

## Research Note

# Evaluation of *Staphylococcus aureus* Growth Potential in Ham during a Slow-Cooking Process: Use of Predictions Derived from the U.S. Department of Agriculture Pathogen Modeling Program 6.1 Predictive Model and an Inoculation Study

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### ABSTRACT

The U.S. Department of Agriculture has cautioned against slow cooking meat such that the interior temperature increases from 10°C (50°F) to 54.4°C (130°F) in  $\geq 6$  h. During a commercial ham-smoking process, the ham cold point is typically between 10 and 54.4°C for 13 h, but the ham is subsequently exposed to heating sufficient to eliminate vegetative pathogenic bacteria. Thus, production of heat-stable staphylococcal enterotoxin is the primary biological hazard. For this study, uncooked surface and uncooked ground interior ham were inoculated with a three-strain *Staphylococcus aureus* mixture, exposed to simulated surface and interior slow-cook conditions, respectively, and analyzed periodically using the Baird-Parker agar and 3M Petrifilm Staph Express count plate methods. For the surface and interior conditions, *S. aureus* numbers increased by no more than 0.1 and 0.7 log units, respectively. Predictions derived from actual time and temperature data and *S. aureus* growth values from a computer-generated model (Pathogen Modeling Program 6.1, U.S. Department of Agriculture) were for 2.7 (ham surface) and 9.9 to 10.5 (ham interior) generations of *S. aureus* growth, indicating that use of model-derived growth values would not falsely indicate safe slow cooking of ham. The Baird-Parker method recovered significantly ( $P < 0.05$ ) greater numbers of *S. aureus* than the Petrifilm Staph Express method. For hams pumped with brine to attain (i) 18% (wt/wt) weight gain, (ii)  $\geq 2.3\%$  sodium lactate, (iii)  $\geq 0.8\%$  sodium chloride, and (iv) 200 ppm ingoing sodium nitrite, slow-cooking critical limits of  $\leq 4$  h between 10 and 34°C,  $\leq 5$  h between 34 and 46°C, and  $\leq 5$  h between 46 and 54.4°C could be considered adequate to ensure safety.

In 1999, the U.S. Department of Agriculture (USDA) issued microbiological performance standards to be followed in the cooking of beef and poultry products (3). The USDA has also published a compilation of product interior time and temperature combinations validated to meet the performance standards (4). This compilation, known in the meat and poultry industry as Appendix A, is legally applicable to certain beef and poultry products, but has also been used as a guide for cooking pork products. Appendix A cautions processors against slow-cooking processes in which the interior of the product increases from 10°C (50°F) to 54.4°C (130°F) in greater than 6 h. Although slow-cooking procedures are not expressly forbidden, the onus for safety validation is on processors using them. Under the mandatory hazard analysis critical control point (HACCP) system, validated time and temperature parameters would likely serve as critical limits for the critical control point of cooking in a slow-cooking process. Thus, studies are needed to examine the safety of slow-cooking pro-

cedures and possibly validate critical limits for their use under the HACCP system.

One common industry slow-cooking procedure is the smoking of large bone-in and boneless hams. During this process, the ham interior is eventually exposed to lethal temperatures ( $\geq 54.4^\circ\text{C}$ ) for times far longer than necessary to meet the performance standards for destruction of *Salmonella* spp. (4). There is little historical evidence to suggest that growth of spore-forming pathogenic bacteria during slow cooking of ham is a significant hazard. For example, out of 680 reported foodborne illness outbreaks caused by pathogenic bacteria from 1999 to 2001, only 19 and 76 were caused by *Bacillus cereus* and *Clostridium perfringens*, respectively (8). Of these few outbreaks, pork was a potential vehicle in only two *C. perfringens* outbreaks and ham was never a potential vehicle. Furthermore, the presence of added sodium chloride and relatively high concentrations of sodium nitrite appear likely to minimize the risk of germination, growth, and toxigenesis by *B. cereus* (6, 16) and germination, growth, and sporulation-linked toxigenesis by *C. perfringens* (13, 15, 17). In contrast, there is an association between ham and outbreaks of *Staphylo-*

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*coccus aureus* intoxication. During the same 3-year period cited above, 63 of the 680 outbreaks were caused by *S. aureus*, with 11 involving ham (8). Thus, the most significant biological hazard in the slow cooking of hams is production of heat-stable enterotoxin by *S. aureus*. Intoxication resulting from growth and enterotoxin production by *S. aureus* in foods is estimated to account for 1.3% of U.S. food-borne illness cases, but a high proportion of cases is believed to be sporadic and unreported (14). The intoxication rarely results in hospitalization and is even less likely to cause death.

There are two plausible ways in which hams could be contaminated with *S. aureus* before cooking. First the surface of the ham could be contaminated as a result of slaughter, fabrication, or handling. A USDA baseline microbiological survey of 2,112 market hogs from 1995 to 1996 found that 16.0% of the carcasses were contaminated with *S. aureus*, with a mean level of 1.92 log CFU/cm<sup>2</sup> for samples testing positive (2). Humans, including food handlers (1, 10, 12), have been identified as frequent carriers of *S. aureus*, and the ham surface could become contaminated with this organism during processing and handling (7). The second possible route of contamination is via the brine solution that is pumped throughout the ham using long needles. If the brine solution or pumping apparatus was contaminated with *S. aureus*, the organism could be introduced into the ham interior. Likewise, *S. aureus* cells on the ham surface could be carried into the muscle interior by the needles during pumping. In the process examined in the present study, the cure solution is prepared and refrigerated prior to pumping the ham. The present study considered potential *S. aureus* contamination of both the ham surface and interior.

An initial evaluation of the potential for *S. aureus* growth during slow cooking is possible using actual product time and temperature data and *S. aureus* growth values from a computer-generated predictive model. The USDA Agricultural Research Service has developed such models, collectively known as Pathogen Modeling Program 6.1 (PMP 6.1; USDA Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, Pa.), for several foodborne pathogens. Although the models are based on data from studies using isothermal conditions in a laboratory medium, the senior authors have frequently used these models as a primary tool in evaluating meat and poultry processing critical limits and deviations. However, computer-generated predictive models are not acceptable as the sole means of validating critical limits. Other methods, such as inoculation studies, are needed to fully validate critical limits.

The objectives of the present study were (i) to evaluate a typical ham slow-cooking procedure for *S. aureus* growth potential using *S. aureus* growth values from the PMP 6.1 model and an inoculation study, and (ii) if the slow-cooking procedure was determined to be safe, to recommend potential critical limits for validation.

## MATERIALS AND METHODS

**Ham processing parameters.** At a commercial meat processing facility, hams were pumped at a target level of 18% using

(for 709 kg of pumped hams) a mix of 94.6 kg of water, 20.3 kg of sodium lactate, 7.4 kg of sodium chloride, 7.6 kg of sucrose, 2.4 kg of a commercial curing salt containing 6.25% sodium nitrite, 0.4 kg of sodium ascorbate, and proprietary amounts of phosphates and seasonings. Three bone-in hams, weighing between 6 and 7.5 kg, were shipped in refrigerated insulated containers to the laboratory immediately after pumping.

**Preparation of the ham for laboratory studies.** Each ham was stored at 5°C until prepared for laboratory studies. To prepare samples, ham was placed on a surface previously treated with 70% ethanol and a flame-sterilized knife was used to slice off a 1.2-cm exterior layer. The exterior layer was cut into 5 cm by 5 cm by 1.2 cm standardized pieces and placed in sterile petri plates for the exterior inoculation study. Next, sections of ham muscle were aseptically removed to reach the center. Interior muscle was aseptically removed and ground using a meat grinder with a plate having 4.0-mm holes (Model MG8912, Univex, Salem, N.H.) and previously treated with 70% ethanol. Ground interior meat (5 g) was transferred to each of several sterile 25-ml plastic tubes (Falcon brand, Fisher Scientific, Itasca, Ill.). Tubes were manually shaken to force meat to the bottom of the tube and minimize air pockets. All samples were stored at 5°C until used.

**Inoculum preparation.** A cocktail was made using three strains of *S. aureus*. Strains ATCC 25923 and ATCC 12600 were obtained from the American Type Culture Collection (Manassas, Va.), and FRI 1007 was provided by Dr. Amy Wong (Food Research Institute, University of Wisconsin–Madison). Cultures were maintained at –20°C in brain heart infusion (BHI) broth (Difco, Becton Dickinson, Sparks, Md.) containing 10% (wt/vol) glycerol (Fisher). Working cultures, maintained at 4°C on BHI agar (Difco, Becton Dickinson) plates, were prepared monthly from frozen stock. To obtain working cultures, each strain was cultured twice at 35°C in BHI broth, streaked to a BHI agar plate, incubated for 18 to 24 h at 35°C, and observed for culture purity. For each experiment, fresh cultures were prepared by transferring a loopful of growth from the working culture plate to 9 ml of BHI broth and the inoculated tubes were incubated at 35°C for 20 to 24 h. Cocktails were prepared by vortex mixing each culture, combining the cultures into a 50-ml conical tube (Falcon brand, Fisher), and centrifuging at 5,000 × *g* for 10 min. The supernatant was decanted and cells were resuspended to the original volume in Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, Wis.). The cocktail was then serially diluted in BPD and plated on Baird-Parker agar (B-P; Difco, Becton Dickinson) and 3M Petrifilm Staph Express (PF-SE; 3M Microbiology, St. Paul, Minn.) for initial enumeration. The PF-SE plates were incubated at 35°C for 24 h, and the B-P plates were incubated for 48 h at the same temperature. If only red-to-purple colonies were observed on a PF-SE plate after 24 h, they were counted as presumptive *S. aureus*. If more than one type of colony was observed, a thermonuclease disk was applied to the PF-SE plate and plates were reincubated at 35°C for 1 to 3 h. Colonies displaying thermonuclease activity, as indicated by a pink halo on the thermonuclease disk after the second incubation period, were counted as presumptive *S. aureus*. Circular, smooth, convex, and gray to jet-black colonies surrounded by an opaque zone on B-P were counted as presumptive *S. aureus*.

**Inoculation and simulation of ham slow-cooking conditions.** Surface ham pieces were inoculated with 0.1 ml of a 1:1,000 dilution (BPD) of the *S. aureus* cocktail. The inoculum was spread evenly over the meat surface using a sterile bent plastic spreader (Daigger, Vernon Hills, Ill.). Each petri plate (no lid)

TABLE 1. Use of *Staphylococcus aureus* growth values from the PMP 6.1 predictive model to estimate *S. aureus* growth during slow cooking of ham<sup>a</sup>

Time elapsed (h)	Length of interval (h)	Actual temp (°C) <sup>b</sup>	Assigned temp (°C)	Lag time from model (h)	% lag phase calculated <sup>c</sup>	Generation time from model (h)	Generations calculated
Surface runs 1–3							
0.5	0.5	22.2–37.2	37.2	1.8	27.8 (27.8)	0.6	— <sup>d</sup>
1.0	0.5	37.2	37.2	1.8	27.8 (55.6)	0.6	—
1.5	0.5	37.2–47.2	37.2	1.8	27.8 (83.4)	0.6	—
3.5	2.0	47.2	42	2.3	16.6 (100)	0.6	2.7
Interior run 1							
2.0	2.0	8.8–22.8	22.8	5.8	34.5 (34.5)	1.8	—
3.0	1.0	22.8–27.2	27.2	3.1	32.2 (66.8)	1.1	—
4.0	1.0	27.2–33.9	33.9	1.9	33.2 (100)	0.7	0.4 (0.4)
5.0	1.0	33.9–36.1	36.1	1.8	— <sup>e</sup>	0.6	1.7 (2.1)
7.0	2.0	36.1–41.7	37.2	1.8	—	0.6	3.4 (5.5)
8.0	1.0	41.7–43.9	41.7	2.2	—	0.6	1.6 (7.1)
10.0	2.0	43.9–47.8	42	2.3	—	0.6	3.3 (10.4)
Interior run 2							
2.0	2.0	6.0–21.0	21.0	8.2	24.3 (24.3)	2.3	—
3.0	1.0	21.0–28.0	28.0	2.8	35.7 (70.0)	1.0	—
4.0	1.0	28.0–33.0	33.0	1.9	30.0 (100)	0.7	0.8 (0.8)
5.0	1.0	33.0–35.5	35.5	1.8	—	0.6	1.4 (2.2)
7.0	2.0	35.5–41.0	37.2	1.8	—	0.6	3.4 (5.6)
8.0	1.0	41.0–44.0	41.0	2.1	—	0.6	1.6 (7.2)
10.0	2.0	44.0–47.0	42	2.3	—	0.6	3.3 (10.5)
Interior run 3							
2.0	2.0	10.0–20.5	20.5	9.1	22.0 (22.0)	2.5	—
3.0	1.0	20.5–27.5	27.5	3.0	33.3 (55.3)	1.1	—
4.0	1.0	27.5–32.0	32.0	2.0	44.7 (100)	0.7	0.2 (0.2)
5.0	1.0	32.0–35.0	35.0	1.8	—	0.6	1.4 (1.6)
7.0	2.0	35.0–40.0	37.2	1.8	—	0.6	3.4 (5.0)
8.0	1.0	40.0–43.0	40.0	2.0	—	0.6	1.6 (6.6)
10.0	2.0	43.0–46.0	42	2.3	—	0.6	3.3 (9.9)

<sup>a</sup> Growth parameters obtained for the following environmental conditions: aerobic growth, pH 6.5, 2.5% water-phase sodium chloride, and 150 ppm added sodium nitrite. Time and temperature data used were based on actual commercial conditions.

<sup>b</sup> Air temperature for surface studies; temperature of interior ground ham for interior studies.

<sup>c</sup> Value is percentage of lag time for the given time interval with cumulative percentage of lag time in parentheses.

<sup>d</sup> Absence of value indicates that the calculated lag phase had not ended. When a value is provided, it is the number of generations for a given time interval with the cumulative number of generations in parentheses.

<sup>e</sup> No value provided because lag phase was completed in preceding time interval.

containing the inoculated ham exterior piece was placed in a 22°C incubator. To simulate time and temperature data obtained for the commercial process of interest, the incubator temperature was increased after 30 min to 37°C, followed by another increase to 47°C after 1.5 h. This temperature was maintained for an additional 2.0 h. The ground interior ham in each tube was inoculated with 0.1 ml of *S. aureus* cocktail (diluted 1:1,000 in BPD) by using a pipette tip to insert inoculum in the center of the ground meat. To simulate time and temperature data obtained for the commercial process of interest, the samples remained at room temperature for 2 h before being placed in a 26.7°C water bath (Precision Reciprocal Shaking Bath, Precision Scientific, Chicago, Ill.). Subsequent increases in temperature occurred hourly. To monitor temperature, a calibrated thermometer was placed in the center of 5 g of uninoculated ground ham in the same type of tube used for the inoculated ham. This tube was placed in the water bath among the other tubes. Both experiments were done in triplicate (one trial per ham) with duplicate samples analyzed at each sampling time in each trial.

#### Enumeration of inoculum organisms before and during slow cooking.

To evaluate survival of *S. aureus* during the slow-cooking ham procedure, samples were analyzed at periodic intervals. Each ham surface piece was placed in a sample bag, and 99 ml of BPD was added before stomaching for 2 min on medium using a Stomacher 400 lab blender (Fisher). To analyze each ham interior sample, ground ham was aseptically removed from the Falcon tube to a sample bag and weighed and BPD was added to make a 1:10 dilution. Samples were stomached for 2 min on medium using a Stomacher 400 lab blender. For both interior and exterior samples, subsequent dilutions were made in BPD and plated to B-P and PF-SE. The initial dilution was transferred to three B-P plates (0.3, 0.3, and 0.4 ml) and one PF-SE plate. All subsequent dilutions were transferred to one plate each of B-P and PF-SE. Plates were incubated as previously described, typical colonies were counted, and log CFU was calculated for each sample. To confirm that colonies counted were *S. aureus*, one presumptive colony from each plating method from each trial was further tested for cell morphology, Gram reaction, and coagulase activity.

Throughout the study, all presumptive colonies were confirmed as *S. aureus*.

**Deriving predictions from *S. aureus* growth values from the PMP 6.1 model.** For each 0.5 to 2 h interval of the ham-cooking simulation, the *S. aureus* growth values from the PMP 6.1 predictive growth model were used to predict the proportion of *S. aureus* lag phase that would elapse or the number of generations of *S. aureus* growth that would occur. To obtain the growth values, the following environmental conditions were used in the model: aerobic growth (chosen because aerobic growth is more rapid than anaerobic), pH 6.5 (typical prepumping value for hams; curing causes a drop of about 0.2), 2.5% water-phase sodium chloride (calculated from worst case values of 1.8% sodium chloride and 68% moisture), and 150 ppm added sodium nitrite (highest value allowed in the model). The model covers the temperature range of 10 to 42°C. For each time interval with temperatures below the optimum for *S. aureus* growth (34°C), the maximum temperature for the interval was assumed to have occurred for the entire interval. For time intervals between 34°C and the maximum temperature for *S. aureus* enterotoxin production (46°C) (12), the lowest growth temperature in the interval was assumed to have occurred for the entire interval. It was assumed that *S. aureus* was initially in lag phase (which is plausible because both the ham and the cure solution are refrigerated) and that the lag time at each given temperature during heating was the same as for the corresponding temperature during an isothermal experiment. Based on these assumptions, the proportion of lag phase elapsed during each time interval was obtained from the model and the cumulative proportion of lag phase was calculated (see Table 1). Similarly, when the entire lag phase elapsed, the number of growth generations predicted during each time interval was determined from the corresponding isothermal model information and the cumulative number of growth generations was calculated.

**Statistical analyses.** For the ham exterior data, mean log CFU per piece of meat was calculated for each sampling time, along with standard deviation. The mean and standard deviation of log CFU per gram were calculated for the ground interior ham for each sampling time. For each experiment, the paired *t* test (release 12, Minitab, Inc., State College, Pa.) was done to determine whether a significant ( $P < 0.05$ ) difference existed between the B-P and PF-SE enumeration methods.

## RESULTS AND DISCUSSION

In both the ham surface and ham interior experiments, there was a small but statistically significant ( $P < 0.05$ ) difference between the two methods used for *S. aureus* enumeration. On average, the B-P method recovered slightly more cells than the PF-SE method. A previous study of inoculated smoked fish, mozzarella cheese, and Parmesan cheese found a similar difference between the two methods for mozzarella cheese samples stored at 4°C for 28 and 42 days (11). However, there was no difference between the B-P and PF-SE methods for mozzarella cheese samples stored for 0 or 14 days or for the Parmesan cheese and smoked fish. It is possible that some environmentally stressed *S. aureus* cells are unable to grow on the PF-SE plate, causing the difference observed in the present study.

Contamination of the ham interior with *S. aureus* is less likely to occur than surface contamination. However, interior contamination poses a greater potential food safety

TABLE 2. Values for *Staphylococcus aureus* in ground interior ham meat during simulation of a commercial slow-cooking process<sup>a</sup>

Time (h)	Target temp (°C) <sup>b</sup>	Actual temp (°C)	Log CFU/g	
			B-P	PF-SE
0	5.0	8.3 (2.1)	3.0 (0.1)	2.9 (0.1)
2	21.1	21.5 (1.3)	3.0 (0)	2.8 (0)
3	26.7	27.7 (0.3)	2.9 (0.1)	2.9 (0.1)
4	32.2	32.9 (0.9)	3.0 (0.1)	2.9 (0.1)
5	35.0	35.6 (0.6)	3.2 (0.5)	2.9 (0.3)
6	37.8	38.5 (0.5)	2.9 (0.1)	3.1 (0.5)
7	40.6	40.8 (0.8)	2.9 (0.1)	3.0 (0.2)
8	43.3	43.7 (0.6)	2.9 (0.1)	2.9 (0.1)
9	45.6	46.0 (0.5)	3.0 (0.2)	2.9 (0.2)
10	46.7	46.8 (0.8)	3.4 (0.4)	3.4 (0.5)
11	47.8	47.7 (0.6)	3.7 (0.7)	3.4 (0.7)
12	49.4	49.0 (1.1)	3.4 (0.8)	3.0 (0.8)
13	52.8	52.9 (0.3)	3.7 (1.1)	3.0 (1.1)
14	54.4	54.8 (0.8)	2.8 (1.5)	2.0 (1.2)

<sup>a</sup> Values are means ( $n = 3$ ) and standard deviations in parentheses. *S. aureus* was enumerated by plating on Baird-Parker agar (B-P) and the 3M Petrifilm Staph Express Count plate (PF-SE).

<sup>b</sup> Based on typical ham interior temperature during a commercial slow-cooking process.

hazard because the ham interior heats much more slowly than the surface. Predictions made from *S. aureus* growth values from the PMP 6.1 computer-generated model were for 9.9 to 10.5 generations, roughly 3 log units, of growth for *S. aureus* exposed to the temperature conditions in each of the three trials. This level of growth would result in enterotoxin production sufficient to cause illness, about  $10^6$  cells, if *S. aureus* numbers prior to growth were in the 3-log range (5). The inoculation studies showed that the predictions based on *S. aureus* growth values from the PMP 6.1 model, as applied to this situation, were extremely conservative. Observed increases in *S. aureus* numbers did not exceed 0.7 log units (Table 2), between two and three generations of growth, for the period during which the ham was within the temperature range supporting *S. aureus* growth. It is likely that the ham composition was not as suitable for growth as the broth-based media used in obtaining data for the computer-generated model. Interestingly, during two of the three trials, *S. aureus* numbers began to decline during hour 14 of the experiment, which suggests that death of the organism begins between 52.5 and 54°C. This decrease accounted for the noticeably higher standard deviation observed for hour 14 data (Table 2).

Surface contamination of ham with *S. aureus* is more likely to occur than interior contamination, but the ham surface is exposed to suitable growth temperatures for a much shorter time than the interior. Predictions based on *S. aureus* growth values from the PMP 6.1 model were for 2.7 generations of *S. aureus* growth under temperature conditions to which the ham surface was exposed. This level of growth would only result in production of illness-causing amounts of enterotoxin if initial numbers of *S. aureus* were extremely high. The inoculation studies showed no signif-

TABLE 3. Values for *Staphylococcus aureus* on exterior pieces of ham during simulation of a commercial slow-cooking process<sup>a</sup>

Time (h)	Incubator temp (°C) <sup>b</sup>	Log CFU/piece	
		B-P	PF-SE
0	5	2.6 (0.1)	2.5 (0.1)
0.5	37	2.7 (0.1)	2.6 (0.1)
1	37	2.7 (0.1)	2.6 (0.1)
1.5	47	2.7 (0.1)	2.6 (0.1)
2.0	47	2.4 (0.2)	2.3 (0.1)
2.5	47	2.4 (0.1)	2.3 (0.2)
3 (n = 2)	47	2.3 (0.4)	2.2 (0.3)
3.5 <sup>c</sup>	47	2.5 (0.1)	2.5 (0.3)

<sup>a</sup> Values are means (n = 3) and standard deviations in parentheses. *S. aureus* was enumerated by plating on Baird-Parker agar (B-P) and the 3M Petrifilm Staph Express Count plate (PF-SE).

<sup>b</sup> Based on typical smokehouse temperature in a commercial ham slow-cooking process.

<sup>c</sup> Experiment ended after 3.5 h because smokehouse temperature exceeded 54.4°C.

icant increase in *S. aureus* numbers during the heating period (Table 3).

For hams having a composition similar to those used in this study, the inoculation study results clearly showed that slow-cooking critical limits for product internal temperature of  $\leq 4$  h between 10 and 34°C,  $\leq 5$  h between 34 and 46°C, and  $\leq 5$  h between 46 and 54.4°C will not allow growth of *S. aureus* to levels where enough toxin is produced to cause illness. Thus, the critical limits are safe and could be validated for use in HACCP plans. Key compositional levels that should be met for pumped hams cooked according to these critical limits would be a weight gain of 18% (wt/wt),  $\geq 2.3\%$  sodium lactate,  $\geq 0.8\%$  sodium chloride, and 200 ppm sodium nitrite (the legal maximum). However, since brine has been shown to be a source of bacterial contamination (9), processors should frequently change the brine and clean and sanitize the brine container and pumping needles. Periodic microbiological testing is recommended to verify that the cure solution and pumping needles are not contaminated with high numbers of *S. aureus*. It is clear from our results that the *S. aureus* growth values from the PMP 6.1 model can be safely used to evaluate the safety of ham slow-cooking procedures, although such use of the values will greatly overestimate *S. aureus* growth.

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