Microbiology, Vol. 74, No. 2, 2005, pp. 159–163. Translated from Mikrobiologiya, Vol. 74, No. 2, 2005, pp. 191–197.

EXPERIMENTAL ARTICLES

Conditions That Influence Bacterial Luminescence in the Presence of Blood Serum

D. G. Deryabin and E. G. Polyakov

Orenburg State University,
pr. Pobedy 13, Orenburg, 460352 Russia

Received June 10, 2004; in final form, November 11, 2004

Abstract—Conditions that influence the luminescence of natural and recombinant luminescent bacteria in the presence of blood serum were studied. In general, blood serum quenched the luminescence of the marine *Photobacterium phosphoreum* and the recombinant *Escherichia coli* strains harboring the luminescent system genes of *Photobacterium leiognathi*, but enhanced the luminescence of the soil bacterium *Photorhabdus luminescens* Zm1 and the recombinant *E. coli* strain harboring the *lux* operon of *P. luminescens* Zm1. The quenching effect of blood serum increased with its concentration and the time and temperature of incubation. The components of blood serum that determine the degree and specificity of its action on bacterial luminescence were identified.

Key words: bioluminescence, *Photobacterium phosphoreum, Photorhabdus luminescens, Escherichia coli*, blood serum.

At present, natural and recombinant luminescent bacteria have become an efficient tool for the biotesting of soils, waters, and industrial wastes [1], as well as for the rapid determination of the toxicity of newly synthesized chemical compounds and pharmaceutical preparations [2]. The biotesting is based on the evaluation of the functional state of the bacterial luminescent system, which responds to many abiotic factors with a proportional decrease in the intensity of luminescence. Luminescent test systems favorably differ from other indicator systems in fast response, accuracy, and sensitivity to the integral action of pollutants [3].

The widely known luminescent test systems are Microtox [4] and LUMIStox [5], the principal ingredients of which are lyophilized viable *Vibrio fischeri* cells. In Russia, similar bioluminescent test systems are produced from the marine *Photobacterium phosphoreum* at the Institute of Biophysics, Siberian Division, Russian Academy of Sciences, Krasnoyarsk [6]. The use of transgenic *Escherichia coli* strains harboring the luminescent system genes of *P. leiognathi* allowed the testing procedure to be considerably simplified [7]. Researchers from Moscow State University devised a series of bacterial biosensors under the trademark Ecolum, which represent recombinant *E. coli* strains harboring the *lux* operon of marine photobacteria or the soil luminescent bacterium *Photorhabdus luminescens* Zm1. Luminescence controlled by the *lux* operon of the latter bacterium is distinguished by a higher optimum temperature of luminescence [8].

The cloning of the *lux* operons in *E. coli* strains, which are opportunistic commensals of mammalian colons, made it possible to solve some technical problems in the biotesting of abiotic factors and to create conditions for the use of bioluminescent analysis in studying the resistance of a macroorganism's fluids to infections [9]. In this case, the luminescent system may not only indicate the presence of toxic agents, but also reports on certain events (such as cell death) because of a disorder in the luminescent system [10].

All this prompted us to study the effect of blood serum on bacterial luminescence, with special emphasis on the effect of the nature of bacterial biosensors, incubation conditions, and the individual components of blood serum on the intensity of the luminescent reaction.

MATERIALS AND METHODS

Experiments were carried out with the natural luminescent marine isolate *Photobacterium phosphoreum* B-17 677f (the principle of the commercial preparation Microbiosensor B-17 677f, produced by the Institute of Biophysics in Krasnoyarsk); the soil luminescent bacterium *Photorhabdus luminescens* Zm1 (a generous gift from V.S. Danilov and A.P. Zarubina, Moscow State University); and three recombinant *Escherichia coli* strains (Z905, Ecolum-5, and Ecolum-8). Strain Z905, carrying the luminescent system genes of *Photobacterium leiognathi* [7], was obtained from the Institute of Biophysics. Ecolum-5 and Ecolum-8 (harboring the same genes of *P. leiognathi* and the *lux* operon of *P. luminescens* Zm1 [8], respectively) were obtained from Moscow State University.

To study the effect of blood serum on the spontaneous luminescence of these microorganisms, we used a pool of the blood sera of 20 healthy persons and

0026-2617/05/7402-0159

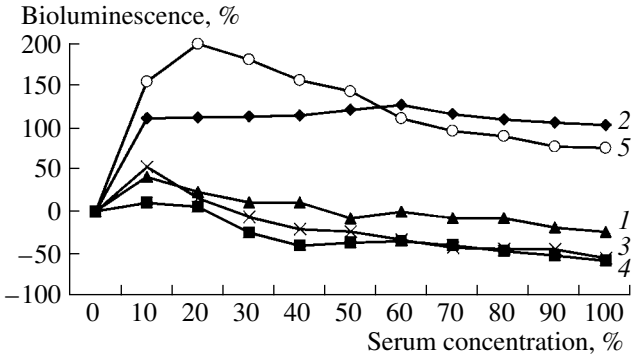

Fig. 1. The effect of various blood serum concentrations on the luminescence of natural and recombinant luminescent bacteria (incubation temperature 22–24°C; incubation time 30 min): (*1*) *P. phosphoreum* B-17 677f; (*2*) *P. luminescens* Zm1; (*3*) *E. coli* Z905; (*4*) *E. coli* (Ecolum-5); and (*5*) *E. coli* (Ecolum-8).

50 individual human sera, which were characterized with respect to the content of bactericidal agents (the whole complement; the complement components C1 through C5; lysozyme; beta-lysine; immunoglobulins A, M, and G [11]; and specific antibodies to *E. coli* and *P. phosphoreum* [12]). The integral bactericidal activity of blood sera (BABS) was evaluated nephelometrically [13] with the test strain *E. coli* K-12 TG1, which served as the recipient during the derivation of the aforementioned recombinant luminescent strains.

The effect of blood sera on the intensity of bacterial luminescence was studied in discrete and kinetic modes. In the discrete mode of measurements, 50-μl aliquots of a cell suspension containing luminescent bacteria at a concentration of 10^9 CFU/ml were mixed with equal volumes of dilutions containing from 100 to 10% (with a 10% step) of blood serum in 0.85% NaCl. In the control, the blood serum was replaced with artificial sea water (in the case of *P. phosphoreum*) or 0.85% NaCl (in the case of *P. luminescens* and *E. coli* strains). In temperature experiments, all the components of the reaction mixtures were preliminarily heated to a required temperature (4, 22–24, and 37°C). The reaction mixtures were incubated for 10, 20, 30, 40, 50, and 60 min, after which the reaction was stopped by adding 10 volumes of the respective saline solution. The intensity of luminescence was measured with a BLM-8820 bioluminometer (Nauka, Krasnoyarsk). In the kinetic mode of measurements, all the concentrations and proportions between the reaction components were the same, but the total reaction volume was raised to 1 ml and the intensity of bioluminescence was measured with the luminometer equipped with a recorder. The intensity of luminescence was calculated by the formula $I_e/I_c \times 100\%$, where I_e and I_c are the intensities of luminescence in the experimental and control cuvettes, respectively.

All the experiments were performed at least in five replicates. The results were statistically processed with the aid of the SPSS program. The effect of various factors on the intensity of bacterial luminescence was studied by variance analysis. The effect of the individual components of blood serum on bioluminescence was studied in factorial experiments.

RESULTS AND DISCUSSION

In the discrete mode of measurements of the luminescence of the natural isolate *P. phosphoreum* B-17 677f (incubation temperature 22–24°C; incubation time 30 min), 10% blood serum caused the maximum stimulation (by 65 ± 5.6%) of bioluminescence (Fig. 1, curve *1*), higher concentrations of blood serum being less efficient or even inhibitory (the undiluted blood serum quenched the luminescence of *P. phosphoreum* B-17 677f by 24.2 ± 3.3%).

At the same time, all the dilutions of blood serum enhanced the luminescence of *P. luminescens* Zm1 by 102 ± 6.7 to 125.8 ± 5.6% (Fig. 1, curve *2*), which can be explained by the specific ecology of this soil luminescent bacterium [14].

The effect of blood serum on the luminescence of the recombinant strain *E. coli* Z905 bearing plasmid pPHL7 with the *lux* operon of *P. leiognathi* was essentially the same (Fig. 1, curve *3*) as in the case of *P. phosphoreum* B-17 677f, which may be due to a similar organization of the luminescent systems and the cell walls of these two bacteria [15]. The same is true for Ecolum-5 (Fig. 1, curve *4*), which is prepared by cloning the *lux* operon in the recipient strain *E. coli* K-12 TG1.

The effect of blood serum on the bioluminescence of Ecolum-8 harboring the *lux* operon of the soil luminescent bacterium *P. luminescens* Zm1 was nearly the same (Fig. 1, curve *5*) as in the case of the donor strain (Fig. 1, curve *2*).

The difference in the effect of blood serum on Ecolum-5 and Ecolum-8 was confirmed in the kinetic experiments. The Ecolum-5 preparation bearing the luminescent system of *P. leiognathi* responded to blood serum with a small (12.1 ± 4.6%) and short-term (about 5 min) increase in bioluminescence, followed by a rapid decline to a steady-state level (Fig. 2a). In contrast, the Ecolum-8 preparation bearing the luminescent system of *P. luminescens* Zm1 responded to blood serum with a profound stimulation of bioluminescence (by up to 40.1 ± 7.7%), which attained a maximum after a period of time correlating positively with the height of the serum concentration.

It should be noted that a similar bell-shaped dynamics of luminescence was observed earlier for the recombinant *E. coli* strain bearing the luciferase gene of the insect *Pyrophorus plagiophthalamus* [16]. In the latter case, the enhanced level of bioluminescence was accounted for by the facilitated diffusion of the substrate to luciferase through the damaged cytoplasmic membrane. This may be true for the bioluminescence of

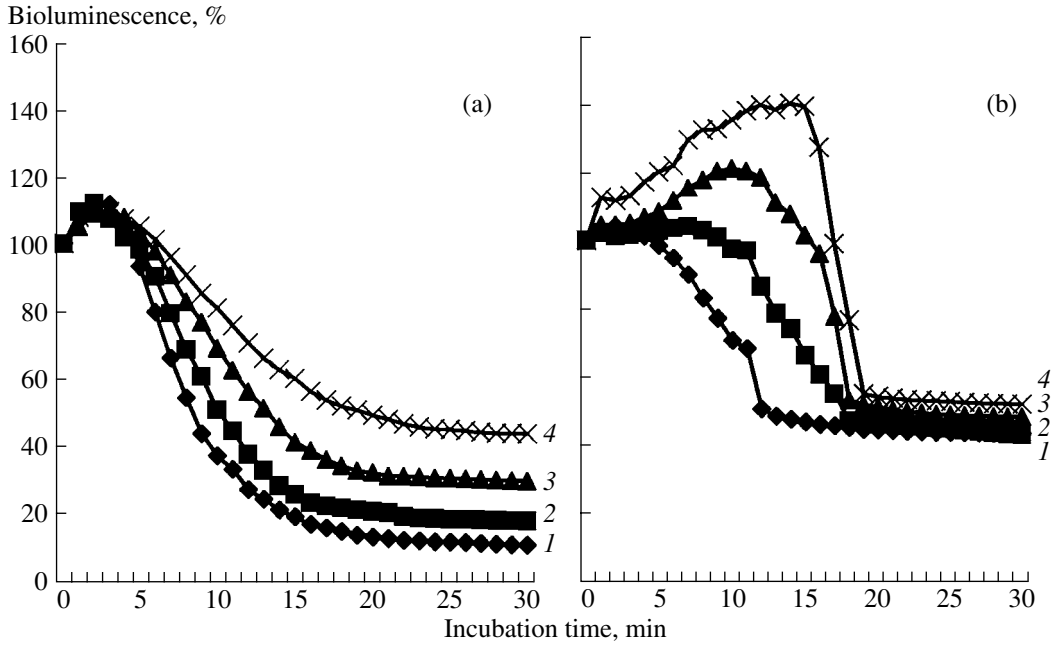

Fig. 2. Changes in the dynamics of the luminescence of (a) *E. coli* (Ecolum-5) and (b) *E. coli* (Ecolum-8) in response to various blood serum concentrations (incubation temperature 37°C): (*1*) 100%, (*2*) 50%, (*3*) 25%, and (*4*) 10%.

Ecolum-8 as well. In any case, it is clear that strain differences in the response of luminescent bacteria to blood serum are determined by not only the structure of their cell walls, but also by the molecular organization of their luminescent systems.

The study of the effect of incubation temperature showed that low temperature (4°C) considerably mitigated the quenching effect of high serum concentrations on the luminescence of all the bacterial strains studied.

In contrast, the high incubation temperature (37°C) enhanced the quenching effect of blood serum on the luminescence of bacterial strains, except for that of *P. phosphoreum* B-17 677f (data not presented). It should be noted, however, that the luminescent system of this bacterium is inactive at 37°C [15]. The quenching effect of blood serum at 37°C was maximum in the case of *E. coli* Z905 and Ecolum-5, reaching 95 ± 4.3% at serum concentrations higher than 50% (after 30 min of incubation).

Extending the incubation time enhanced the quenching effect of blood serum on bioluminescence. For instance, the luminescence of *P. luminescens* Zm1 was quenched by high serum concentrations (80–100%) only at sufficiently long incubation times (>30 min) and high incubation temperature (37°C).

The variance analysis of the combined effect of these three factors (serum concentration, incubation time, and incubation temperature) showed that they were responsible for 47–84% of changes in the intensity of bioluminescence in response to blood serum (table). Other factors (such as the organization of the luminescent system, the functional state of bacterial cells, fluctuations in their concentration, etc.) were responsible for 50.1% of changes in the intensity of the luminescence of *P. luminescens* Zm1 and 52.3% of changes in the luminescence of Ecolum-8.

The time of incubation with blood serum was found to be the most important factor for *P. phosphoreum* B-17677f (responsibility for 27.7% of changes in bioluminescence) and *P. luminescens* Zm1 (responsibility for 45.1% of changes in bioluminescence). The temperature of incubation was the most important factor for *P. phosphoreum* B-17 677f and two recombinant *E. coli* strains harboring the *lux* operon of *P. leiognathi* (responsibility for 23.4–65.9% of changes in bioluminescence). The serum concentration was the most important factor (responsibility for 17.6–44.1% of changes in bioluminescence at $P < 0.05$) for four of the five natural and recombinant bacterial strains under study (except for *P. luminescens* Zm1).

These results stimulated further studies into the effect of the individual components of blood serum on bioluminescence. For this purpose, we used 50 individual human sera, which were characterized with respect to the content of bactericidal agents (the whole complement; the complement components C1 through C5; lysozyme; betalysine; immunoglobulins A, M, and G; specific antibodies to *P. phosphoreum* B-17 677f and *E. coli* Z905; as well as the integral BABS evaluated nephelometrically [13] with the test strain *E. coli* K-12 TG1).

Factorial experiments allowed us to determine the three most significant factors, which were responsible for about 62% of the bioluminescence variance (Fig. 3).

The effect of various factors on the quenching of the luminescence of natural and recombinant luminescent bacteria by blood serum (from the data of variance analysis)

Factor	Factor significance (%) for the luminescence of				
	P. phosphoreum B-17677f	*P. luminescens* Zm1	*E. coli* Z905	*E. coli* "Ecolum-5"	*E. coli* "Ecolum-8"
Temperature	23.4*	3.5	46.1*	65.9*	0.9
Incubation time	27.7*	45.1*	1.5	1.1	2.7
Serum concentration	21.8*	1.3	31.6*	17.6*	44.5*
Unidentified factors	27.1*	50.1*	20.8*	15.4	51.9*

* The asterisks mark data for which $P < 0.05$.

The first most significant factor (the proper value 6.667; responsibility for 26.67% of variance) was characterized by a high value of factor weight (0.46) for the quenching of the luminescence of *E. coli* Z905 and by a high level of factor weight (0.26) for bactericidal effect with respect to *E. coli* K-12 TG1. This factor was called the factor of specific action on *E. coli.* It also included the concentrations of specific antibodies to *E. coli* cells (factor weight 0.64), lysozyme (factor weight 0.44), complement (factor weight 0.28), and beta-lysine (factor weight 0.22), which are responsible for the specific reaction of bacteriolysis initiated by the antibodies. The specific feature of this factor was the negative values of factor weights for the quenching of the luminescence of *P. phosphoreum* B-17 677f (factor weight –0.98) and for the antibodies against this bacterium (factor weight –0.53).

The second most significant factor (the proper value 3.516; responsibility for 23.5% of variance) was characterized by the high values of factor weight for such complement components as C1 (factor weight 0.64), C2 (factor weight 0.79), C3 (factor weight 0.60), C4 (factor weight 0.81), and C5 (factor weight 0.76), whereas the other complement components were characterized by either small or negative factor weights. It should be noted that high values of factor weights were demonstrated for both *E. coli* Z905 and *P. phosphoreum* B-17 677f, which allowed this factor to be called *the factor of the nonspecific action of blood serum.* This factor accounts for the membranotropic effect of the complement components.

The third most significant factor (the proper value 2.947; responsibility for 11.79% of variance) was characterized by relatively high values of factor weight (0.12 and 0.20, respectively) for the antibodies against *P. phosphoreum* B-17 677f and the quenching of the luminescence of this bacterium. This factor was called *the factor of specific action on P. phosphoreum.* The specific feature of this factor was a negative value (–0.56) of factor weight for the quenching of the lumi-

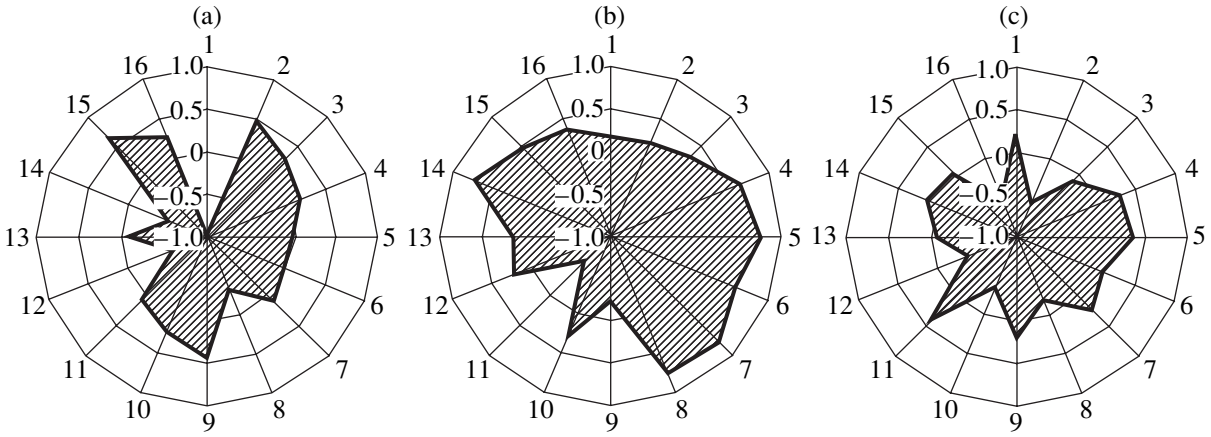

Fig. 3. The structure of factors responsible for the intensity and the specificity of action of blood serum components on the luminescence of *P. phosphoreum* B-17 677f and *E. coli* Z905: (a) factor of specific action on *E. coli*; (b) factor of the activity of complement; (c) factor of specific action on *P. phosphoreum.* Factor components: (1) effect on the luminescence of *P. phosphoreum* B-17 677f; (2) effect on the luminescence of *E. coli* Z905; (3) complement; (4) complement component C1; (5) complement component C2; (6) complement component C3; (7) complement component C4; (8) complement component C5; (9) lysozyme; (10) beta-lysine; (11) IgA; (12) IgM; (13) IgG; (14) antibodies against *P. phosphoreum* B-17 677f; (15) antibodies against *E. coli* Z906; and (16) BABS nephelometry.

nescence of *E. coli* Z905 and the profound bactericidal effect with respect to *E. coli* K-12 TG1.

Thus, the data obtained indicate that there are two primary mechanisms that determine the efficiency of the action of blood serum on bacterial luminescence. The first, specific, mechanism is implemented by the serum antibodies against the test strain, which activate the complement and implicate other components of blood serum in the reaction of bacteriolysis. The second, nonspecific, mechanism lies in the membranotropic action of the complement, which is activated by the lipopolysaccharides of target cells in an alternative way.

These findings can be used to choose an appropriate test strain and to determine optimal reaction conditions for a new bioluminescent method of BABS assay. This method is anticipated to be rapid, sensitive, and applicable for the determination of the infection resistance of not only blood serum, but also of other normal and pathological biofluids that contain particular serum components.

REFERENCES

1. Belkin, S., Microbial Whole-Cell Sensing Systems of Environmental Pollutants, *Curr. Opin. Microbiol.*, 2003, vol. 6, no. 3, pp. 206–212.
2. Roda, A., Guardigli, M., Pasini, P., and Mirasoli, M., Bioluminescence and Chemiluminescence in Drug Screening, *Anal. Bioanal. Chem.*, 2003, vol. 377, no. 5, pp. 826–859.
3. Kaiser, K.L. and Esterby, S.R., Regression and Cluster Analysis of the Acute Toxicity of 267 Chemicals to Six Species of Biota and the Octanol/Water Partition Coefficient, *Sci. Total Environ.*, 1991, vol. 109–110, pp. 499–514.
4. Blaise, C., Forghani, R., Legault, R., and Guzzo, J., Dubow MS. A Bacterial Toxicity Assay Performed with Microplates, Microluminometry and Microtox Reagent, *BioTechniques*, 1994, vol. 16, no. 5, pp. 932–937.
5. Zieseniss, K. and Grabert, E., A Novel Method for Determining Chronic Toxicity with the LUMIStox Luminescent Bacteria Test, in *Bioluminescence and Chemiluminescence: Fundamental and Applied Aspects*, 1994, pp. 76–78.
6. *Katalog kul'tur svetyashchikhsya bakterii* (Catalog of Fluorescent Bacteria), Rodicheva, E.K., Ed., Novosibirsk: Nauka, SO RAN, 1997.
7. Illarionov, B.A. and Protopopova, M.V., Cloning and Expression of the Fluorescent System Genes of *Photobacterium leiognathi, Mol. Genet. Mikrobiol. Virusol.*, 1987, no. 8, pp. 41–46.
8. Manukhov, I.V., Zavil'gel'skii, G.B., and Danilov, V.S., Cloning of the *luxAB* Genes of the Thermostable Luciferase of *Photorhabdus luminescens* ZM1 in *Escherichia coli* K12, *Biotekhnologiya*, 1999, no. 1, pp. 40–43.
9. Deryabin, D.G. and Polyakov, E.G., Prospects of Using Luminescent Bacteria for the Evaluation of the Bactericidal Activity of Macroorganism's Biofluids, in *Mater. VIII s"ezda Vseross. obshch. mikrobiol., epidemiol. i parazitol.* (Proc. of 8th Congress of the Russian Society for Microbiology, Epidemiology, and Parasitology), Moscow, 2002, vol. 3, p. 253.
10. Virta, M., Lineri, S., Kankaanpaa, P., Karp, M., Peltonen, K., Nuutila, J., and Lilius, E., Determination of Complement-Mediated Killing of Bacteria by Viability Staining and Bioluminescence, *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 2, pp. 515–519.
11. *Immunologicheskie metody* (Immunological Methods), Frimelya, G., Ed., Moscow: Meditsina, 1987.
12. Astaf'ev, D.G. and Mayanskii, A.N., Quantitative Characterization of Human Antibodies against the Peptidoglycans of Various Staphylococci, *Zh. Mikrobiol. Epidemiol. Immunobiol.*, 1987, no. 3, pp. 55–57.
13. Bukharin, O.V. and Sozykin, V.L., Photonephelometric Method for Determining the Bactericidal Activity of Blood Serum, *Faktory Estestvennogo Immuniteta* (Natural Immunity Factors), Orenburg, 1979, pp. 43–45.
14. Ffrench-Constant, R., Waterfield, N., Daborn, P., Joyce, S., Bennett, H., Au, C., Dowling, A., Boundy, S., Reynolds, S., and Clarke, D., *Photorhabdus*: Towards a Functional Genomic Analysis of a Symbiont and Pathogen, *FEMS Microbiol. Rev.*, 2003, vol. 26, no. 5, pp. 433–456.
15. Kuwae, T., Sasaki, T., and Kurata, M., Chemical and Biological Properties of Lipopolysaccharide from a Marine Bacterium *Photobacterium phosphoreum* PJ-1, *Microbiol. Immunol.*, 1982, vol. 26, no. 6, pp. 455–466.
16. Virta, M., Karp, M., Ronnemaa, S., and Lilius, E., Kinetic Measurement of the Membranolytic Activity of Serum Complement Using Bioluminescent Bacteria, *J. Immunol. Methods*, 1997, vol. 201, no. 2, pp. 215–221.