APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1985, p. 730–731
0099-2240/85/030730-02$02.00/0

Vol. 49, No. 3

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

LAI-KING NG,[1] DIANE E. TAYLOR,[1,2]* AND MICHAEL E. STILES[1,3,4]

Departments of Microbiology,[1] Medical Microbiology,[2] Food Science,[3] and Foods and Nutrition,[4] University of Alberta, Edmonton, Alberta, Canada T6G 2E9

Received 19 June 1984/Accepted 30 October 1984

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_2. *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_2.

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 × *g* for 5

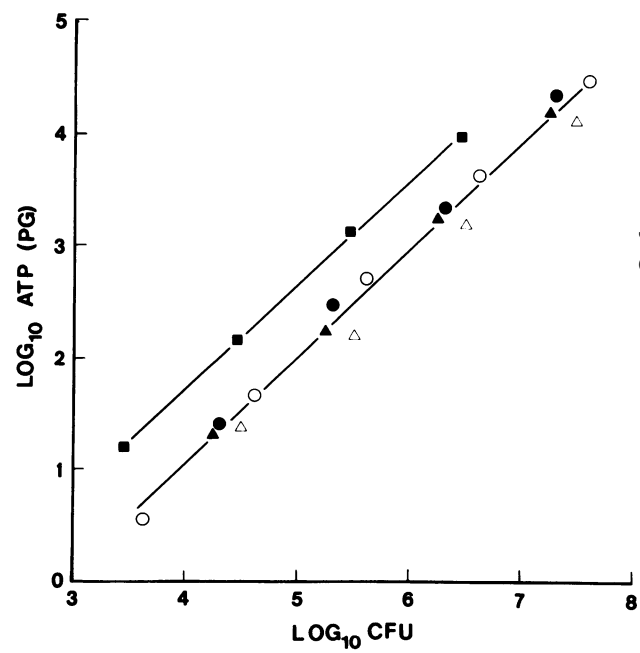

FIG. 1. Linear relationship between cell numbers and ATP levels in broth cultures of *E. coli* and *Campylobacter* spp. Symbols: ●, *C. coli* in KB; ▲, *C. coli* in BB; ○, *C. jejuni* in KB; △, *C. jejuni* in BB; and ■, *E. coli* in NB.

* Corresponding author.

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

Sample	Centrifugation		Filtration	
	RLU/100 μl	CFU/100 μl	RLU/100 μl	CFU/100 μl
Untreated	45,400	4.3×10^7	53,000	3.6×10^7
Supernatant or filtrate	8,400	1.3×10^6	10,100	<10
Resuspended culture[a]	39,200	1.1×10^7	46,000	

[a] 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 × *g* for 10 min) has been reported for several bacteria (8). Filtration with a 0.22-μm-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 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.

TABLE 2. ATP content in bacterial cells

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

We thank J. A. Robertson for her suggestion that the bioluminescence assay might be useful for estimating *Campylobacter* cell numbers. We thank M. E. Stemler for technical assistance and advice on bioluminescence reagents.

L.-K.N. was supported by an Alberta Heritage Foundation for Medical Research (AHFMR) studentship, and D.E.T. was supported by an AHFMR scholarship. The Biocounter M2010 was purchased with funds from AHFMR.

LITERATURE CITED

1. **Alexander, D. N., G. M. Ederer, and J. M. Matsen.** 1976. Evaluation of an adenosine 5'-triphosphate assay as a screening method to detect significant bacteriuria. J. Clin. Microbiol. **3**:42–46.
2. **Beckers, B., and H. R. M. Lang.** 1983. Rapid diagnosis of bacteraemia by measuring bacterial adenosine triphosphate in blood culture bottles using bioluminescence. Med. Microbiol. Immunol. **172**:117–122.
3. **Chappelle, E. W., and G. V. Levin.** 1968. Use of the firefly bioluminescent reaction for rapid detection and counting of bacteria. Biochem. Med. **2**:41–52.
4. **Hamilton, R. D., and O. Holm-Hansen.** 1967. Adenosine triphosphate content of marine bacteria. Limnol. Oceanogr. **12**:319–324.
5. **Hysert, D. W., F. B. Knudsen, N. M. Morrison, and G. Van Ghluwe.** 1979. Application of a bioluminescence ATP assay in brewery waste water treatment studies. Biotechnol. Bioeng. **21**:1301–1314.
6. **Knowles, E. J.** 1977. Microbial metabolic regulation by adenine nucleotide pools, p. 241–283. *In* B. A. Haddock and W. A. Hamilton (ed.), Microbial energetics. The Twenty-Seventh Symposium of the Society of General Microbiology. Cambridge University Press, Cambridge.
7. **Levin, G. V., J. R. Schrot, and W. C. Hess.** 1975. Methodology for application of adenosine triphosphate determination in wastewater treatment. Environ. Sci. Technol. **9**:961–965.
8. **Lundin, A., and A. Thore.** 1975. Comparison of methods for extraction of bacterial adenine nucleotides determined by firefly assay. Appl. Microbiol. **30**:713–721.
9. **Penner, J. L., and J. N. Hennessy.** 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J. Clin. Microbiol. **12**:732–737.
10. **Sharpe, A. N., M. N. Woodrow, and A. K. Jackson.** 1970. Adenosinetriphosphate (ATP) levels in foods contaminated by bacteria. J. Appl. Bacteriol. **33**:758–767.
11. **Taylor, D. E., S. A. De Grandis, M. A. Karmali, and P. C. Fleming.** 1981. Transmissible plasmids from *Campylobacter jejuni*. Antimicrob. Agents Chemother. **19**:831–835.
12. **Taylor, D. E., R. S. Garner, and B. T. Allan.** 1983. Characterization of tetracycline resistance plasmids from *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob. Agents Chemother. **24**:930–935.