Food Sci. Biotechnol. 20(5): 1351-1359 (2011)
DOI 10.1007/s10068-011-0186-3

RESEARCH ARTICLE

Prevalence of the Levels of *Bacillus cereus* in Fried Rice Dishes and Its Exposure Assessment from Chinese-style Restaurants

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Received: 14 April 2011 / Revised: 1 July 2011 / Accepted: 4 July 2011 / Published Online: 31 October 2011

Abstract This study aimed to identify the exposure pathway for fried rice dishes and evaluate its microbiological quality from Chinese-style restaurants. Exposure pathway for fried rice was assessed in terms of time, temperature, and serving size by phases from preparation to consumer consumption. The microbiological quality of 32 samples was evaluated for the levels of *Bacillus cereus,* aerobic mesophilic plate count (APC), and coliforms. One serving size of fried rice dishes was 352.2 g. The final temperature of fried rice dishes at the consumption point was 66.1°C for cook-to-order restaurants, and 59.8°C for reheat-to-cook type restaurants. The prevalence of *B. cereus* detected in cooked rice at consumption point was 37.5%. Production types, final temperature at cooking, and consumption phases were associated with contamination level of *B. cereus* ($p<0.05$). Therefore, for the prevention of *B. cereus* outbreaks from fried rice dishes, cook-to-order type of production, and rapid consumption after cooking were recommended.

Keywords: fried rice dish, prevalence of *Bacillus cereus,* restaurant, real time-PCR, exposure pathway

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Introduction

Bacillus cereus is found in ubiquitous in water, soil, and air with vegetable cell and endospore form (1). In Korea, *B. cereus* was responsible for 15 foodborne outbreaks (392 patients), accounting 5.5% of the total outbreaks in 2009 from bacteria caused illness (2). *B. cereus* is a pathogenic microorganism showing 2 types of foodborne illness (3). One is an emetic type of foodborne illness associated with the consumption of *kimbab* (4) and *sunshik* (5), whereas the other is diarrheal type associated with sprout vegetables (6). *B. cereus* is underreported in the US because of not tracking by the US FoodNet Program (7). Like the US, foodborne illness outbreaks from *B. cereus* in South Korea have been steadily increasing with underreported, even though with a small scale.

Fried rice dishes are one of the popular menu items in Korea foodservice industry. But there is little research for identifying the prevalence of *B. cereus.* From a study (8) for identifying the prevalence of *B. cereus* of fried rice dishes delivered from Chinese-style restaurants with MYP agar and the API kit (bio-Merieux, Marey l'Etoile, France) for conformation test, the level of *B. cereus* of the dishes indicated as 1.48-3.47 log CFU/g. But any samples did not show *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella*. To reach much clear conclusion for identification of *B. cereus,* the study pointed out that a future study should be conducted using chromgenic *B. cereus* media with PCR (7,8).

For the quantitative microbial risk assessment (QMRA) in fried rice dishes, moreover, data for assessing the exposure during the pathway of the *B. cereus* from production to consumption should be collected (9-11). A QMRA is defined as the technical assessment of the nature and magnitude of a risk caused by a hazard (9). It includes hazard identification, exposure assessment, dose-response

assessment, and risk characterization.

To prevent food poisoning from *B. cereus* in food service operation, strict management for cooking methods, and time-temperature control are necessary (12). For example, maintaining a temperature of more than 63°C, or rapid cooing within 2 h after cooking was recommended. Agata *et al*. (13) also pointed out that for the prevention of emetic food poisoning outbreaks caused by *B. cereus,* the most important factor is to inhibit the bacterial growth through strict temperature control in the whole production process. However, in Korea, there are no data on the exposure pathway and contamination level of *B. cereus* in those dishes.

Therefore, in this study to determine the prevalence of *B. cereus* in fried rice dishes produced in 8 Chinese-style restaurants, the level of *B. cereus* and the exposure assessment were conducted. The specific objectives of this study were (1) to investigate the exposure time and temperature of fried rice dishes during production and consume process, (2) to evaluate the level of aerobic mesophilic plate count (APC), coliforms, and *B. cereus*, and (3) to identify the critical determinants on increases of *B. cereus* through the assessment of production procedure and consumption pathway.

Materials and Methods

Procedures of the study This study conducted as 4 steps. First, a literature review on the prevalence and contamination levels of *B. cereus* in fried rice dishes was done. Second, through on-visit surveys, data were collected in face-to-face interviews with cooks or managers and measured with a thermometer and a watch during production phases. For third step, microbiological tests for fried rice dishes delivered from the surveyed restaurants to the laboratory were done with chromogenic *B. cereus* medium and real time (RT)-PCR system. The last step was for the risk assessment. We tried to identify risk factors in the pathway of production and consumption in fried rice dishes. A preliminary survey was conducted from July 17 to July 23, and the main survey was done from 24 to Aug. 29, 2008.

Collection of samples and measurement for exposure time and temperature Two researchers visited the restaurants around 12:00 (at lunch time) and on-site investigations were conducted. As presented at Table 1, among foods selling at Chinese restaurants, 2 dishes of fried rice and seafood fried rice were selected. Each dish, both with and without a sauce mix was taken. Thus, a total of 32 samples were tested for the microbiological tests. Exposure time and temperature were measured at the production and consumption phases at 8 restaurants. Thereafter, the samples were delivered to the laboratory for microbiological testing and the determination of the food temperature at the delivery points and delivery time. One serving portion of dishes were measured.

Table 1. Variables of exposure pathway

Process	Variable
Preparation	Temperature of precooked rice/Storage condition Temperature of precooked sauce/Storage condition
Cooking	Cooking time Temperature of fried rice dishes Environment temperature
Holding before service	Holding time Temperature of fried rice dishes Environment temperature
Delivery	Temperature of fried rice dishes Delivery time Environment temperature

Enumeration of microorganisms from fried rice dishes The collected samples were treated aseptically and inspected using sterilized instruments. Twenty g of the food samples were placed in 225 mL of sterile 0.1%(w/v) buffered peptone water and homogenized for 2 min in a stomacher (Easymix, BRUZ cedex, France) and serial decimal dilutions were performed. To reduce the variation in analysis, all experiments were done in duplicate.

All samples were analyzed for aerobic mesophilic plate count (APC), coliform, and *B. cereus* based on the guideline of the Korea Food Code (14) and Food & Drug Administration (FDA) (15). Serial decimal dilutions in sterile peptone water were prepared and plated in duplicate using pour plate techniques. The diluted solution was added to 15 mL plate count agar (BD, Detroit, MI, USA) and incubated for 48 h at 35°C. Deoxycholate lactose agar (BD) was used to determine coliform groups. The plates were incubated for 48 h at 35°C. Plates containing purple-red colonies forming units (CFU) were counted.

Chromogenic *B. cereus* media (Oxoid, Basingstoke, UK) were used to detect *B. cereus,* thereafter RT-PCR was used for the confirmation of the microorganism. A 0.2 mL of diluted sample was inoculated onto each of the 5 petri dishes and was dispersed using sterilized spreaders. The media were incubated for 24 h at 30°C. The preliminary number of *B. cereus* was determined after enumeration of the colonies having blue colony. Then, 5 colonies were selected for the conformation of *B. cereus* using GenSpector TMC-1000 PCR system (Samsung Techwin, Seongnam, Korea).

DNA extraction for PCR assays DNA of each strain was extracted from 5 typical colonies of *B. cereus*

Table 2. Sequences of the primers used in this study

Bacteria	Primer[1)]	Sequence (5'→3')	Target gene	Size
Bacillus cereus	BC-F	AAGCACGTCGTTCGATGCT	groEL	45 bp
	BC-R	TTGGTCCAAGCGTTACTTTTACTG		

[1)]F, forward primer; R, reverse primer

chromogenic agar cultures. Colony was suspended in 5 mL sterilized water. One mL of suspended sample cell was taken in 1.5-mL tube and centrifuged at 9,516×*g* for 5 min. The pellets was resuspended in 150 μL of nuclease free water, and boiled at 95°C for 20 min. After cooling of sample for 1 min, it was centrifuged at 9,516×*g* for 10 min.

PCR assays for *B. cereus* using TMC-1000 PCR system For the confirmation test of *B. cereus* with PCR, GenSpector TMC-1000 system (Samsung Techwin) was used. The oligonucleotide primer used in this study was manufactured by Bioneer Company (Daejeon, Korea). Samples were checked for the presence of the gene listed in Table 2. All PCR reactions were performed using previously described conditions (16).

The reaction mixture was prepared with template 4 μL, primer 2 μL, and 2X SYBR Green I Primix Ex Taq 6 μL. Mixture 1 μL was injected into a channel at a silicon chip of GenSpector TMC-1000 PCR system (Samsung Techwin). Parameters were 10 min at 95°C followed by 50× [5 s 95°C, 5 s at 60°C, 5 s at 72°C] and 10 min at 95°C. At the RT-PCR program results, an 'OK' display represented a negative indication of *B. cereus* and 'NG' indicated a positive for *B. cereus*. The value of Tm for *B. cereus* at TMC-1000^{TM} system (Samsung Techwin) was 82.7°C.

pH measurement Ten g of the food samples were mixed with 90 mL of distilled water in a stomacher (Easymix) and then the pH was measured in 1:1 (v/v) dilution distilled water using a pH meter (Orion, Beverly, MA, USA).

Statistical analyses In order to calculate the level of *B. cereus*, the numbers of colonies after incubations were converted into log values. The assessment for the microbiological quality of the surveyed restaurants was completed with the application to the domestic and foreign microbiological standards (14,15). Wilcoxon sum rank test was applied to identity the effects of the production types on the food safety management and Pearson's correlation were tested for the relations between variables. The data were analyzed using the SPSS software (version 12.0; Statistical Package for the Social Science Inc., Chicago, Il, USA).

Results and Discussion

Exposure time and temperature during production and consumption of fried rice As presented at Table 3 for fried rice and seafood-fried rice dishes, exposure assessments including time and temperature during the production process and consumption were done at 8 Chinese-style restaurants on Gyeonggi province in Korea. Through on-site observation, the production types can be classified into 2 types: cook-to-order, and reheat-to-cook. The cook-to-order type is where steamed rice is made in the morning and kept under a warmer (an electric cooker) set to over 60°C. It is then subsequently cooked with oil and vegetables when a customer orders. The other type, reheat-to-cook, is 5 to 20 serving sizes of fried rice with other ingredients, which has par-cooked in advance and held at room temperature. Cooking is completed when a customer orders. Three out of 8 restaurants (37.5%) employed cook-to-order production. The rest (62.5%) employed reheat-to-cook with an average batch size of 10 serving size, with a range from 6 to 15.

In the pre-preparation of fried rice dishes and fried rice with seafood, all 8 restaurant cooks responded that the steamed rice and the sauce on the dish were made fresh every day. Little *et al*. (10) pointed out that 241 samples among 508 rice dishes, that are 47%, were made with cooked rice in advance and 20% of the cooked rice (47/241) exceeded acceptable level of the quality standard for APC, *B. cereus,* and *E. coli* samples. One hundred-sixteen samples among the 241 precooked rice dishes were kept under ambient environment conditions and among them 24% (28/116) did not met the microbial standard. This study presented that large quantities of preheated fried rice was kept under room temperature especially high humid and temperature in cooking area in restaurants. In our study, unlike the previous study, it revealed that all restaurants made fried rice dishes with newly cooked rice each day.

In our study, the temperature of cooked rice being kept in the warmer showed an average temperature of 75.1°C, meanwhile, that of preheated fried rice in the process of reheat-to-cook type was 43.1°C. In the study of the identification of the growth pattern for *B. cereus* in *kimbab* (17), *B. cereus* inoculated on *kimbab* did not grow within

Table 3. Exposure time and temperature during production and delivery of fried rice and seafood-fried rice dishes

Process		Variable[1)]	A	B	C	D	E	F	G	H
Preparation	Rice	Start point of cooking	On the day	On the day	On the day	On the day	On the day	On the day	On the day	On the day
		Storage condition	Rice cooker	Rice cooker	Room	Room	Room	Room	Room	Rice cooker
		FT of cooked rice	72.3°C	78.9°C	41.4°C	42.0°C	50.3°C	29.4°C	43.2°C	74.0°C
		Type of rice	Steamed rice	Steamed ice	Precooked fried rice	Precooked fried rice	Precooked fried rice	Precooked fried rice	Precooked fried rice	Steamed rice
		Quantity of precooked fried rice dishes	-	-	8 portion	10 portion	6 portion	10 portion	15 portion	-
	Sauce	A staring point on cooking	On the day	On the day	On the day	On the day	On the day	On the day	On the day	On the day
		Storage condition	Room	Warmer	Warmer	Warmer	Warmer	Warmer	Warmer	Warmer
		FT	69.7°C	75.5°C	68.0°C	78.3°C	47.5°C	41.4°C	60.7°C	64.9°C
Cooking		ET	29.7°C	32.7°C	30.2°C	37.2°C	27.5°C	43.8°C	34.5°C	30.2°C
		FT: Fried rice	81.1°C	83.2°C	64.0°C	58.3°C	84.4°C	67.7°C	68.1°C	79.8°C
		Seafood-fried rice	81.1°C	88.4°C	60.2°C	64.9°C	84.3°C	62.0°C	65.7°C	74.9°C
		Cooking time: Fried rice	2 min 10 s	2 min 6 s	1 min 30 s	52 s	1 min 47 s	46 s	1 min 15 s	1 min 11 s
		Seafood-fried rice	3 min	2 min 13 s	1 min 29 s	1 min 15 s	1 min 30 s	1 min 19 s	1 min 45 s	1 min 14 s
Holding before service		Holding time	1 min	1 min	1 min	1 min	3 min	1 min 20 s	1 min	1 min 20 s
		ET	33.6°C	26.0°C	27.1°C	26.6°C	24.6°C	27.3°C	29.1°C	27.1°C
		FT: Fried rice	81.1°C	83.2°C	64.0°C	58.3°C	84.4°C	67.7°C	68.1°C	79.8°C
		Seafood-fried rice	81.1°C	88.4°C	60.2°C	64.9°C	84.3°C	62°C	65.7°C	74.9°C
Delivery		Delivery time	10 min	12 min	5 min	6 min	14 min	23 min	11 min	18 min
		FT: Fried rice	68.6°C	70.0°C	60.7°C	51.7°C	74.8°C	57.7°C	54.1°C	59.8°C
		Seafood-fried rice	74.5°C	74.6°C	56.4°C	62.4°C	70.1°C	57.6°C	56.0°C	60.2°C
		ET	29.0°C	26.8°C	24.6°C	23.7°C	26.7°C	24.8°C	24.1°C	27.1°C
		One portion size								
		Fried rice dishes from	-	437.1 g	385.5 g	550.0 g	405.4 g	326.2 g	322.6 g	322.6 g
		Sauce	-	117.8 g	-	265.0 g	73.9 g	115.0 g	140.9 g	140.2 g

[1)]FT, food temperature; ET, environment temperature

1 h, and grew slowly after 2 h, but logarithmically grew after 3 h under 25°C. Given these points, the most risky conditions were found at F restaurant, showing 29.4°C internal temperature of pre-fried rice. It could be a good medium for *B. cereus*.

Only 1 restaurant stored sauce at room temperature (1/8; 12.5%). The other 7 restaurants kept it hot. Hot-holding equipment included: hot-holding steam tables and a double boiler (a handleless pot of sauce sitting in a pot of boiling waters). In the case of warming sauce up in a double boiler, contamination of water in the outer pot with foreign substances was observed, and it was possible to deteriorate the quality of sauce. Of most concern was that, even if the sauce was kept in a warmer, the internal temperature of sauce in 2 restaurants did not meet the recommended FDA standard of over 57°C (indicating ranged 41.4 and 47.5°C). The temperature of the rest was over 57°C whether kept at room temperature or in the warmer.

The cooking of fried rice was done in an average of 1 min 21 s under indoor temperatures of 33.2°C. More specifically, cook-to-order type restaurants cooked for 1 min 49 s under 30.9°C, with the internal temperature of fried rice eventually reaching 81.4°C. Meanwhile, the reheat-to-cook restaurants showed less cooking time (1 min 14 s), less final temperature of foods (68.5°C), and higher indoor ambient temperatures (34.6°C) compared to those of the cook-to-order restaurants. It means that the restaurants employing the reheat-to-cook type of food production were exposed to more vulnerable situations in terms of food safety than that of cook-to-order restaurants. Reheat-to-cook type restaurants could reduce the cooking time by keeping foods pre-cooked, however, given that the time difference was 35 s, the time-saving effect was minimal. Therefore, considering the food safety, the cook-to-order method was more preferable and recommended than the reheat-to-cook.

Cooking time of seafood-fried rice at 8 restaurants was 1 min 42 s, on average. For fried rice with seafood, the cooking time was longer (21 s more) than fried rice. The average cooking time, room temperature of kitchen, and the final temperature of seafood fried rice were 2 min 9 s, 30.9, 81.5°C for cook-to-order and 1 min 27 s, 34.6, 67.4°C for reheat-to-cook, respectively.

The Ministry of Education, Science and Technology (18) suggested the school food safety monitoring tool which is called the 'Hygiene and Safety Checklist for School Foodservice'. It was divided into 3 parts: food production sanitation, facility sanitation, and personal sanitation. According to the guideline, the foods should be heated up to 74°C or more and employees should verify it by measuring the internal temperature of food. In this study, food samples from 4 restaurants among 8 restaurants surveyed did not meet the internal food temperature limit of 74°C right after cooking. In the process of assembly for fried rice with and without seafood, time for packaging and delivery and ambient temperature of packing area were measured. In the assembly stage, the assembly time from 8 restaurants was taken 1 min 20 s on average. Delivery time was calculated by subtracting from the time which researchers reported the deliverer left the restaurant to the time the food was delivered to laboratory. Delivery time was different depending on the distance between the laboratory and the restaurant surveyed, and traffic conditions, but the average delivery time was approximately 12 min. The indoor temperature of the laboratory was 25.6°C, on average. The temperature of fried rice dishes at the consumption point was 66.1°C for cook-to-order, and 59.8°C for reheat-to-cook. In the case of seafood-fried rice, the former was 69.7°C and the latter was 60.5°C. These results indicated that cook-to-order types' restaurants are better controlled in terms of the temperature of foods than reheat-to-cook.

One-serving size of fried rice with/without seafood from 8 restaurants presented at Table 3. The average quantity of fried rice was 395.2 g and the quantity of its sauce was 142.1 g.

Microbiological quality of fried rice dishes Levels of APC, coliforms, and *B. cereus* of fried rice dishes with and without sauce were presented at Table 4. The pH ranged from 6.61 to 8.09, on average of 7.13 for fried rice dishes. Except only 1 restaurant samples not detected in APC, 7 restaurants indicated the contamination levels with APC for fried rice dishes as 2.90 to 4.93 log CFU/g, on average with 3.73 log CFU/g, indicating a fairly good condition. In 3 samples out of 8, meanwhile, coliforms detected with range of 1.65 to 2.88 log CFU/g on average of 2.05 log CFU/g. It represented that a need for the actions for employees' food handling practices. *B. cereus* were not detected in 5 restaurants' samples among 8 and contamination levels of *B. cereus* from the other restaurants reached from 3.08 to 4.68 log CFU/g with average of 4.08 log CFU/g, indicating comparatively high when we compared with the levels of coliforms.

With the addition of the sauce on the fried rice, the pH of the dishes showed a tendency to decrease as pH 6.46. With the sauce on the rice dishes, excluding of A restaurant sample, microbial quality of samples had tendencies of lower, indicating 3.84 log CFU/g for APC, 2.23 log CFU/g for coliforms, and 4.51 log CFU/g for *B. cereus* on average.

The pH of seafood fried rice dishes had a tendency of higher than those of fried rice, showing the range from pH 6.17 to 7.56, on average pH 7.16. Only A restaurant's samples did not detect APC and the others ranged between 2.7 and 4.27 log CFU/g with the average of 3.35 log CFU/

Table 4. Microbiological quality of fried rice dishes with and without sauce

(unit: log CFU/g)

Sample	Fried rice dishes				Fried rice dishes with sauce				Seafood fried rice dishes				Seafood fried rice with sauce			
	pH	ACC[1)]	Coliforms	*B. cereus*	pH	ACC	Coliforms	*B. cereus*	pH	ACC	Coliforms	*B. cereus*	pH	ACC	Coliforms	*B. cereus*
A	7.05	ND[2)]	ND	ND	6.34	ND	ND	ND	7.05	ND	ND	ND	6.53	ND	ND	ND
B	6.61	4.31	2.88	ND	6.31	4.11	1.70	ND	6.61	4.31	2.88	ND	6.17	4.11	2.79	ND
C	6.64	3.55	2.00	4.49	6.25	3.99	3.81	4.57	6.64	3.55	2.00	4.49	6.64	3.60	1.98	ND
D	6.99	4.92	1.78	3.08	6.11	4.79	2.60	4.27	6.99	4.92	1.78	3.08	5.88	3.66	1.54	4.13
E	7.15	3.65	ND	ND	5.78	3.38	ND	ND	7.15	3.65	ND	ND	6.02	3.60	ND	ND
F	7.01	3.18	1.65	4.68	6.44	3.77	1.62	4.68	7.01	3.18	1.65	4.68	6.33	3.92	1.81	4.26
G	7.48	3.61	ND	ND	6.32	3.80	1.98	ND	7.48	3.61	ND	ND	5.90	5.15	4.60	ND
H	8.09	2.90	1.95	ND	8.15	3.02	1.65	ND	8.09	2.90	1.95	ND	6.75	3.06	2.30	1.30
Average[3)]	7.13	3.73	2.05	4.08	6.46	3.84	2.23	4.51	7.13	3.73	2.05	4.08	6.28	3.87	2.50	3.23

[1)]Aerobic colony count
[2)]Not detected
[3)]Average was calculated with excluding of not detected samples.

Table 5. Detection rate and out-of-compliance rate of *B. cereus*, aerobic mesophilic plate count (APC), and coliform by production types (unit: %)

	Classification[1)]	Overall (*n*=8)	Cook-to-order restaurant (*n*=3)	Reheat-to-cook restaurant[2)] (*n*=5)
B. cereus	Detection rate	37.50	8.3	55.0***
	Out-of-compliance rate[3)] (Acceptable level 4 log CFU/g)	31.3	0.0	50.0***
APC	Detection rate	87.5	66.7	100.0*
	Out-of-compliance rate[3)] (Acceptable level 5 log CFU/g)	3.1	0.0	5.0
Coliforms	Detection rate	65.6	66.7	65.0
	Out-of-compliance rate[3)] (Acceptable level <2 log CFU/g)	25.0	41.7	15.0

[1)]Microbial guidelines for *B. cereus* and APC applied with Korean Food Code (14) and for coliforms with Sorberg *et al.* (20)
[2)]*p<0.05, ***p<0.001
[3)]Detection rate of samples exceeded the standard.

g, reporting that better microbiological quality than that of fried rice dishes. Coliforms in seafood fried rice were indicated more than those of fried rice, showing the range of 1.7-3.19 log CFU/g (mean: 2.45 log CFU/g). Five samples did not detect *B. cereus*, the others' detected level was on the average of 4.82 log CFU/g, showing less quality than *B. cereus* in fried rice. In adding sauce on seafood fried rice, the lower pH (pH 6.28), more APC (3.87 log CFU/g), coliforms (2.50 log CFU/g), and less *B. cereus* (3.23 log CFU/g) indicated compared to the samples without sauce.

Quality comparison of the fried rice dishes between cook-to-order and reheat-to-cook With applying the standards of the KFDA (14) and Public Health Laboratory Service (PHLS) ready-to-eat in the UK (19) on the above microbiological results of fried rice and seafood-fried rice from 8 restaurants (Table 5), detection rate of *B. cereus* was 37.5% (12/32), and out-of-compliance rate which exceeded the standard limits of 4 log CFU/g for *B. cereus* was 31.3% (10/32). Detection rate of APC was 87.5% (28/32), but the only 3.1% (1/32) exceeded the quality limit of 5 log CFU/g. In the case of coliforms, now that there is no quality standard for coliforms in Korea and the UK, Sorberg *et al*. (20) criteria of less than 2 log CFU/g for acceptable were applied. Detection rate was calculated as 65.6% (21/32) and out-of compliance rate of samples was 25%.

Comparing out-of-compliance rate between cook-to-order and reheat-to-cook restaurants, cook-to-order restaurants controlled better the quality of APC and *B. cereus* except coliforms than reheat-to-cook restaurants. Especially in *B. cereus*, the detection rate and out-of-compliance rate of the cook-to-order production type was 8.3% (1/12), and 0.0% (0/12), respectively, whereas detection rate of reheat-to-cook restaurants was 55.0% (11/20) and out-of-compliance rate 50% (10/20). Therefore, cook-to-order production type was a significantly better way for controlling *B. cereus* than that of ready-to-heat restaurants (p<0.001). In the case of APC, similar results revealed and cook-to-order was a significantly better quality-control method (p<0.05), indicating 66.7% (8/12) of prevalence rate for cook-to-order and 0% of out-of-compliance rate. However, in the result of coliforms detection rate was fairly high both cook-to-order and reheat-to-cook restaurants, showing 66.7 and 65.0%, respectively. Contrary to our expectation, moreover, the out-of-compliance rate of cook-to-order restaurants (41.7%) was higher than that of reheat-to-cook (15.0%), even though there was no significant difference. During the on-site investigation, all restaurant except A and E restaurants were observed non-hygienic practices of kitchen employee, serving foods in undried dishes and a dirty delivery box. So, these might be medium for the cross-contamination after cooking. Therefore, even though the fried rice cooked with higher temperature, coliforms could exist on it after a cross-contamination. Compared with Little *et al*. (10) reported that out of compliance rate of *B. cereus* exceeding 4 log CFU/g was 1.4% (7/508) of cooked rice (cooked rice) and that of APC exceeding 5 log CFU/g was 13.6% (69/508), in our results, non-compliance rate of *B. cereus* indicated fairly high, but APC fairly low.

Based on the above results, we recommend that the cook-to-order method is more desirable production type for the ensuring the microbial quality of fried rice dishes, and efforts for improving proper handling practices of work employees should be cultivated regardless of the production type. Similar results were suggested in the previous study (8,21) that, even *B. cereus* of fried rice under circumstance of highly heated-up during a production, it can survive with longer keeping in room temperature (8,21). Put together, based on the above results, it considered that a cooking method of large quantity of preparation in advance is the critical risk factor for affecting the growth of *B. cereus.*

Table 6. Correlations among time-temperature and microbial levels of fried rice dishes (*n*=32)

Variable	APC	Coliforms	*B. cereus*
Final temp. of fried rice at preparation phase	-0.46**[1)]	-0.04	-0.68**
Environmental temp at cooking phase	0.34	0.21	0.63***
Final temp of fried rice at cooking phase	-0.43*	-0.33	-0.71***
Cooking time of fried rice at cooking phase	-0.60***	-0.24	-0.54**
Holding time of fried rice before service	0.07	-0.33	-0.24
Environmental temp. at consumption	-0.78***	-0.27	-0.20
Final temp. of fried rice at consumption	-0.43*	-0.33	-0.71***
Delivery time	0.01	-0.07	-0.03
Aerobic mesophilic plate count (APC)	1.00	0.60***	0.33
Coliforms	0.60***	1.00	0.27
B. cereus	0.33	0.27	1.00

[1)]*p<0.05, **p<0.01, ***p<0.001

Factors affecting the microbiological quality of fried rice dishes The final temperature of cooked rice and cooking time were negatively correlated with levels of APC (p<0.05) and *B. cereus* (p<0.05), whileas no significant correlation with coliforms (Table 6). APC was negatively correlated with the final food temperature at preparation phase (r= –0.46, p<0.01), cooking phase(r= –0.43, p<0.05), service (r= –0.43, p<0.05), and delivery (r= –0.46, p<0.01). It also negatively correlated with cooking time of fried rice dishes (r= –0.60, p<0.001), but had no correlation with holding time in service and delivery time. Level of *B. cereus* on fried rice dishes also negatively correlated with final temperature of cooked rice at preparation (r= –0.68, p<0.001), cooking (r= –0.71, p<0.001), and service (r= –0.71, p<0.001) showing that more strong negative correlation than APC. Notable thing is that higher environmental temperature (range: 27.5-43.8) in cooking area, higher level with *B. cereus* of the dishes (r=0.63, p<0.001).

Based on the above results, we found that, for controlling *B. cereus* and APC, exposure temperature was more important factor than the exposure time during production and consumption pathway. *B. cereus*, especially, can be more easily monitored by time and temperature compared with APC and coliforms, meaning that it can be controlled by cooking at high temperature with longer time and by averting room temperature holding. In addition, a controlling method for the coliforms in fried rice which known as weak at temperature over 60°C is more an effective with hygienic food handling practices of employees especially during distribution and assembly phase rather strict time and temperature management.

In conclusion, fried rice dishes are one of the most popular food items in Korea, however there are no data on the exposure pathway and contamination level of *B. cereus* in those dishes. Therefore, this study identified exposure pathway for fried rice dishes and evaluated its microbiological quality and the relationships among exposure factors. One serving size of fried rice dishes was 352.2 g. Two types of production system were employed at 8 restaurants: cook-to-order and reheat-to-cook. In 5 restaurants which reheat pre-cooked rice dishes, mean temperature of the dishes after the reheating phase was 68.5°C, and the final cooking time was 1 min 14 s. Meanwhile, mean temperature of fried rice dishes in 3 cook-to-order type restaurants was 81.4°C with 1 min 49 s of cooking time. Average meal assembly time was measured as 1 min 20 s. Average delivery time from restaurant to place of consumption was approximately 12 min. The mean temperature of fried rice dishes at the consumption point was 66.1°C for cook-to-order restaurants, and 59.8°C in counterpart restaurants with reheating process. The prevalence of *B. cereus* detected in cooked rice at consumption point was 37.5%. Especially, 31.3% of samples contaminated higher than the microbial standard in the guidelines set out in the Korea Food Code for *B. cereus* of 3 log CFU/g. The percentage of samples showing more than 5 log CFU/g of APC was 3.1%. Out-of-compliance of coliforms was 65.6% of samples. From the result of the relations among the microbiological quality and exposure pathway, we draw a conclusion that for controlling the level of *B. cereus*, restaurants need to employ the cook-to-order production type which can be easily monitored time and temperature than reheat-to-cook and conduct continuous trainings making employees practice hygienic food handlings during assembly and delivery foods.

Acknowledgments This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund; KRF2008-331-C00318). We also thank the staff in the Samsung Everland Inc. and the participated restaurants for their supports.

References

1. Johnson KM, Nelson CL, Busta FF. Influence of temperature on germination and growth of spores of emetic and diarrheal strains of *Bacillus cereus* on a broth medium and in rice. J. Food Sci. 48: 286-287 (1983)
2. Korea Food & Drug Administration. Statistics for foodborne illness outbreaks in 2010. Available from: http://e-stat.kfda.go.kr/. Accessed Dec 30, 2010.
3. Martinez S, Borrajo R, Franco I, Carballo J. Effect of environmental parameters on kinetics of *Bacillus cereus* (ATCC 7004) after mild heat treatment. Int. J. Food Microbiol. 117: 223-227 (2007)
4. Bahk GJ, Todd ECD, Hong CH, Oh DH, Ha SD. Exposure assessment for *Bacillus cereus* in ready-to-eat *kimbab* selling at stores. Food Control 18: 682-688 (2007)
5. Cho YS, Jung EY, Lee MK, Yang CY, Shin DB. Survival, isolation, and characterization of *Bacillus cereus* from *sunshik*. J. Microbiol. Biotechn. 23: 343-347 (2008)
6. Valero M, Hernández-Herrero LA, Giner MJ. Survival, isolation, and characterization of a psychrotrophic *Bacillus cereus* strain from a mayonnaise-based ready-to-eat vegetable salad. Food Microbiol. 24: 671-677 (2007)
7. Ankolekar C, Rahmati T, Labbe RG. Detection of toxigenic *Bacillus cereus* and *Bacillus thureingensis* sopres in U.S. rice. Int. J. Food Microbiol. 12: 460-466 (2009)
8. Chang HJ, Lee JH. Prevalence of *Bacillus cereus* from fried rice dishes and monitoring guidelines for risk management. Korean J Soc. Food Cookery Sci. 25: 45-53 (2009)
9. McElroy DM, Jaykys LA, Foegending MP. A quantitative risk assessment for *Bacillus cereus* emetic disease associated with the consumption of Chinese-style rice. J. Food Safety 19: 209-229 (1999)
10. Little CL, Mitchell RT, Barnes J. Microbiological quality of take-away cooked rice and chicken sandwiches: Effectiveness of food hygiene training of the management. Commun. Dis. Public Health 5: 289-298 (2002)
11. Lake R, Hudson A, Cressey P. Risk Profile: *Bacillus* spp. in Rice. A Crown Research Institute, New Zealand. pp. 1-40 (2004)
12. Gilbert RJ, Stringer MF, Peace TC. The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. J. Hyg. -Cambridge 73: 433-444 (1974)
13. Agata N, Ohta M, Yokoyama K. Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. Int. J. Food Microbiol. 73: 23-27 (2002)
14. Korea Food & Drug Administration. Food Code. Available from: http://www.foodnara. go.kr /portal/site/kfdaportal/infotelegram/. Accessed May 10, 2010.
15. Food and Drug Administration. Food Code 2009: US Public Health Service. US Dept. of Health and Human Service, Washington, DC, U.S. Available from: http://www.fda.gov/Food/FoodSafety/RetailFoodProtection/FoodCode/FoodCode2009/default.htm. Accessed May 10, 2010.
16. Kim JS, Lee GG, Kwon JY, Kwon ST. The development of rapid real-time PCR detection system for *Vibrio parahaemolyticus* in raw oyster. Soc. Appl. Microbiol. 46: 649-654 (2008)
17. Jin SS. Development of model for predictive growth of *Bacillus cereus* and *Staphylococcus aureus* in *kimbab*. MS thesis, Kwangwon University, Chuncheon, Korea (2005)
18. The Ministry of Education, Science, and Technology. The Sanitation Management Guidebook for the School Foodservice Operations. 3rd ed. The Ministry of Education, Science and Technology, Seoul, Korea. pp. 112-114 (2007)
19. Gilbert RJ, de Louvois J, Donovan T, Little C, Nye K, Ribeiro CD, Richards J, Roberts D, Bolton FJ. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. Commun. Dis. Public Health 3: 163-167 (2000)
20. Solberg M, Buckalwe JJ, Chen CM, Schaffner DW, O'Neil K, McDowell J, Post LS, Boderck M. Microbiological safety assurance system for food service facilities. Food Technol. -Chicago 44: 68-73 (1990)
21. Rosenquist H, Smidt L, Andersen SR, Jensen GB, Wilcks A. Occurrence and significance of *Bacillus cereus* and *Bacillus thuringiensis* in ready-to-eat food. FEMS Microbiol. Lett. 250: 129-136 (2005)