Journal of Food Protection, Vol. 72, No. 11, 2009, Pages 2236–2242

Transfer of *Bacillus cereus* Spores from Packaging Paper into Food

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MS 09-065: Received 10 February 2009/Accepted 1 May 2009

ABSTRACT

Food packaging papers are not sterile, as the manufacturing is an open process, and the raw materials contain bacteria. We modeled the potential transfer of the *Bacillus cereus* spores from packaging paper to food by using a green fluorescent protein–expressing construct of *Bacillus thuringiensis* Bt 407Cry$^-$ [pHT315Ω(*papha3-gfp*)], abbreviated BT-1. Paper (260 g m^{-2}) containing BT-1 was manufactured with equipment that allowed fiber formation similar to that of full-scale manufactured paper. BT-1 adhered to pulp during papermaking and survived similar to an authentic *B. cereus*. Rice and chocolate were exposed to the BT-1–containing paper for 10 or 30 days at 40 or 20°C at relative air humidity of 10 to 60%. The majority of the spores remained immobilized inside the fiber web; only 0.001 to 0.03% transferred to the foods. This amount is low compared with the process hygiene criteria and densities commonly found in food, and it does not endanger food safety. To measure this, we introduced BT-1 spores into the paper in densities of 100 to 1,000 times higher than the amounts of the *B. cereus* group bacteria found in commercial paper. Of BT-1 spores, 0.03 to 0.1% transferred from the paper to fresh agar surface within 5 min of contact, which is more than to food during 10 to 30 days of exposure. The findings indicate that transfer from paper to dry food is restricted to those microbes that are exposed on the paper surface and readily detectable with a contact agar method.

Extending the shelf life of processed foods and raw materials requires minimizing the microbial load of the products and protection toward contamination. Paper manufacturing is an open process, and the raw materials (e.g., wood, straw, starch, mineral pigments) contain bacteria, and thus, the products are not free of microorganisms. During paper manufacturing, the drying end temperatures can reach 140°C *(32)*. This reduces the viability of microorganisms in the paper. The survivors consist mainly of bacteria forming heat-stable endospores *(15, 25, 31)*. Food packaging paper has been reported to contain from less than 50 to up to 10^5 viable spores per g, *Bacillus* and *Paenibacillus* being the most prevalent genera *(25, 31)*. Five to 10% of the spores in paper represent the *Bacillus cereus* sensu lato sp. *(25, 29, 32)*.

Among the spore-forming bacterial species regularly found in paper, *B. cereus* is the only one classified as a pathogen causing infection in humans *(5),* and it is a common causative agent connected to foodborne illness *(28)*. Its transfer from the paper to packaged food is therefore of interest concerning food safety. Reports of such studies are, to our knowledge, not found in scientific literature. A reason for this possibly is the fact that most foodstuffs packaged in paper, e.g., rice, dehydrated potato products, herbs, and spices, indigenously contain viable *B. cereus (2, 8, 16, 20)* in quantities higher than those amounts found in paper, making it difficult to assess transfer of *B. cereus* from paper to food.

The aim of this project was to measure quantitatively the transfer of *B. cereus* spores from packaging paper to foods. This knowledge is needed for assessing whether the spores in packaging could cause risk for food safety. A green fluorescent protein (GFP)–labeled derivative of *Bacillus thuringiensis* was constructed and used to prepare paper containing the labeled strain. The paper was made by using a technique that allows for fiber formation similar to that of industrially manufactured food packaging paper. The transfer of the labeled spores from the packaging to food was then studied, using rice and chocolate as recipient foods.

MATERIALS AND METHODS

Preparation of the GFP-expressing *B. thuringiensis* strain Bt 407Cry$^-$ [pHT315Ω(*papha3-gfp*)]. The strain, herein designated as BT-1, contains a transcriptional fusion between the constitutive promoter p*aph*A3 and the *gfp* gene, encoding GFP. The *gfp*-expressing plasmid was obtained by cloning the *gfp-mut1* gene (obtained by PCR using the pNF8 plasmid as template *(10)*) downstream of the *aphA3* promoter, which was PCR amplified from the pGDG783 plasmid *(11)*. The pHT315 shuttle vector, used for the cloning of these DNA sequences, confers resistance to ampicillin (100 μg/ml, in *Escherichia coli*) and to erythromycin (10 μg/ml, in *Bacilli*) *(6)*. The pHT315Ω(*papha3-gfp*) plasmid was transformed into the Bt-407 strain by electroporation, as previously described *(19)*. This GFP construction is part of a larger panel of

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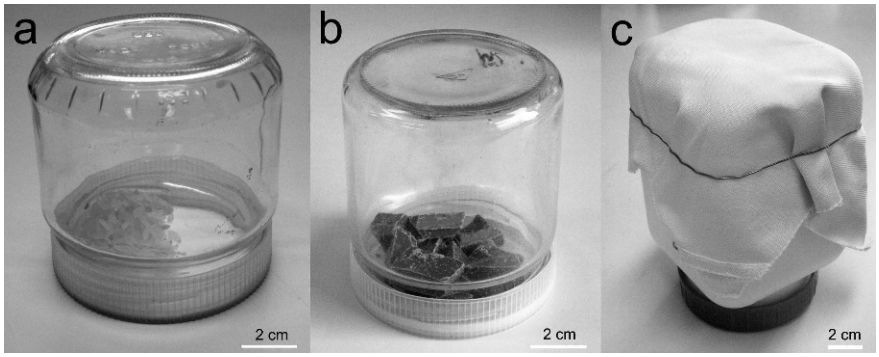

FIGURE 1. *Exposure chambers for measuring transfer of* Bacillus cereus *from paper to food. Disks fitting on the inside area of the lids of the chambers were cut from the paper sheets. The diameter of the disk was slightly larger than the opening of the jar to exclude food contact with the cutting edge of the paper. At low relative air humidity, rice and chocolate were exposed to BT-1–containing paper in glass jars (a, b). At high humidity, plastic jars were used (c). A window was cut into the bottom of the jar and covered with a sterile cloth to allow equilibration of the moist air between the jar and the incubation hood.*

gfp fusion from the laboratory of the INRA *(23),* the results of which will be published elsewhere.

Preparation of the bacterial spores. Strain BT-1 was grown in the sporulation medium described by Agaisse and Lereclus *(1)* for 3 days at 37°C on a rotary shaker (160 rpm), and then allowed to finalize sporulation and to achieve spore liberation stationary for 7 days at 22 ± 2°C. The spores were collected from 0.9 liters of the sporulated culture by centrifugation, washed four times with sterile Milli-Q purified water (Millipore, Billerica, MA), and then suspended in 20 ml of water. The suspension was divided into aliquots containing 10^8 or 10^{10} viable spores each.

Preparation of the paper containing BT-1 spores. The stock for papermaking was prepared by re-pulping 100 g of dry paper in 20 liters of drinking water. The stock was prepared separately for each individual sheet of paper. BT-1 spores (0, 10^8, or 10^{10} viable spores per 20 liters of stock to prepare one sheet) were dispensed into the stock in the head box. The sheets of paper were prepared from the stock by using a Dynamic Sheet Former (Allimand S.A., Rives Cedex, France), which allows the preparation of oriented sheets with properties similar to paper products made by commercial machines. The principles of dynamic sheet formation described by Neagu et al. *(22)* are, in short, as follows. The sheet is built by projection of the stock (or fiber suspension) on a wire inside a centrifuge drum. The stock is sprayed on walls of the drum by a nozzle that moves up and down. The centrifugal force makes the fibers settle in a particular direction and aids dewatering of formed sheets. In our experiment, the formed sheets were removed from the drum and dried between two sheets of filter paper by using subsequent pressures of 100, 200, and 300 kPa. After each pressure, the sheets of filter paper were replaced, and the formed sheet was finally heat dried (15 min at 85°C). The prepared sheets (1,800 cm^2, 260 g/m^2) were individually packed in black plastic bags and stored at 22 ± 2°C.

Measurement of the viable count of BT-1 spores in the paper. The content of viable spores in the BT-1–spiked paper was measured by the most-probable-number (MPN) method *(13)* or by the colony-counting method *(14),* except that erythromycin (10 μg/ml) was added as the selective agent in lieu of polymyxin B. For the MPN method, the initial suspension was prepared by homogenizing 1 g of paper per 100 ml of sterile drinking water by using a blender (Waring Products, Torrington, CT). Initial suspensions (5 ml) were heated in a water bath (10 min at 80°C) to inactivate vegetative bacteria. Tenfold serial dilutions (in sterile drinking water) were used to inoculate (eight parallels) a 10× volume of tryptic soy broth (TSB; Scharlau Chemie S.A, Barcelona, Spain) containing 10 μg/ml erythromycin (Sigma, St. Louis, MO). Fluorescent growth was read after 2 days at 37°C and 160 rpm shaking with a rotary shaker, using a fluorometric plate reader (excitation wavelength of 485 nm, emission wavelength of 535 nm; Victor[3], Perkin-Elmer, Waltham, MA). The number of fluorescent cultures (indicating growth of BT-1) was converted into amount of viable spores by using the Thomas formula *(21).*

For colony counting, the sheets were homogenized (1 g of paper per 100 ml) with the Ultra Turrax T25 laboratory homogenizer (IKA Werke GmbH and Co., Staufen, Germany) in Ringer's solution (Merck KGaA, Darmstadt, Germany) for 1 min at 20,500 rpm. The resulting slurry was heated (10 min at 80°C), serial 10-fold dilutions plated on nutrient agar (Merck KGaA) with erythromycin (10 μg/ml), and the fluorescent colonies counted after 2 days at 37°C. Spore content and count of total heterotrophs were measured from samples of industrial paper similarly, except that erythromycin was not used, and slurry was not heated when used for measuring total heterotrophs.

Measurement of the transfer of spores from paper into packaged food. Ten grams of dry rice (Risella long-grained rice, Herba Ricemills S.L.U., Spain) was dispensed in exposure chambers consisting of jars of glass or plastic (Fig. 1). The exposure chambers with rice were turned upside down and incubated for 10 days at 40°C ± 1°C *(3)* at the relative air humidities of 10 and of 60%, with intermittent agitation (160 rpm, 37°C, 5 cycles of 30 min within the exposure time of 10 days). After the exposure time, the numbers of viable fluorescent spores in the disks of paper and in the rice (initial suspension was 10 g of rice homogenized in 30 ml of sterile water) were separately counted with MPN method above described. The lowest 10× dilution was prepared with 500 μl of the initial suspension, with 5 ml in three parallels, and subsequent 10× dilutions in eight parallels. Fluorescent growth was confirmed by inspecting slide preparations under an epifluorescence microscope. The detection limit with this method (MPN method when growth was observed only in one of the parallels) was 30 transferred spores per exposure chamber.

Transfer of BT-1 spores from the paper into chocolate was analyzed similar to rice, except that 42 g of milk chocolate (Fazer Sininen, Cloetta Fazer Makeiset Oy, Vantaa, Finland), cut into ca. 10 pieces of 1 by 1 by 2 cm, was placed in each exposure chamber.

TABLE 1. *Retention of spores of* Bacillus thuringiensis *and of* Bacillus cereus *from the papermaking stock into the paper*[a]

Wt (g/m^2)	Stock vol (liters)	Code[b]	Spores spiked in the stock, viable count/sheet	Spores found ($\times 10^4$)		% of input	Source/ reference
				Per g^{-1} ($\pm$SD) of paper	Per 100 cm^{-2} of paper		
Paper made by the dynamic sheet former technology; the spores were detected by the MPN method[c]							
260	20	L0	0	<0.06	<0.2	0	This study
260	20	L1	10^8	8.2 $\pm$ 0.44	21	4	This study
260	20	L2	10^8	3.2 $\pm$ 0.21	8.3	2	This study
260	20	H1	10^{10}	150 $\pm$ 17	390	1	This study
260	20	H2	10^{10}	290 $\pm$ 10	760	1	This study
Paper made by the dynamic sheet former technology; the spores were detected by colony counting							
260	20	L3	10^8	3.8 $\pm$ 0.63	9.9	2	This study
260	20	L5	10^8	3.5 $\pm$ 0.93	9.1	2	This study
260	20	L6	10^8	6.5 $\pm$ 1.5	17	3	This study
Paper prepared as hand sheets, spiked with a sporulated culture of *B. cereus* NS61; viable spores were measured by colony counting[d]							
475	0.6		0	3 $\pm$ 0.1	14		*12*
475	0.6		2.5×10^7	13 $\pm$ 0.5	62	2.0	*12*
475	0.6		1.3×10^8	60 $\pm$ 0.6	285	1.8	*12*

[a] Each sheet of paper was prepared from individually prepared stock of pulp slurry. The stock was spiked with spores of *B. thuringiensis* BT-1 or *B. cereus* NS61. The sheets of paper prepared by the dynamic sheet former technology were each 1,800 cm^2, with a dry weight of 47 g.

[b] Indicating individual sheet of paper manufactured with dynamic sheet former technology.

[c] MPN results are averages from three parallel samplings from each sheet.

[d] The results for handmade paper (80 cm^2 each, 3.8 g) from stock spiked with *B. cereus* NS61 were calculated from the data reported by Hoornstra et al. *(12)* of experiments carried out in the same laboratory used in the present study.

The exposure was for 30 days at 20 $\pm$ 1°C at a relative humidity of 30 or 60%. The chambers with the chocolate were rotated upside down 10 times (in glass jars at 30% humidity) or shaken by hand for 10 s (in plastic jars at 60% humidity) on five occasions during the exposure time. At the end of the exposure time, the initial suspension was prepared by homogenizing the chocolate in 300 ml of warm (37°C), sterile drinking water. For MPN method assays, 5 ml (three parallels), 500 μl (three parallels), and 20 μl (eight parallels) of the initial suspension was grown in 10× volume of TSB containing 10 μg of erythromycin per ml. The fluorescent outgrowth was recorded as above.

Contact agar imprinting. The transfer of BT-1 spores from the paper into agar medium was analyzed by the direct surface agar contact method *(9),* modified as follows. Pieces of the paper (5 by 5 cm, aseptically cut) were pressed onto the nutrient agar with 10 μg/ml erythromycin and aseptically removed after 5 min (22 $\pm$ 2°C) of contact. The fluorescent colonies were counted from plates after 2 days of growth at 37°C. Growth from the edges of the paper was excluded from counting by omitting colonies located within 2 mm from the edge. Transfer from the samples of industrially manufactured paper was measured similarly, omitting erythromycin from the nutrient agar.

Other. The food contact areas of paper or paperboard were measured from packaged foods sold at three large supermarkets (of independent ownership) in the Helsinki area in December 2008.

RESULTS

Retention of GFP-labeled *B. thuringiensis* spores from the papermaking stock into the paper. BT-1 spores in the paper were measured from homogenized samples by the MPN method or plate counting. Strain BT-1 expressed strong green fluorescence, both as the vegetative cells and as spores, and was very stable; no loss of fluorescence was noted after maintaining it for 1 month in the absence of erythromycin. The retention of spores from the stock in the sheets of paper is shown in Table 1. An average of 2.4% $\pm$ 0.4% (10^8 dispensed in the stock, $n = 5$) or 1.0% $\pm$ 0.1% (10^{10} dispensed in the stock, $n = 2$) of the BT-1 spores were retained into the paper. Retention of the GFP-labeled *B. thuringiensis* strain BT-1 was identical (2.4% $\pm$ 0.4%) to that measured for hand sheets prepared with *B. cereus* strain NS61 (1.8 to 2.0% of the input). The majority of the spores (>97% of input) was not retained in the paper but remained in the wire water. It appears that the GFP-labeled *B. thuringiensis* BT-1 sorbed or adhered to paper fibers to the same extent as did strain NS61, representing *B. cereus* sensu stricto, isolated from a paper-manufacturing environment *(24)*. Therefore, the labeled strain likely is a good model for studying possible transfer from packaging paper to food.

Transfer of GFP-labeled spores from the paper to food. The transfer of BT-1 spores was measured, with rice and chocolate as recipient foods. They represent different types of foods commonly packed in noncoated packaging paper. The areas of the paper surface exposed to the food were 4.0 and 0.95 cm^2/g of food. Most paper-packaged products in the consumer markets have food contact areas within this range (Table 2).

Rice was exposed to BT-1–containing paper for 10 days at 40°C at two different relative air humidities of 10 and 60%. Transfer of BT-1 spores to rice from the paper

TABLE 2. *Surface areas of paper per gram of food packaged for the consumer market; interior surfaces of the packages in direct contact with food were measured*

Packaged food	Amt (g)	Paper area exposed to food (cm^2/g)	
		Range	Avg (n)[a]
Starches (corn, potato, barley)	400–500	1.25–2.15	1.71 (4)
Flour	400–2,000	0.32–1.35	0.83 (3)
Rice	500–1,000	0.90–1.27	1.06 (4)
Flakes, couscous, semolina, oatmeal	350–1,000	0.80–2.08	1.36 (9)
Chips, cookies, crackers	125–600	1.76–3.63	2.68 (8)
Mueslis, breakfast cereals	375–500	1.73–5.22	3.74 (9)
Mashed potato powder	210–214	3.12–3.36	3.24 (2)
Sugar (cubes, granulated)	500–1,000	0.51–0.87	0.69 (2)
Malt extract for home brewed beer	1,000	0.63	0.63 (1)
Table salt	1,000	0.51	0.51 (1)
Artificial sweetener	90	8.07	8.07 (1)
Tea bags	35–44 (net)	9.89–11.5	10.7 (2)
Tea	60–200	2.35–3.70	3.15 (3)

[a] *n,* number of packages measured.

containing 3×10^4 or 8×10^4 BT-1 spores g^{-1} was detected in 1 of 8 exposures at 10% relative air humidity, and in none of the 8 exposures at 60% relative air humidity (Table 3). Thus, only 1 of the executed 16 exposures resulted into detectable transfer into rice. This translates to an MPN of two transferred spores from 100 cm^2 of paper to rice, corresponding to 0.002% of the total count of BT-1 spores in the paper.

From the paper containing an extremely high density of BT-1 spores, 2×10^6 or 3×10^6 g^{-1}, transfer exceeding the detection limit was found in 9 out of the 16 exposures (Table 3), equivalent to an MPN of 55 transferred BT-1 spores per 100 cm^2 of paper, or 0.001% of the contained spores.

Chocolate was exposed for 30 days at 20 ± 1°C. No transfer was observed from the paper with 3×10^4 spores g^{-1} at a relative humidity of 30% (Table 3). This corresponds to an MPN of fewer than 13 transferred spores per 100 cm^2 of paper, equaling 0.02% of the total spore count in the paper. When paper containing a 100 times higher amount of BT-1 spores, 3×10^6 g^{-1}, was used for exposures, the detected transfer (Table 3) corresponded to an MPN of 180 transferred spores per 100 cm^2, or 0.002% of total BT-1 spore content in the paper.

When chocolate was exposed at higher air humidity, 60%, transfer was observed in three of the six parallel exposures from paper containing 3×10^4 or 8×10^4 BT-1 spores g^{-1} (Table 3). This corresponds to an MPN of 24 transferred BT-1 spores from 100 cm^2, equal to 0.03% of the total count of BT-1 spores in that paper. Transfer into chocolate was observed in all parallel exposures ($n = 5$) from the paper containing 2×10^6 or 3×10^6 spores g^{-1}. The observed transfer corresponds to an MPN of 1,000 transferred spores from 100 cm^2, equaling 0.03% of the total BT-1 spore content in the paper.

Transfer of BT-1 spores from paper by contact agar imprinting. This was investigated with nutrient agar, with an exposure time of 5 min and temperature of 22 ± 2°C. Transfer of the BT-1 spores to the moist, freshly poured agar from sheets ($n = 3$, 18 pieces per sheet) of paper containing 4 to 16×10^4 BT-1 spores g^{-1}, was 40 to 80 CFU/100 cm^2, corresponding to 0.03 to 0.1% of the total count of BT-1 spores in the paper (Table 4). The paper containing 10^6 viable spores per g could not be measured with this method because of the excessive growth.

It thus appears that the proportion of BT-1 spores that transferred from the paper surface to rice and to chocolate (0.001 to 0.03%) at air humidities of 10 to 60% in 10 to 30 days was not higher than the proportion (0.03 to 0.1%) transferred from the paper to moist agar surface in 5 min of contact. This can be interpreted to mean that both methods measured the same spores, i.e., those that were surface exposed.

Transfer of bacteria from industrial paper by contact agar imprinting. Table 5 shows contact agar imprinting measurements done on examples of industrially manufactured paper (normal trade ware). The majority of the heterotrophic bacterial load (8×10^3 to 7×10^5 per 100 cm^2 of the paper) consisted of heat-stable bacterial spores (Table 5). The fraction that transferred into the contact agar was 0.01 to 0.02% of the total amount of viable bacteria or spores present in the paper. These figures were close to those found (0.03 to 0.1%) for the experimental BT-1–labeled paper. This indicates that bacterial transfer results obtained with laboratory made paper apply also for industrial paper.

DISCUSSION

In this study, we raised the question whether viable *B. cereus* spores in packaging paper would transfer to packaged foods, and if yes, to what extent. We focused on noncoated packaging paper used for dry foods. Liquids and moist foods are usually packed in paper coated with polyethene or some other protective layer. Transfer was studied at different relative air humidities, because humidity

TABLE 3. *Transfer of BT-1 spores from paper to rice and to chocolate; results of four parallel exposure chambers per sheet, with exceptions indicated*[a]

Exposing paper		BT-1 received by the recipient food from 100 cm^2 of paper							
		Rice[b]				Chocolate[b]			
		BT-1 MPN count[c]		% of transfer from paper		BT-1 MPN count		% of transfer from paper	
Code	BT-1 content ($\times 10^4$ in 100 cm^2)	10% air humidity	60% air humidity	10% air humidity	60% air humidity	30% air humidity	60% air humidity	30% air humidity	60% air humidity
L1	21	<17[d]	<17[d]	<0.009[d]	<0.009[d]		23		0.01
L2	8.3	17	<17[d]	0.02	<0.02[d]	<15[d]	29[e]	<0.02	0.03
H1	390	17	57	0.0004	0.001		1,400		0.04
H2	760	85	110	0.001	0.001	170[e]	640[e]	0.002	0.008

[a] The papers used to expose rice and chocolate are described in Table 1.
[b] Contact time was 10 days at 40°C for rice and 30 days at 20°C for chocolate, with occasional agitation. The contact area of the paper was 40 cm^2 (1.0 g dry weight), and the amounts of food were 10 g of rice and 42 g of chocolate.
[c] After exposure, the GFP-labeled BT-1 spores in rice and in chocolate were counted by the MPN method.
[d] Lower limit of detection.
[e] Number of exposure chambers with chocolate: at 30% relative humidity, $n = 5$ for H2; at 60% relative humidity, $n = 2$ for L2 and $n = 1$ for H2.

TABLE 4. *Estimation of surface-exposed BT-1 spores in the paper*[a]

Code	BT-1/100 cm^2 (2.5 g) of the paper (CFU $\times 10^3$)[b]	Transfer of BT-1 from paper to agar surface	
		CFU/100 cm^{-2}	% of transfer
L3	99	79	0.1
L5	91	39	0.04
L6	170	56	0.03

[a] The papers used to expose rice and chocolate are described in Table 1. Pieces 50 by 50 mm were cut from the sheets (18 pieces per sheet) of paper containing the BT-1 spores (Table 1). The pieces were pressed onto freshly poured nutrient agar surface, one piece per agar plate, and removed after 5 min of contact time. The exposed agar plates were counted for fluorescent colonies after 2 days at 37°C. Colonies growing from cutting edges (<2 mm from the edge) were excluded from counting.
[b] Analyzed from paper homogenates by plate counting.

dramatically affects the bonding, tensile, stretch, and tearing strengths of paper *(7)*. A GFP-expressing derivative of a *B. thuringiensis* strain was constructed to distinguish the spores transferred from the paper package from those already present in the food. This strain, BT-1, adhered to wood pulp during the process of papermaking and survived through the process of papermaking, similar to the authentic wood isolate *B. cereus* NS61 *(12, 24)*.

In all conditions used, the transfer of BT-1 spores from package to rice and chocolate remained between 0.001 and 0.03%. To be able to measure this small migration, we introduced BT-1 spores into the paper in densities exceeding 100- to 1,000-fold the amounts of the total *B. cereus* group bacteria found in industrially produced paper *(25, 26, 31, 32)*.

It thus appears that only a small fraction of the spores in paper was mobilized by food contact; the bulk of the spores remained immobilized inside the fiber web. The BT-1–spiked paper was manufactured with equipment that allows fiber formation similar to that in full-scale manufactured paper. No adhesives (sizing), wet-strength agents, or other papermaking additives *(17)* were used when BT-1–spiked paper was manufactured. Thus, the explanation for spore immobilization within the fiber web must be found in the physical properties of the paper-containing fiber materials *(7)*. In an earlier study *(30)*, we observed that the inherent rigidity of the paper web prevented microorganisms from moving, even when the paper was heavily wetted.

The amount of spores transferred from paper in extended food contact was no higher than that transferred in 5 min of contact to a fresh agar surface. This indicates that only the microbes that are exposed on the paper surface have the potential to transfer to food. The direct agar contact method could thus be used as an indicator of the number of germs in paper that can transfer to food.

GFP-labeled bacteria have successfully been used in many studies to monitor the interactions between the

TABLE 5. *Transfer of bacteria from industrial paper in contact to nutrient agar*[a]

	Paper tested				Transferred from the paper to agar surface	
			CFU × 10^3/100 cm^2			
Code[b]	Wt (g m^{-2})	Grade[c]	Total heterotrophs	Spores	CFU/100 cm^2	% proportion of total heterotrophs transferred
1/2008	240	FG	7.9	2.4	0.3 (315)[d]	0.004
2/2008	290	FG	8.6	7.0	1.4 (252)	0.02
3/2008	290	FG	7.5	16	1.9 (252)	0.03
4/2008	210	FG	2	2.1	0.5 (1,000)	0.03
5/2008	250	NFG	130	85[e]	11 (609)	0.008
6/2008	410	NFG	720	660[e]	10 (378)	0.001

[a] Pieces 50 by 50 mm were cut from the papers. The pieces, 15 to 18 per test paper, were pressed onto freshly poured nutrient agar surface, one piece per agar plate, and removed after 5 min of contact time. Colonies on the exposed agar plates were counted after 2 days at 37°C.
[b] Indicating individual sample of industrial paper.
[c] FG, food grade; NFG, non–food grade.
[d] Only one colony was transferred from an analyzed area of 315 cm^2.
[e] Counted after 24 h.

bacterium and its environment *(18, 27)*. The construct used in this paper is a part of several transcriptional promoter fusions that were made to follow expression of genes during infection of the Bt407Cry$^-$ strain in insect larvae *(23)*. The *B. thuringiensis* BT-1, described in this article, or the transformation of Bt bioinsecticidal strains with the pHT315Ω(*papha3-gfp*) plasmid, could be used to model the passage of *B. thuringiensis,* not only from package to food, but also the transfer from the harvested crop through food processing chains.

Heat-stable spores in dry foods are important for the food safety after foods are prepared with water. Process hygiene criteria for *B. cereus* in sensitive dried foods, such as infant and dietary foods, were defined by the European Commission *(4)* as m = 50 (satisfactory) and M = 500 (acceptable) of presumptive *B. cereus* spores per gram. The contact area between packaging paper and the food ranges from 0.5 to 10 cm^2 g^{-1} (Table 2). Food packaging paper (250 g/m^2) may contain ≤10^3 *B. cereus* group spores per g *(24),* i.e., ≤30 spores per cm^2. Measurements described in this article indicated that 0.001 to 0.03% of these spores were transferable to food. The highest observed transfer (0.03%) could increase the spore content of 1 g of food by 0.1 spores. This is low compared with the process hygiene criteria or densities commonly found in rice (3.6 to 4.6 × 10^2 CFU *B. cereus* g^{-1} rice *(2)*) and does not endanger food safety.

ACKNOWLEDGMENTS

We thank Anna Peltola for laboratory assistance, Marja Pitkänen for paper technical advice, and Nicolas Fortineau for the gift of the pNF8 plasmid. We also thank the reviewers for their professional feedback. This work was supported by the Finnish Agency for Technology and Innovation (MECO II project, TEKES), EnSTe Graduate School (Ph.D. scholarship to Jaakko Ekman), and the Academy of Finland (CoE grant 118637 ''Photobiomics'').

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