. coli*, the ATP content was 5.21 fg per cell. Other values for *E. coli* ATCC 11229 were 1.6 to 12.9 fg per cell (2).

Based on the linear relationship between cell numbers and ATP levels, the luciferin-luciferase reaction can be used to estimate cell numbers of *Campylobacter* spp. However, the sensitivity for *Campylobacter* cells is lower than the sensitivity for *E. coli* cells. This may be due to the quenching effect of the *Campylobacter* growth media or to lower levels of ATP per cell. Standard curves or internal standardization for determining cell numbers yielded similar results. This

TABLE 2. ATP content in bacterial cells

Culture	Medium	ATP (fg/cell)	
		Standard curve	Internal standardization
<i>E. coli</i>	NB	5.21	5.51
<i>C. coli</i>	BB	1.82	2.06
<i>C. coli</i>	KB	2.11	2.37
<i>C. jejuni</i>	BB	1.83	0.89
<i>C. jejuni</i>	KB	1.69	1.11

indicated that cell turbidity and growth metabolites excreted into the medium had a negligible effect on RLU readings, as compared with the quenching effect of the growth medium.

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