Bacillus coagulans, FRR B666, as a Potential Biological Indicator Organism†

A. T. JONES* and I. J. PFLUG* ˣ

University of Minnesota, Department of Food Science and Nutrition, Minneapolis, Minnesota

ABSTRACT: *Bacillus coagulans, FRR B666, was evaluated as a potential biological indicator organism. The organism was grown on two types of sporulation media and the spores were tested for wet heat resistance. Further tests were carried out to determine if replicate spore crops exhibited similar heat resistance characteristics. Survivor curve tests were carried out to determine the behavior of* B. coagulans *spores when heated in Water for Injection (USP), 0.0153* M *Butterfield's buffer, and three representative parenteral solutions. Large numbers of spores were produced using relatively simple sporulation media. Aliquots of replicate spore crops grown on the same lot of medium had almost identical heat destruction characteristics. The semilogarithmic survivor curves in most solutions were straight lines. The results indicate that* B. coagulans, *FRR B666, is a stable and reliable biological indicator organism. Reproducibility of survivor curves and the low variability among replicate samples in all solutions tested are characteristics that suggest that* B. coagulans *has attributes of a potentially excellent biological indicator organism.*

Introduction

Drugs, biological products, and devices used in the health industry must be adequately sterilized. Because microorganisms die exponentially or logarithmically during the sterilization process, the level of sterility is best expressed on a probabilistic basis. For injectable drugs, the fluid paths of injectable foods and drugs, and for implantable devices, the probability of a nonsterile unit (PNSU) should be less than one in one million units (PNSU $\leq 10^{-6}$). It is not possible to measure this level of sterility directly; therefore, indirect methods of assuring the adequacy of sterilization processes must be used. One method is the use of biological indicators (BIs); these are calibrated, resistant bacterial spores in a measurement system. Selkon et al. (1) have shown that biological indicators, if properly calibrated and used, are effective tools for sterilization process validation and monitoring. Except in an overkill situation, the destruction characteristics of biological indicators must be related to the product sterilization process design, which should be based on the numbers and microbial flora of the product.

It would be convenient to have an ideal biological indicator organism that could be used in all situations. However, each product represents a unique microbial environment. Because environmental factors vary from product to product, no one organism can effectively meet the diverse BI requirement of all sterilization cycles. Therefore, several biological indicator organisms are needed so that a wide range of thermal resistance conditions can be validated and monitored. At present the sterilization microbiologist is in need of an aerobic organism with a heat resistance between that of *Clostridium sporogenes* and *Bacillus stearothermophilus*. It is possible that FRR B666, a heat-resistant *B. coagulans* variant, will meet this need.

B. coagulans, FRR B666, was originally isolated in Australia from heat-processed cabbage and designated as Organism 320 (2). Warth (3) reported that this organism is an atypical strain of *B. coagulans* and requires heat activation at greater than 100 °C.

B. coagulans was first described by Hammer (4). Berry (5) and Becker and Pederson (6) describe *B. coagulans* as a heat-resistant, spore-forming organism capable of growing in an acid food. It is the heat resistance of organism FRR B666, as described by Murrell and Warth (7), that gives it potential as a biological indicator.

This paper reports a study to evaluate *B. coagulans*, FRR B666, as a biological indicator organism. Included are the results of studies to determine: (*a*) the effect of different sporulation media on spore crop production and on the characteristics of the spores, (*b*) if replicate spore crops exhibit the same response to a heat stress, and (*c*) the effect of different heating solutions on the heat resistance of the spores. In addition, by specifically defining conditions for growth and recovery of this organism, information relevant to the use of *B. coagulans* as a biological indicator will be provided.

Materials and Methods

Experimental Plan

Five spore crops of *B. coagulans* were grown on two different formulations of media. All five spore crops were tested under similar environmental conditions for heat re-

† Scientific Journal Series No. 11,626, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

Received September 16, 1980. Accepted for publication March 10, 1981.

* Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN 55108.

ˣ Author to whom inquiries should be directed.

Reprinted from Journal of PARENTERAL SCIENCE AND TECHNOLOGY, May/June 1981

TABLE II. *D* (115 °C) and IR Values for *Bacillus coagulans* Spores Heated in Water for Injection (USP)

Experiment Number	Spore Code	Equivalent Heating Times, min	N_0	*D* (115 °C), min	95% CI *D* Value, min	95% CI as % of *D* Value	IR
		Spores Grown on Soya Peptone Agar Plus Spore Salts					
8317A	BC1	3, 8, 13, 18, and 23	1.2×10^6	6.94	6.33–7.68	19	0.92
8346B	BC3	3, 10, 17, 24, and 31	9.9×10^7	6.83	6.38 –7.36	14	0.94
8346A	BC2	3, 10, 17, 24, and 31	1.1×10^8	6.77	6.32–7.30	14	0.95
9040A	BC2	3, 10, 17, 24, and 31	1.1×10^8	6.75	6.46–7.06	9	0.96
		Spores Grown on Nutrient Agar Plus $MnSO_4$					
8345A	BC4	3, 10, 13, 24	2.8×10^6	4.72	4.41–5.09	14	1.05
9177A	BC5	3, 8, 13, 18, and 23	8.7×10^6	4.51	4.11–5.00	20	1.06
9183A	BC5	3, 8, 13, 18, and 23	1.0×10^7	4.46	4.20–4.74	12	1.06

the screw-cap test tube miniature retort system of heating. Five suspending solutions were evaluated.

Inoculum for each test was prepared by adding 1 ml of the *B. coagulans* spore suspension to a dilution bottle containing 100 ml of the appropriate solution. The inoculated bottle was shaken 30 times before filling procedures were started and again after every six tubes had been filled. Five-milliliter aliquots of inoculated solution were deposited into 18 × 150-mm screw-cap test tubes. The prepared tubes were held in ice water until heated. Three replicate tubes were heated at each time, and three heat-shocked tubes were used to determine the initial number of spores per tube. The heat-shock tubes were placed in a boiling water (100 °C) bath for 15 min. After heating, the tubes were cooled and held in ice water until recovery procedures were started.

Heating times were corrected for the lag in heating and cooling to equivalent minutes. The lag correction factor for 18 × 150-mm screw-cap test tubes containing 5 ml of solution heated in the miniature retort system was 2.0 mins.

To assay for survivors, a 0.1-ml aliquot of the spore suspension in each tube was diluted in 0.0003 *M* Butterfield's phosphate buffer (8). Duplicate 0.01-, 0.1-, or 1.0-ml aliquots of the dilution were pipetted into 100-mm diameter plastic petri plates. Where no dilution was necessary, duplicate 0.01-, 0.1-, or 1.0-ml aliquots of the heated spore suspension were directly plated. One lot of Tryptic Soy agar was used as the recovery medium for all experiments. Plates were incubated at 50 °C for 72 hr. Colonies were counted with the aid of a Bactronic colony counter.

Data Analysis

The data, in terms of number of surviving organisms per tube as a function of equivalent minutes, were analyzed with the aid of a survivor curve computer program. The *D* value was calculated using survivor data for heated spores only (the initial number, N_0, data was not included in the *D* value analysis). The *D* value, 95% confidence interval (CI) of the *D* value, and the intercept ratio (IR) were calculated for all the experiments. The IR value is defined as the logarithm of the zero time intercept of the regression line divided by the logarithm of the N_0.

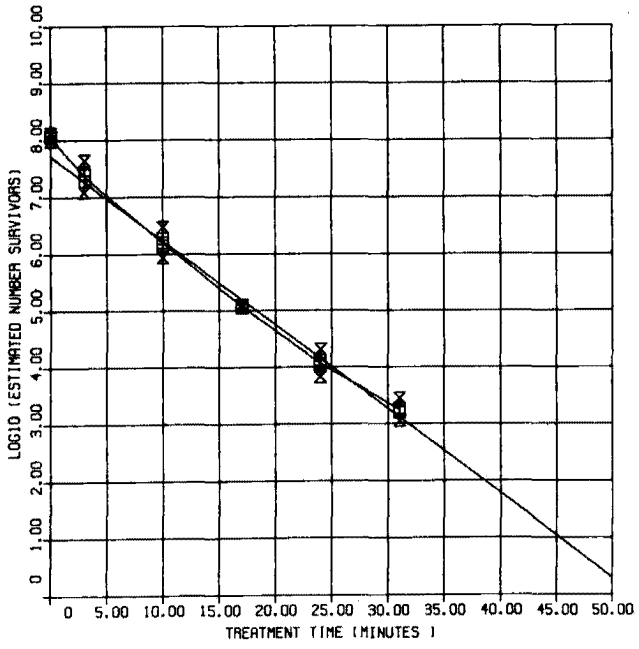

Figure 1—Survivor curve for spores (BC2) grown on Soya Peptone Agar and heated in Water for Injection (USP) at 115.0 °C.

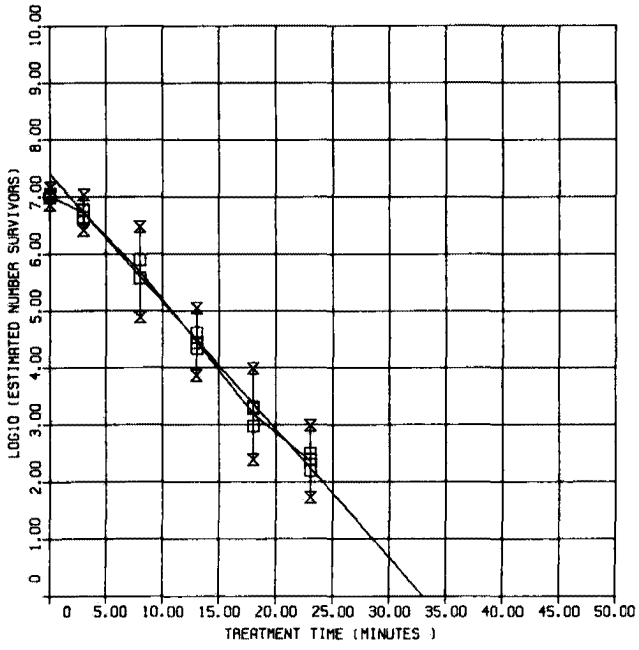

Figure 2—Survivor curve for spores (BC5) grown on nutrient agar and heated in Water for Injection (USP) at 115.0 °C.

sistance using a miniature retort system for heating. One spore crop was then used for heat resistance determination in five different solutions: Water for Injection (USP); dextrose, 5% in Water for Injection (USP); lactated Ringer's; normal saline, 0.9%; and 0.0153 *M* Butterfield's buffer (8) in Water for Injection (USP).

Sporulation Media and Spore Crop Growth Procedures

Two media were used for the production of *B. coagulans* spores. The first medium was Soya Peptone agar plus spore salts (2) which was recommended by Warth (3) for the growth of large numbers of *B. coagulans*, FRR B666, spores. The second medium employed was commercial nutrient agar plus manganese sulfate (5 ppm).

The composition of Soya Peptone plus spore salts agar is as follows.

	Quantity per Liter of Medium
Soy Peptone	5 g
Casamino acids	5 g
Yeast extract	1 g
Agar	15 g
Distilled water	1000 ml
Salts	
Dibasic sodium phosphate (Na_2HPO_4)	0.71 g
Sodium chloride (NaCl)	0.29 g
Magnesium sulfate ($MgSO_4$)	15.05 mg
Calcium chloride ($CaCl_2$)	5.55 mg
Manganese sulfate ($MnSO_4$)	0.76 mg
Ferrous sulfate ($FeSO_4$)	0.76 mg

Outlined below is the step-wise procedure for the production of high-yield, heat resistant *B. coagulans* spore crops (in our laboratory inoculation and harvesting of the spore crops is carried out in a Class 100 clean room).

1. Approximately 10^7 *B. coagulans* spores are suspended in 5 ml of Water for Injection (USP) in an 18 × 150-mm screw-cap test tube.

2. The spore suspension is heat-activated in a boiling water bath, approximately 100 °C for 1 hr (2), followed by cooling in an ice water bath.

3. Agar slants of the appropriate medium (20 ml in 25 × 150-mm tubes) are inoculated with 0.1 ml of the heat-activated spore suspension. The slants are placed in a horizontal position with the caps off for 20 min to allow the inoculum to dry on the surface of the slant.

4. The agar-slant tubes are loosely capped and vertically incubated at 50 ± 2 °C for 48 hr.

5. Microscopic examination of the growth should show at least 30–40% sporulation at the end of 48 hr.

6. The condensate that has collected at the bottom of the slant is removed, using care not to disturb the growth on the agar surface.

7. The growth is washed from the surface of the agar using 2 ml of Water for Injection (USP) and transferred to an 18 × 150-mm screw-cap test tube.

8. The suspension is heated in a boiling water bath, approximately 100 °C for 15 min, and cooled in an ice water bath.

9. Plates (15 × 100 mm) containing about 35–40 ml of the appropriate medium are inoculated with 0.2 ml of the heated suspension. The inoculum is spread over the agar surface using a sterile glass rod.

10. The inoculated plates are left uncovered for 20 min to allow the inoculum to dry on the surface of the agar.

11. The inoculated plates are covered, inverted, stacked two high on metal trays, and incubated at 50 ± 2 °C for 48 hr. (The incubator in our laboratory operates at approximately 12% relative humidity.)

12. After 48 hr, microscopic examination of the growth should show at least 50% sporulation.

13. The plates are held at 4 °C for at least 4 hr before harvesting procedures are carried out.

14. The plates are assembled in groups of five and the harvesting of the growth on one group of plates is completed before proceeding to the next group.

15. Two milliliters of Water for Injection (USP) are added to each plate in a group.

16. A sterile cotton swab is used to loosen the growth from the surface of the agar. One swap is used for five plates to keep spore loss on the swab to a minimum.

17. The suspended growth is transferred by pipette from the plate to a sterile centrifuge tube or bottle.

18. As soon as harvesting of the spores from all the plates is completed, the suspension is centrifuged at 1500 × *g*, the supernate is poured off, and the spore pellet resuspended in Water for Injection (USP).

19. The spore suspension is centrifuged four times at 3000 × *g* or until the supernate appears clear.

20. The spore crop is then insonated for 3 min using a sonic probe (125 watts) operating at 60% maximum power intensity.

21. The insonated spore suspension is washed and centrifuged repeatedly at 3000 × *g* until the supernate is clear.

22. The cleaned spores are suspended in Water for Injection (USP) and stored at 4 ± 2 °C.

Heat Resistance Test Procedures

All heat resistance tests were carried out at 115 °C using

TABLE I. Abundance of Growth and Numbers of *Bacillus coagulans* Spores

Sporulation Medium	Spore Crop	Abundance of Observed Growth	Number of Viable Spores per Plate[a]
Soya Peptone Agar plus spore salts	BC1	Heavy	1.4×10^9
Soya Peptone Agar plus spore salts	BC2	Heavy	1.4×10^9
Soya Peptone Agar plus spore salts	BC3	Heavy	1.2×10^9
Nutrient Agar Lot F9DEXC plus 5 ppm $MnSO_4$	BC4	Light	6.2×10^7
Nutrient Agar Lot F9DEXC plus 5 ppm $MnSO_4$	BC5	Light	9.0×10^7

[a] *Plates 100 mm in diameter.*

TABLE III. *D* (115 °C) and IR Values for *Bacillus coagulans* Spore Crop BC2 Heated at 115 °C in Five Solutions

Experiment[a] Number	Heating Medium	*D* Value, min	95% CI min	95% CI as % of *D* Value	IR
8346A	Water for Injection (USP)	6.77	6.32–7.30	14	0.95
9040A	Water for Injection (USP)	6.75	6.46–7.06	9	0.96
	Mean	6.76			0.96
9036A	Dextrose 5% in water	4.90	4.58–5.28	14	1.04
9051A	Dextrose 5% in water	4.85	4.50–5.26	16	1.05
	Mean	4.88			1.04
9023A	Lactated Ringer's	8.33	7.86–8.86	12	0.97
9043A	Lactated Ringer's	8.64	8.24–9.03	9	0.99
	Mean	8.48			0.98
9033A	Normal saline, 0.9%	9.25	8.84–9.71	9	0.98
9047A	Normal saline, 0.9%	8.99	8.22–9.92	19	0.98
	Mean	9.12			0.98
9022A	0.0153 *M* Butterfield's buffer in Water for Injection (USP)	9.68	9.24–10.2	10	0.97
9029A	0.0153 *M* Butterfield's buffer in Water for Injection (USP)	9.80	9.46–10.2	7	0.98
	Mean	9.74			0.98

[a] N_0's for all experiments ranged from 5.7×10^7 to 1.1×10^8 spores per unit. Equivalent heating times of 3, 10, 17, 24, and 31 min for all experiments.

Results

Effect of Sporulation Media on the Characteristics of B. coagulans, *FRR B666 Spores*

Microscopically, there were no apparent differences in the spores grown on any lot or kind of sporulation medium. The macroscopic appearance of the growth on both sporulation media was smooth, shiny, and off-white in color. However, the abundance of growth and the number of resistant spores produced per plate, shown in Table I, varied with the sporulation media used. The growth on nutrient agar appeared similar to that on the Soya Peptone agar but was less abundant and averaged 6.0% of the number of spores produced on the Soya Peptone agar.

Survivor curve tests were carried out at 115 °C with the spores suspended in Water for Injection (USP). The experimental parameters as well as a summary of the *D* values, 95% CI of the *D* values, and the IR values are presented in Table II.

The heat resistance characteristics of the spore crops varied with sporulation medium. All three spore crops (BC1, BC2, and BC3) grown on Soya Peptone agar plus spore salts showed very similar heat resistance characteristics. The *D* (115 °C) values ranged from 6.75 to 6.94 min. The shape of the survivor curves was close to a straight line with IR values ranging from 0.92 to 0.96. Variation among replicate samples at each heating time was very small.

Duplicate survivor curve tests for BC2 spores (grown on Soya Peptone agar) were carried out about 2 months apart; the *D* and IR values were approximately the same in both tests. The shapes of the semilogarithmic survivor curves were almost identical with corresponding *D* (115 °C) values of 6.77 and 6.75 min for the two tests.

The 95% CIs of the *D* values for the survivor curves for the three spore crops produced on Soya Peptone agar overlapped, indicating that replicate spore crops of this organism with very similar heat resistance characteristics can be grown using Soya Peptone agar plus spore salts. A representative survivor curve (Spores BC2) is shown in Figure 1.

Two spore crops, BC4 and BC5, were grown using the same lot of nutrient agar plus 5 ppm $MnSO_4$. The mean *D* (115 °C) values for the two spore crops were 4.72 and 4.48 min, respectively. The shape of the survivor curves was close to a straight line with intercept ratio values of 1.05 and 1.06. The 95% confidence intervals of the *D* values for Spore Crops BC4 and BC5 overlap, indicating that replicate spore crops were produced with very similar heat resistance characteristics. A representative survivor curve (Spores BC5) is shown in Figure 2.

Heat Resistance of B. coagulans, *FRR B666, Spores in Five Solutions*

Survivor curve tests were carried out at 115 °C using Spore Crop BC2 grown on Soya Peptone plus spore salts medium. Tests were carried out with spores suspended in five solutions: Water for Injection (USP); dextrose, 5% in Water for Injection (USP); lactated Ringer's solution; normal saline (0.9%); and 0.0153 *M* Butterfield's buffer made up with Water for Injection (USP). The results expressed as *D* values, 95% CI of the *D* values, and the IR values are summarized in Table III.

The heat resistance was the lowest when the spores were suspended in dextrose 5% in Water for Injection (USP). The mean *D* (115 °C) value was 4.88 min. The survivor curves were concave downward.

The *D* value increased by 38% when the spores were heated in Water for Injection (USP). The mean *D* (115 °C) value was 6.76 min. The shape of the survivor curve changed to slightly concave upward with a mean IR value of 0.96.

The heat resistance of the spores was the largest when the spores were suspended in one of the solutions containing ions. The mean *D* (115 °C) values were 8.48, 9.12, and 9.74 min in lactated Ringer's, normal saline (0.9%), and 0.0153

M Butterfield's buffer, respectively. The shape of all the survivor curves was very close to a straight line through N_0, with IR values ranging from 0.97 to 0.99. When the spores were heated in 0.0153 *M* Butterfield's buffer in water, the *D* (115 °C) values were clearly larger than when the spores were heated in lactated Ringer's solution. The *D* (115 °C) values in normal saline (0.9%) do not appear different from the *D* (115 °C) values in either lactated Ringer's or 0.0153 *M* Butterfield's buffer.

For the five solutions in which the spores were evaluated, the variability in the response of the spores among replicate samples at each heating time was small. Duplicate survivor curves were carried out and the *t* test run on the data showed no significant difference between the duplicate tests.

Discussion

Five spore crops of *B. coagulans*, FRR B666, were grown on two types of sporulation medium. The Soya Peptone plus spore salts medium produced a larger number of viable spores per plate than nutrient agar Lot F9DEXC plus $MnSO_4$. The results suggest that the ability to produce a certain number of spores may be a property of the specific medium system.

Spore crops grown using the same medium were similar in heat resistance (Table II), as well as having semilogarithmic survivor curves of almost identical shapes. This suggests that given one kind and lot of sporulation medium and one growth procedure protocol, replicate spore crops can be grown that are similar in heat resistance, as well as shape of the semilogarithmic survivor curve. This is an important factor in the selection of a biological indicator organism.

The replicate spore crops, which yielded similar *D* and IR values, were grown using the same spore growing protocol and the same medium to the extent that ingredients were identical (from the same bottle of nutrient agar, etc.). The same medium does not mean just a product with the same generic name or the same named product from a single supplier, but means from the same bottle or manufacturers' lot.

B. coagulans spores are sensitive to environmental conditions; this is shown by the variation of the *D* (115 °C) value and survivor curve shape in the different solutions (Table III). It has been observed that the heat resistance of bacterial spores is higher in solutions with ions than in solutions without ions. Gauthier et al. (9) found that for *B. stearothermophilus* spores, changes in heat resistance paralleled changes in the ionic concentration of the solution in which the spores were heated; however, the maximum *D* value did not occur at the maximum ionic concentration. Regarding the ionic composition of the five solutions evaluated in this study: no major ions are present in either Water for Injection (USP) or dextrose 5% in Water for Injection (USP). The 0.0153 *M* Butterfield's buffer in Water for Injection (USP) is a laboratory-prepared solution containing 15.5 mEq of both K^+ and $H_2PO_4^-$ per liter of solution. Lactated Ringer's has 130 mEq Na^+, 4 mEq K^+, 3 mEq Ca^{2+}, 109 mEq Cl^-, and 28 mEq lactate^{2-} per liter of solution. Normal saline (0.9%) contains 154 mEq of both Na^+ and Cl^- per liter of solution.

Our results may show that there is an optimum ion concentration associated with maximum heat resistance, since the *B. coagulans* spores exhibited the highest heat resistance when heated in 0.0153 *M* Butterfield's buffer in Water for Injection (USP), which has the lowest ion content of the three ionic solutions.

The difference between the two nonionic solutions, dextrose 5% in Water for Injection (USP) and Water for Injection (USP), is the 5% dextrose. Dextrose in this concentration appears to decrease the thermal resistance of the *B. coagulans* spores. This effect also has been shown to exist for both *B. stearothermophilus* spores (10, 11) and *B. subtilis* spores (12).

Summary and Conclusions

1. In these preliminary studies we were able to produce large numbers of *B. coagulans* spores rather easily on two relatively simple sporulation media.
2. The performance of replicate spore crops produced on either Soya Peptone agar plus spore salts or nutrient agar plus $MnSO_4$ was very reproducible.
3. *B. coagulans* spores appear to be very sensitive to the solution in which they are heated; both the heat resistance and the shape of the resulting semilogarithmic survivor curve are influenced by the solution in which the spores are heated.
4. Although the *B. coagulans* spores are very sensitive to the solution in which they are heated, they are very stable; duplicate survivor curves yielded almost identical *D* and IR values, and the variability among replicate samples of spores heated under similar conditions was small.
5. The overall characteristics of *B. coagulans* spores in these preliminary tests make it a potentially excellent biological indicator organism.

Acknowledgments

We thank Dr. Warth of CSIRO of Australia for supplying the original spore strain used in this study. The technical assistance of Yvonne Heisserer, John Kao, Janet Rowles, and Geraldine Smith, in carrying out the tests, analyzing the data, and developing this manuscript is gratefully acknowledged.

This work was supported in part by a grant from the Burroughs Wellcome Company, Greenville, NC.

References

1. Selkon, J. B., Sisson, P. R., and Ingham, H. R., "The use of spore strips for monitoring the sterilization of bottled fluids," *J. Hyg.*, **83**, 121-125 (1979).
2. Ohye, D. F., and Murrell, W. G., "Formation and structure of the spore of *Bacillus coagulans*," *J. Cell Biol.*, **14**, 111-123 (1962).
3. Warth, A. D., Personal communication, 1977.
4. Hammer, B. W., "Bacteriological studies on the coagulation of evaporated milk," *Iowa Agr. Exp. Stn. Res. Bull.* 19, 1915.
5. Berry, R. N., "Some new heat resistant acid-tolerant organisms causing spoilage in tomato juice," *J. Bacteriol.*, **25**, 72-73 (1933).
6. Becker, M. E., and Pederson, C. S., "The physiological characters of *Bacillus coagulans*," *J. Bacteriol.*, **59**, 717-725 (1950).

7. Murrell, W. G., and Warth, A. D., "Composition and heat resistance of bacterial spores," *Spores III*, Campbell, L. L., and Halvorson, H. O., Eds. American Society for Microbiology. Washington, DC, 1965, pp. 1-24.
8. Butterfield, C. T., "The selection of dilution waters for bacteriological examination," *Public Health Rep.*, **48**, 681-691 (1933).
9. Gauthier, C. A., Smith, G. M., and Pflug, I. J., "Effect of phosphate buffer concentration on the heat resistance of *Bacillus stearothermophilus* spores suspended in parenteral solutions," *Appl. Environ. Microbiol.*, **36**, 457-464 (1978).
10. Pflug, I. J., Smith, G. M., Scheyer, M., and Chapman, P. A., "Thermal resistance of *Bacillus stearothermophilus* spores suspended in parenteral solutions," *Bull. Parenteral Drug Assoc.*, **30**, 128-138 (1976).
11. Pflug, I. J., and Smith, G. M., 1977. "Survivor curves of bacterial spores heated in parenteral solutions," in *Spore Research 1976*, Barker, A. N., Wolf, J. Ellar, D. J., Dring, G. J., and Gould, G. W. Eds., Academic Press, London.
12. Caputo, R. A., Odlaug, T. E., Wilkinson, R. L., and Mascoli, C. C., "Biological validation of a sterilization process for a parenteral product—fractional exposure method," *J. Parenteral Drug Assoc.*, **33**, 214-221 (1979).

RESUMEN: Se evaluó el Bacilo coagulans FRR B666 como un posible indicador biológico. El organismo fué cultivado en dos tipos de medios de esporulación y se probó la resistencia de las esporas al calor húmedo. Se llevaron a cabo pruebas adicionales para determinar si las cosechas de esporas replicadas exhiben caracteristrsticas similares de resistencia al calor. Se llevaron a cabo pruebas de las curvas de supervivencia para determinar el comportamiento de las esporas de B. coagulans cuando calentadas en Agua para Inyecciones (FEU), solución tampón de Butterfield a 0.0153 M y tres soluciones parenterales representativas. Se produjeron un gran número de esporas usando un medio de esporulación relativamente simple. Partes alícuotas de las cosechas de esporas replicadas cultivadas en el mismo lote del medio tenían características casi idénticas en cuanto a la destrucción por calor. En la mayoría de las soluciones, las curvas de supervivencia semilogarítmicas eran rectilíneas. El resultado indica que B. coagulans FRR B666 es un indicador biológico estable y seguro. La reproducibilidad de las curvas de supervivencia y la poca variabilidad entre las muestras de replicàción en todas las soluciones probadas son características que sugieren que B. coagulans tiene los atributos de un organismo que potenciamente es un excelente indicador biológico.