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NEW DECONTAMINATION SURROGATE MICROORGANISMS

FIELD OF THE INVENTION

The present invention relates to the validation of decontamination processes, and in particular new indicator organisms, as well as mixtures of these microorganisms, used to validate decontamination processes.

STATE OF THE ART

Pasteurization processes are applied in multiple fields, both for equipment sterilization or for food decontamination, in particular dry food. These processes consist of specific decontamination/sterilization steps, or are the concomitant result of a step of a treatment process, such as a step of cooking a food, for example the roasting of products of plant origin.

Products of plant origin, such as almonds or spices, are often contaminated by pathogenic microorganisms present in the environment where they are grown, stored and used which requires a decontamination step before their use for human consumption. Often, this decontamination is performed as a temperature treatment of these foods, such as cooking, roasting or drying. However, certain pathogens may be resistant to certain decontamination conditions and it is necessary to make sure, before implementing the process that the decontamination objective will be achieved.

This validation cannot be carried out with pathogenic microorganism due to the risks of contamination. For this, so-called "surrogate" indicator microorganisms are used, the behavior of which under the treatment conditions must be similar to that of the pathogenic organism. Preferably, the surrogates will be selected to be more resistant to the treatment conditions than the pathogens, without in as much having a behavior that is too different from that of these target pathogens.

These surrogates are generally specific to a particular pathogen in a decontamination process, such as for example *Enterococcus faecium* (ATCC 8459) recommended for the validation of pasteurization processes for almonds likely to be contaminated by pathogenic salmonella.

Surrogates are not necessarily the microorganisms phylogenetically closer to the target pathogens, such as for example the genus *Citrobacterium*, a genus evolutionarily closer to *Salmonella*, is not described as a surrogate for this pathogen. Thus, as a surrogate for *Salmonella*, some have used *Geobacillus stearothermophilus* (ATCC 12980) for the

validation of a feed extrusion process for animals (Okelo et al., 2006 and 2008), *Enterococcus faecium* (NRRL B-2354) for the pasteurization of liquids (Annous & Kozempel, 1998), almonds (ABC, 2007) or food extrusion (Bianchit, 2014), *Pantoea agglomerans* (SPS 2F-1) for the roasting of almonds (ABC, 2007), *Pantoea dispersa* for the treatment of fresh food by electron beam irradiation (Fudge & al., 2016), *Pediococcus spp.* and *Pediococcus acidilactici* for the preparation of beef jerky (Borowski et al, 2009), the preparation of dried turkey (Williams, 2010) or the feed extrusion for animals (Ceylan and Bautista, 2015), and *Staphylococcus carnosus* (CS-299) for the preparation of ground beef and frankfurter batter (Vasan et al., 2014).

As surrogates for *Clostridium botulinium*, some have used *Clostridium sporogenes* (PA3679, 3676 and 3678) for low-acid foods (Wallace et al. 2006)

As surrogates for *Listeria monocytogenes*, some have used *Listeria innocua* in a pasteurization process for frankfurters (Sommers et al., 2008) or *Escherichia coli* K12 in an irradiation sterilization process for cantaloupes (Rodriguez et al., 2006).

Various non-pathogenic *E. coli* have been described as surrogates for *E. coli* O157:H7 in the treatment of juice (Gurtler, 2010) or beef (Garcia Hernandez et al., 2015).

While the strain *Enterococcus faecium* (NRRL B-2354) has been used for the validation of thermal processes for a plurality of low water activity foods, this strain shows a much higher thermal resistance than a large number of pathogens, particularly *Salmonella*.

In addition, apart from *Enterococcus faecium*, the strains identified in the state of the art are at the level of laboratory tests, often in the form of extemporaneous liquid suspensions, which are not very versatile as for the carriers used and not very suitable for large-scale industrial use that requires the availability of large amounts of viable forms of ready-to-use surrogates on multiple carriers.

There remains a need for surrogates better adapted to decontamination processes and to target pathogens, that may be used alone or in mixtures, which have resistance behaviors closer to the target pathogen(s) and provide more relevant information about the decontamination process so as to validate processes that are more energy efficient and better respect the structural and/or organoleptic properties of the treated products; in particular adapted to industrial use. The inventors have highlighted a plurality of groups of non-pathogenic microorganisms that meet this need.

DESCRIPTION OF THE INVENTION

The present invention relates to a process for monitoring a decontamination process wherein the decontamination process is implemented in the presence of at least one indicator microorganism, or a mixture of indicator microorganisms, and the behavior of said at least one indicator microorganism is observed during said decontamination process, characterized in that the indicator microorganism is a non-pathogenic microorganism selected from non-pathogenic *Enterobacteriaceae* of the genus *Enterobacter* selected from the species *Enterobacter hormaechei* and *Enterobacter mori*, of the genus *Erwinia* selected from the species *Erwinia persicina* or of the genus *Pantoea* selected from the species *Pantoea agglomerans* and *Pantoea calida*, and mixtures thereof.

The present invention also relates to indicator microorganisms likely to be used as surrogates in a process for monitoring a decontamination process, selected from *Enterobacter hormaechei* CNCM I-5058, *Pantoea agglomerans* CNCM I-5059, *Enterobacter mori* CNCM I-5060, *Pantoea calida* CNCM I-5061, *Erwinia persicina* CNCM I-5062, *Erwinia persicina* CNCM I-5063, *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055, *Pantoea calida* CNCM I-5056.

It also relates to a kit for monitoring a decontamination process, comprising at least one indicator microorganism according to the invention and a suitable carrier for its use in the decontamination process.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a process for monitoring a decontamination process wherein the decontamination process is implemented in the presence of at least one indicator microorganism, or a mixture of indicator microorganisms, and the behavior of said at least one indicator microorganism is observed during said decontamination process, characterized in that the indicator microorganism is a non-pathogenic microorganism selected from non-pathogenic *Enterobacteriaceae* of the genus *Enterobacter* selected from the species *Enterobacter hormaechei* and *Enterobacter mori*, of the genus *Erwinia* selected from the species *Erwinia persicina* or of the genus *Pantoea* selected from the species *Pantoea agglomerans* and *Pantoea calida*, and mixtures thereof.

"Microorganism" means a set of a plurality of individual microorganisms of the same species. Preferably, the microorganisms are suitable for industrial use, that is to say that they may be produced in large quantities by fermentation, up to at least 10^{10} CFU/g, more preferably up to at least 10^{11} CFU/g.

It will be cited particularly bacteria selected from the non-pathogenic species *Enterobacter hormaechei*, *Enterobacter mori*, *Erwinia persicina*, *Pantoea agglomerans*, *Pantoea calida*, preferably capable of being produced industrially, more particularly selected from the following species, deposited at the CNCM according to the Budapest Treaty: *Enterobacter hormaechei* CNCM I-5058, *Pantoea agglomerans* CNCM I-5059, *Enterobacter mori* CNCM I-5060, *Pantoea calida* CNCM I- 5061, *Erwinia persicina* CNCM I-5062, *Erwinia persicina* CNCM I-5063, *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055, *Pantoea calida* CNCM I-5056.

According to a first embodiment of the invention, at least one indicator microorganism is selected from the non-pathogenic *Enterobacteriaceae* of the genus *Pantoea*, of the genus *Enterobacter* or of the genus *Erwinia* and the mixtures thereof defined above.

Advantageously, a mixture of at least 2 indicator microorganisms is used (multiplex validation), in particular at least 2 indicator microorganisms selected from the non-pathogenic *Enterobacteriaceae* of the genus *Pantoea*, of the genus *Enterobacter* or of the genus *Erwinia* and the mixtures thereof defined above. According to a more specific embodiment of the invention, the mixture comprises at least one indicator microorganism selected from the non-pathogenic *Enterobacteriaceae* of the genus *Pantoea* and at least one indicator microorganism selected from the non-pathogenic *Enterobacteriaceae* of the genus *Enterobacter* or of the genus *Erwinia* such as defined above.

The advantage of these non-pathogenic indicator microorganisms is that they show greater resistance to the implementation conditions of various decontamination processes than that of at least one target pathogenic organism. These target pathogenic organisms are microorganisms responsible for contamination, in particular the pathogenic bacteria of the genera *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Campylobacter*, *Cronobacter*, etc. The aim of the decontamination process that is the subject of the monitoring is to eliminate all of these pathogens if they were to be present in the treated product.

Advantageously, the indicator microorganisms have a thermal resistance greater than *Salmonella* under low aw conditions and are packaged on an inert matrix.

"Low aw" preferably means a water activity below 0.85 (CAC/RCP 75-2015, Codex Alimentarius).

"Inert matrix" preferably means a suitable carrier for the preservation and use of indicator microorganisms, in particular in dry form. The carrier is inert, that is to say does not interact with the metabolism of bacteria in dry form enabling optimal preservation over time.

In the decontamination process, the indicator microorganism will be used in a suitable form, corresponding to the form of the target pathogen likely to be present in the product to be decontaminated, in particular in vegetative form and/or dry vegetative form.

"Dry form" or "dry vegetative form" means vegetative bacteria that have undergone a drying process enabling them to be preserved for a determined period without altering their resistance features.

The indicator microorganisms produced by fermentation are then dried for their preservation according to techniques known to the skilled person, such as lyophilization, atomization or drying.

The decontamination processes generally comprise one or more steps of pasteurization, drying, extrusion, roasting, cooking, sterilization, autoclaving and steam treatments.

These processes are well known to the skilled person, particularly pasteurization, drying, extrusion, roasting, cooking, sterilization, autoclaving, steam treatments, pulsed light, high-pressure treatments, or irradiation, gas sterilization (EtO, ppo, ozone) and disinfectants (bleach, peracetic acid, etc.), in particular for the treatment of natural or manufactured products, such as nuts, aromatic herbs, seeds, spices, food powders, pet and livestock feed, cereals, etc.

The surrogates, and surrogate mixtures, according to the invention may be used, according to the food and processes selected, to validate the decontamination of pathogens such as *Salmonella*, *Escherichia coli*, *Bacillus*, *Listeria*, *Campylobacter*, *Cronobacter sakazakii*, etc.

To this end, the indicator microorganism will be used with a suitable carrier, well known to the skilled person, preferably inert, for example with cryoprotectants such as

maltodextrin and/or milk powder and solid carriers such as talc, silica and/or activated carbon. The carrier may also comprise a marker that makes it possible to easily find the contaminated products (for example a visible or UV/IR-fluorescent dye), in particular any marker making it possible to distinguish the contaminated areas from others (magnetic, isotopic, chemical marking, etc.).

The use of a suitable carrier makes it possible to standardize the use of microorganisms on various matrices by providing both better stability of the microorganisms and avoiding the need to validate the stability of each indicator microorganism on each carrier after inoculation. It facilitates the implementation of the process according to the invention.

The invention also relates to a dry composition comprising an indicator microorganism and a suitable carrier, such as defined above and below.

The composition advantageously comprises an indicator microorganism content of at least 10^{10} CFU/g of dry composition.

The dry composition is advantageously a powder that has a water activity equal to or less than 0.3.

These compositions are prepared according to methods known by the skilled person by mixing according to the usual techniques, the indicator microorganisms in a dry form with the carrier, in the desired proportions. According to another embodiment, the indicator microorganisms are mixed with the suitable carrier, the mixture then being dried for its preservation.

In general, the indicator microorganisms with their carrier are added to the products to be decontaminated in suitable amounts to enable the verification of the efficacy of the decontamination process.

The microorganisms and their carrier may, if necessary, undergo a treatment prior to the decontamination, similar to that undergone by the product to be decontaminated, that is to say that will mimic the known product contamination processes. For example, in the case of natural products that are ground (particularly spices) it is possible to grind them after adding indicator microorganisms on their carrier to produce powders while recreating the classic natural product contamination conditions.

The monitoring process according to the invention may be implemented prior to any implementation of a decontamination process on the product to be decontaminated, in order to validate the efficacy of the decontamination process (validation process). It

may also be implemented during the decontamination operations on the product to be decontaminated, such as an indicator of decontamination or as an indicator of conformity of implementation of the decontamination process (monitoring process).

The process according to the invention, whether it concerns a validation or monitoring process may be implemented under the responsibility of the person carrying out the decontamination or also under that of an inspection or accreditation body.

The monitoring microorganisms will advantageously be supplied in kit form, with their carrier for use, and if necessary a set of instructions.

Observation of the behavior of the indicator microorganism generally consists of monitoring the presence of viable individuals, during the decontamination process and/or at its end. The methods used are known to the skilled person: counting of colonies on agar and/or molecular methods such as PCR and/or qRT-PCR, or microorganism detection tests such as immunology tests, for example the tests using SPR technologies, such as those developed by PRESTODIAG, or also phage-based detection tests.

The present invention also relates to an indicator microorganism likely to be used as a surrogate in a process for monitoring a decontamination process, selected from *Enterobacter hormaechei* CNCM I-5058, *Pantoea agglomerans* CNCM I-5059, *Enterobacter mori* CNCM I-5060, *Pantoea calida* CNCM I-5061, *Erwinia persicina* CNCM I-5062, *Erwinia persicina* CNCM I-5063, *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055, *Pantoea calida* CNCM I-5056. It relates more particularly to these isolated microorganisms, or in vegetative form and/or dry vegetative form. The invention also relates to a mixture of microorganisms comprising at least 2 of the species of microorganisms above, in all of their 2-by-2 combinations, up to a mixture comprising the 10 species of microorganisms above. The invention also relates to a composition comprising a microorganism above or a mixture of said microorganisms and a suitable carrier, in particular an inert carrier such as defined above.

According to a preferred embodiment of the invention, the surrogate is selected from *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055, *Pantoea calida* CNCM I-5056 or a mixture of surrogates comprising at least one surrogate selected from *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055, *Pantoea calida* CNCM I-5056.

The invention also relates to a kit for monitoring a decontamination process, characterized in that it comprises at least one microorganism above and a suitable carrier

such as defined above for its use in the decontamination process, and if necessary a set of instructions.

The invention also relates to the use of at least one microorganism selected from the non-pathogenic *Enterobacteriaceae* of the genus *Enterobacter*, of the genus *Erwinia* or of the genus *Pantoea* such as defined above, or a kit according to the invention, for monitoring a decontamination process, particularly for prior validation or monitoring during the process.

DESCRIPTION OF THE FIGURES

Figures 1 to 4 show the resistance curves of various surrogate microorganisms in comparison with the pathogens *Salmonella* and *Cronobacter sakazakii*.

Figures 5 to 7 show the cell destruction dynamics of indicator microorganisms according to the invention, of the reference strain *E. faecium* ATCC 8459 and of 4 *Salmonella* serotypes on various carriers.

EXAMPLES

Example I. Isolation and selection of microorganisms.

1. Isolation of environmental Enterobacteriaceae

A 0.5 g sample of dry products was placed in a 1.5 mL Eppendorf tube and heat treated in a dry bath for 15 min at 95°C. After cooling to room temperature, 1 mL of concentrated PBS (aw = 0.950) was added before performing a mixture for 30 s thanks to a vortex. Successive dilutions to the 10^{th} were prepared in the PBS (aw = 0.995) before spreading on Violet Red Bile Glucose Agar (VRBG) in an amount of 100 µL per dish. After incubation at 37°C for 24-48 h, the colonies were isolated on Tryptic Soya Agar (TSA) and incubated again at 37°C for 24 h.

2. Identification of environmental Enterobacteriaceae

Amplifications of 16S rDNA of each isolate were performed directly by sub-culturing the colonies in the PCR mix. The primers used were: 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGH TAC CTT GTT ACG ACT T-3'). To carry out the PCR reactions, the "Taq core kit" (Quiagen, France) was used. Briefly, the PCR mix (50 µL per reaction) for a colony consists of 0.5 µM of each primer, of 0.2 mM of dNTP mix, of 0.75 U of Taq polymerase and of 1 X buffer containing $MgCl_2$. The amplification was verified by 1% agarose gel electrophoresis before sequencing the PCR products by the Sanger

method. The sequences obtained were used to search the NCBI database (BLASTn) and thus the isolates were able to be identified. Twelve isolates were then selected.

3. Thermal challenge

a. Strain culture conditions

All of the cultures were stored in Tryptic Soy Broth (TSB, Sigma- Aldrich) with 20% glycerol (Sigma-Aldrich) at -80°C. To restart the cultures, the bacteria were inoculated on TSA for 24 h at 37°C then five colonies of each bacterium were sub-cultured in 50 mL of TSB before being incubated at 37°C for 8 h. These bacterial suspensions are then diluted in 50 mL of fresh TSB to achieve an optical density (OD) of 0.01 at 600 nm. Cultures in stationary growth phase are thus obtained after 20 h at 37°C.

b. Inoculation of milk powder

For each bacterium, the 50 mL of cultures are centrifuged (3400 g, 10 min at 25°C) then washed twice in 25 mL of PBS. Finally, a last centrifugation is performed, the supernatant is removed and the pellets are weighed. The milk powder (26% of fat) is added on each pellet with a ratio of 1:20 (m_{pellet}:m_{powder}) and the whole is homogenized with the aid of a mortar. An inoculated milk powder is thus obtained.

c. Drying process

For the inoculated milk powder, airtight containers, containing saturated salt solutions making it possible to monitor the activity of the water and therefore the relative humidity of the atmosphere, are used. Lithium chloride, potassium acetate, potassium carbonate and sodium bromide were used to obtain a water activity of 0.11, 0.25, 0.44 and 0.58. The atmospheres thus obtained are maintained under convection using a fan. For each strain, the inoculated powder is spread in Petri dishes (approximately 5 g per dish). These dishes are then placed without lids in the airtight containers for 16 h to reach equilibrium water activity. All drying was performed at room temperature.

d. Heat treatment

0.1 g of dried inoculated milk powder is placed in a 0.2 mL tube and treated at various temperatures (85°C, 90°C, 95°C and 100°C) for a given time (0 s, 30 s, 60 s, 90 s, 120 s, 150 s and 180 s) thanks to a thermocycler before being cooled to 4°C. The samples are rehydrated by adding 1 mL of PBS before vortexing for 30 s. A CFU count was carried out after incubation on TSA for 24 h at 37°C. The results are expressed as $\log_{10}(N/N_0)$, where N is the CFU after treatment and N_0 is the initial CFU of the milk powder before treatment (t = 0 s).

Species	Deposit No.	D-value
Enterococcus faecium	ATCC 8459	23.69
Enterobacter hormaechei	CNCM I-5058	4.36
Pantoea agglomerans	CNCM I-5059	1.81
Enterobacter mori	CNCM I-5060	3.95
Pantoea calida	CNCM I-5061	2.03
Erwinia persicina	CNCM I-5062	1.74
Erwinia persicina	CNCM I-5063	1.96
Pantoea agglomerans	CNCM I-5054	1.27
Pantoea agglomerans	CNCM I-5055	1.33
Pantoea calida	CNCM I-5056	1.14
Salmonella Typhimurium	DSM 10506	1.21
Cronobacter sakazakii	PAC 103183T	1.13

Example II. Validation of a decontamination process.

The technology the most commonly used in the decontamination sector, in agri-food and pharmaceutical industries, remains the autoclaves. It is thus possible to heat the product in a chamber, while static or in motion, simply by steam condensation on the latter. The product may then be dried by a combination of heating and placing in a vacuum. There are hundreds of autoclave manufacturers worldwide, a certain number of which work on the pasteurization of dry food products.

The classic pasteurization cycle of this equipment consists of the following steps:

- Phase 1: Air elimination. A plurality of cycles is carried out in order to eliminate as much air as possible. This step is necessary to make it possible for steam to penetrate through the product.
- Phase 2: Heating. Steam is injected in the aim of heating the product. The enclosure of the chamber is also heated by electrical resistances to prevent any condensation phenomenon.
- Phase 3: Pasteurization. Once the product has reached a target temperature, there is a holding stage at this temperature. The treatment time-temperature pair is defined

upstream of the validation work. The time-temperature pair is crucial for the efficacy of the treatment.

- Phase 4: Drying. The steam is removed by vacuum drying.
- Phase 5: Aeration. The chamber is ventilated by a stream of filtered air at atmospheric pressure

The product is then removed from the chamber in the production direction, it cannot come into contact with untreated material. According to the cycle selected, its end of process temperature varies from 30 to 50°C. The product is not packaged until it has returned to room temperature because any bagging when too hot could cause a development of germs.

The in situ validation of a decontamination process generally comprises three main steps:

- Preparatory phase: process evaluation, risk assessment, model germ qualification, development of the in situ validation protocol
- Execution phase: inoculation of the product to be tested, execution of validation "batches", sample recovery
- Synthesis phase: counting of model germs, writing of the analysis report and/or validation report

During the development of the validation protocol, the following, among other things, are defined:

- Whether or not it is necessary to carry out a pre-treatment of the product to be tested (for example: irradiation)
- The number of validation batches as well as the duration of each validation batch to be carried out
- The amount of product to be inoculated (from 25 g to > 10 t depending on the decontamination processes and on the validation method selected)
- The desired level of inoculation as well as the amount of model germ to be used
- The method for inoculating the product with the model germ (there are various possibilities, including inoculation in the laboratory, directly in the factory, with a service provider, etc.)
- The sampling method at the end of the production line (including, among other things, the number and the size of the samples)

- The method for counting the model germ (among other things: selective or non-selective medium)

The answers to these various questions essentially depend on three parameters: type of process to be validated, target pathogen, product to be inoculated.

Thus, the validation "kit" supplied may vary in particular in:

- Mixing level of the model germ with the product to be tested (the model germ may be supplied in concentrated form to be inoculated or in pre-mixed form with the product)
- Concentration level in model germ
- Amount supplied (from a plurality of kg/tens of kg for the concentrated form to a plurality of tons for the pre-mixed version)

Example III. Production of indicator microorganisms by fermentation

Pre-culture

The pre-culture of the surrogate microorganism must be started between 16 h and 24 h before the fermentation. The Erlenmeyer flask containing the culture medium is inoculated with surrogate microorganism according to a ratio of 1:5. The pre-culture is incubated at 37°C under agitation conditions at 150 rpm.

Fermentation process

The culture starts when the entire pre-culture has been inoculated in the fermenter. The agitation, aeration and substrate addition conditions are the following:

- pH maintained by a base throughout the culture
- Temperature maintained at 37°C by heating the double-walled enclosure and/or jacket, and throughout the culture
- Oxygen saturation of the initial medium before inoculation ($pO_2 > 90\%$)
- Agitation (rpm): 200-500.
- Aeration (L/min): 1-3.

At the end of the culture, the culture medium of the fermenter is transferred to sterile bottles in order to recover all of the biomass.

Biomass recovery and preparation in dry form

The biomass is recovered by centrifugation or by another technique such as ultrafiltration that enables us to separate the cells of the culture medium.

The cryoprotectant, once sterilized is added according to a volume ratio of 1:1 to the biomass and the whole is frozen at -80°C for at least 24 h in view of a potential lyophilization.

Example IV. Use of indicator microorganisms on various dry carriers

The objective of these examples is to show the thermal destruction kinetics of various indicator microorganisms and to compare them to that of *Salmonella* in various low water activity products.

Microorganisms tested

Four various *Salmonella* serotypes (Senftenberg, Enteritidis, Typhimurium and Mbandaka), inoculated individually or in cocktail form, are used as control strains for comparison with the model microorganisms tested.

The thermal resistance of two different indicator microorganisms is tested:

- *Enterobacter hormaechei* CNCM I-5058, dry preparation.
- *Pantoea agglomerans* CNCM I-5055, dry preparation.

The strain *Enterococcus faecium* (ATCC® 8459™) was used as reference strain because it is widely used as a biological tracer in validation of treatment processes for dry food products

Inoculation protocols

Two different methods are used to inoculate the various products:

- Liquid inoculation of pathogens: broth cultures of four *Salmonella* serotypes prepared the day before are used independently to inoculate the products produced (paprika powder, milk powder and macadamia nuts). After inoculation the product is placed under a type II biological safety enclosure in order to balance its water activity.
- Dry inoculation of indicator microorganisms: the various products (paprika powder, milk powder and macadamia nuts) were inoculated independently with the surrogate microorganisms in dry form following a production process by fermentation and stabilization by lyophilization. No resting time is required after the inoculation with the model microorganisms in dry form, which results in very little alteration of the properties of the product (aw, moisture percentage, etc.), which enables faster use of the inoculated matrices.

Results

The cell destruction dynamics at 90°C and 100°C of the strain *E. hormaechei* CNCM I-5058, the reference strain *E. faecium* ATCC 8459 and the 4 *Salmonella* serotypes on the paprika powder is shown in Figures 5A (treatment at 90°C) and 5B (treatment at 100°C). The results show in all cases a behavior of the model microorganism *E. hormaechei* CNCM I-5058 always closer to all of the *Salmonella* serotypes, thereby confirming its nature as a surrogate germ well adapted to the target pathogen on the product in question.

The cell destruction dynamics at 100°C and 110°C of the strain *P. agglomerans* CNCM I-5055, the reference strain *E. faecium* ATCC 8459 and the 4 *Salmonella* serotypes in cocktail form, on the macadamia nuts is shown in Figures 6A (treatment at 100°C) and 6B (treatment at 110°C). The results show a behavior of the model microorganism *P. agglomerans* CNCM I-5055 closer to all of the *Salmonella* serotypes, thereby confirming its nature as a surrogate germ well adapted to the target pathogen on the product in question.

The cell destruction dynamics at 85°C and 100°C of the strain *P. agglomerans* CNCM I-5055, the reference strain *E. faecium* ATCC 8459 and the 4 *Salmonella* serotypes in cocktail form on the skimmed milk powder is shown in Figures 7A (treatment at 85°C) and 7B (treatment at 100°C). The results show a behavior of the model microorganism *P. agglomerans* CNCM I-5055 closer to all of the *Salmonella* serotypes, thereby confirming its nature as a surrogate germ well adapted to the target pathogen on the product in question.

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Patentkrav

1. Fremgangsmåde til kontrol af en dekontamineringsfremgangsmåde hvor dekontamineringsfremgangsmåden implementeres under tilstedeværelsen af mindst en indikatormikroorganisme og adfærden af nævnte mindst ene indikator-mikroorganisme observeres under nævnte dekontamineringsfremgangsmåde, **kendetegnet ved, at** den mindst ene indikatormikroorganisme er valgt fra ikke-patogene *Enterobacteriaceae* af slægten Pantoea valgt fra arterne *Pantoea agglomerans* og *Pantoea calida,* af slægten *Enterobacter* valgt fra arterne *Enterobacter hormaechei* og *Enterobacter mori,* eller af slægten *Erwinia* valgt fra arterne *Erwinia persicina* og blandinger deraf.

2. Fremgangsmåde ifølge krav 1, **kendetegnet ved, at** indikator-mikroorganismerne anvendes i tør vegetativ form.

3. Fremgangsmåde ifølge et af kravene 1 eller 2, **kendetegnet ved, at** indikatormikroorganismerne anvendes tørre på en inert bærer.

4. Kontrolfremgangsmåde ifølge et af kravene 1 til 3, **kendetegnet ved, at** indikatormikroorganismerne valgt fra ikke-patogene *Enterobacteriaceae* er valgt fra *Enterobacter hormaechei* CNCM I-5058, *Pantoea agglomerans* CNCM I-5059, *Enterobacter mori* CNCM I-5060, *Pantoea calida* CNCM I-5061, *Erwinia persicina* CNCM I-5062, *Erwinia persicina* CNCM I-5063, *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055, *Pantoea calida* CNCM I-5056 og blandinger deraf.

5. Kontrolfremgangsmåde ifølge et af kravene 1 til 4, **kendetegnet ved, at** den mindst ene indikatormikroorganisme er valgt fra *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055, *Pantoea calida* CNCM I-5056 og blandinger deraf.

6. Fremgangsmåde ifølge krav 1, kendetegnet ved, at der anvendes en blanding af mindst 2 indikatormikroorganismer.

7. Fremgangsmåde ifølge et af kravene 1 til 6, **kendetegnet ved, at** dekontamineringsfremgangsmåden omfatter et eller flere trin til pasteurisering, tørring, ekstrudering, ristning, kogning, sterilisering, autoklavering og dampbehandlinger.

8. Kontrolfremgangsmåde ifølge et af kravene 1 til 7, **kendetegnet ved, at** kontroldekontamineringsfremgangsmåden har til hensigt at fjerne en eller flere patogene målmikroorganismer valgt fra *Salmonella, Escherichia coli, Bacillus, Listeria, Campylobacter, Cronobacter sakazakii.*

9. Indikatormikroorganismer som er egnede til at anvendes som erstatninger i en fremgangsmåde til kontrol af en dekontamineringsfremgangsmåde, **kendetegnet ved, at** de er valgt fra *Enterobacter hormaechei* CNCM I-5058, *Pantoea agglomerans* CNCM I-5059, *Enterobacter mori* CNCM I-5060, *Pantoea calida* CNCM I-5061, *Erwinia persicina* CNCM I-5062, *Erwinia persicina* CNCM I-5063, *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055 og *Pantoea calida* CNCM I-5056.

10. Blanding af indikatormikroorganismer, **kendetegnet ved, at** den omfatter mindst 2 arter af mikroorganismer ifølge krav 9.

11. Tør sammensætning **kendetegnet ved, at** den omfatter mindst en indikator-mikroorganisme ifølge krav 9 og en inert bærer.

12. Sammensætning ifølge krav 11, **kendetegnet ved, at** den omfatter et indhold af indikatormikroorganismer på mindst 10^{10} CFU/g af den tørre sammensætning.

13. Tør sammensætning ifølge et af kravene 11 eller 12, **kendetegnet ved, at** den mindst ene indikatormikroorganisme er valgt fra *Pantoea agglomerans* CNCM I-5054, *Pantoea agglomerans* CNCM I-5055 og *Pantoea calida* CNCM I-5056.

14. Kit til kontrol af en dekontamineringsfremgangsmåde, **kendetegnet ved, at** det omfatter mindst en mikroorganisme ifølge krav 9 og en bærer, som er

egnet til dens anvendelse i dekontamineringsfremgangsmåden.

15. Anvendelse af mindst en mikroorganisme ifølge krav 9 eller af en blanding ifølge krav 10 eller af en tør sammensætning ifølge et hvilket som helst af kravene 11 til 13 eller af et kit til kontrol ifølge krav 14 til kontrol af en dekontamineringsfremgangsmåde.

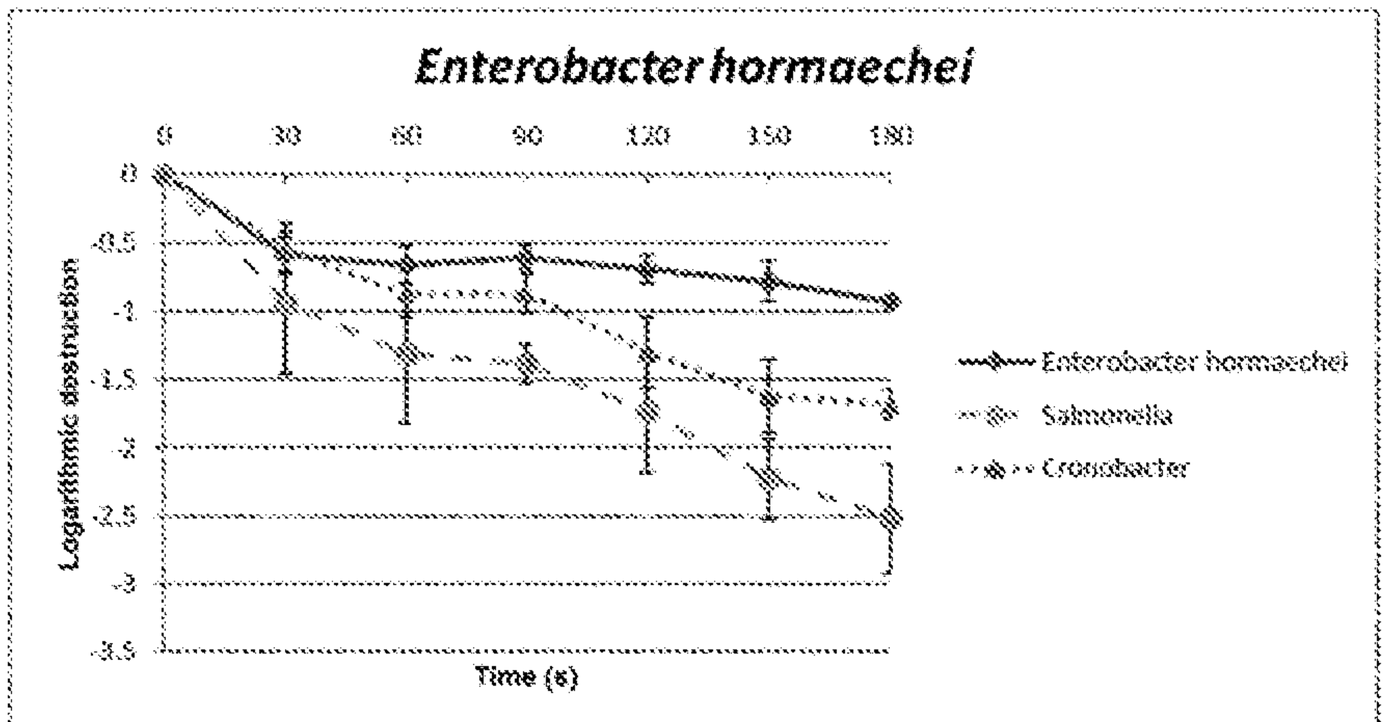
Enterobacter hormaechei
0
30
60
90
120
150
180
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
Logarithmic destruction
Time (s)
Enterobacter hormaechei
Salmonella
Cronobacter

Fig. 1

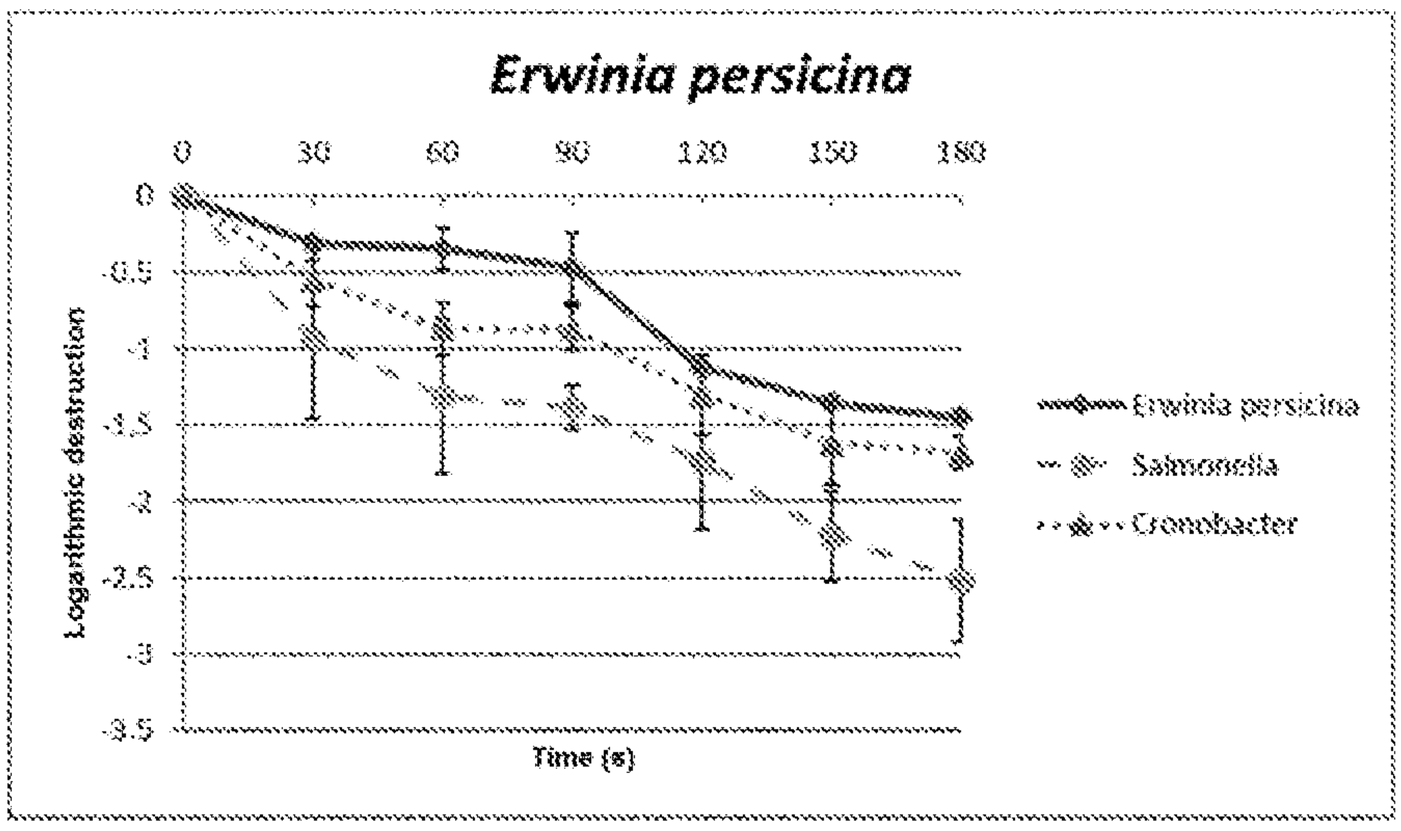
Erwinia persicina
0
30
60
90
120
150
180
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
Logarithmic destruction
Time (s)
Erwinia persicina
Salmonella
Cronobacter

Fig. 2

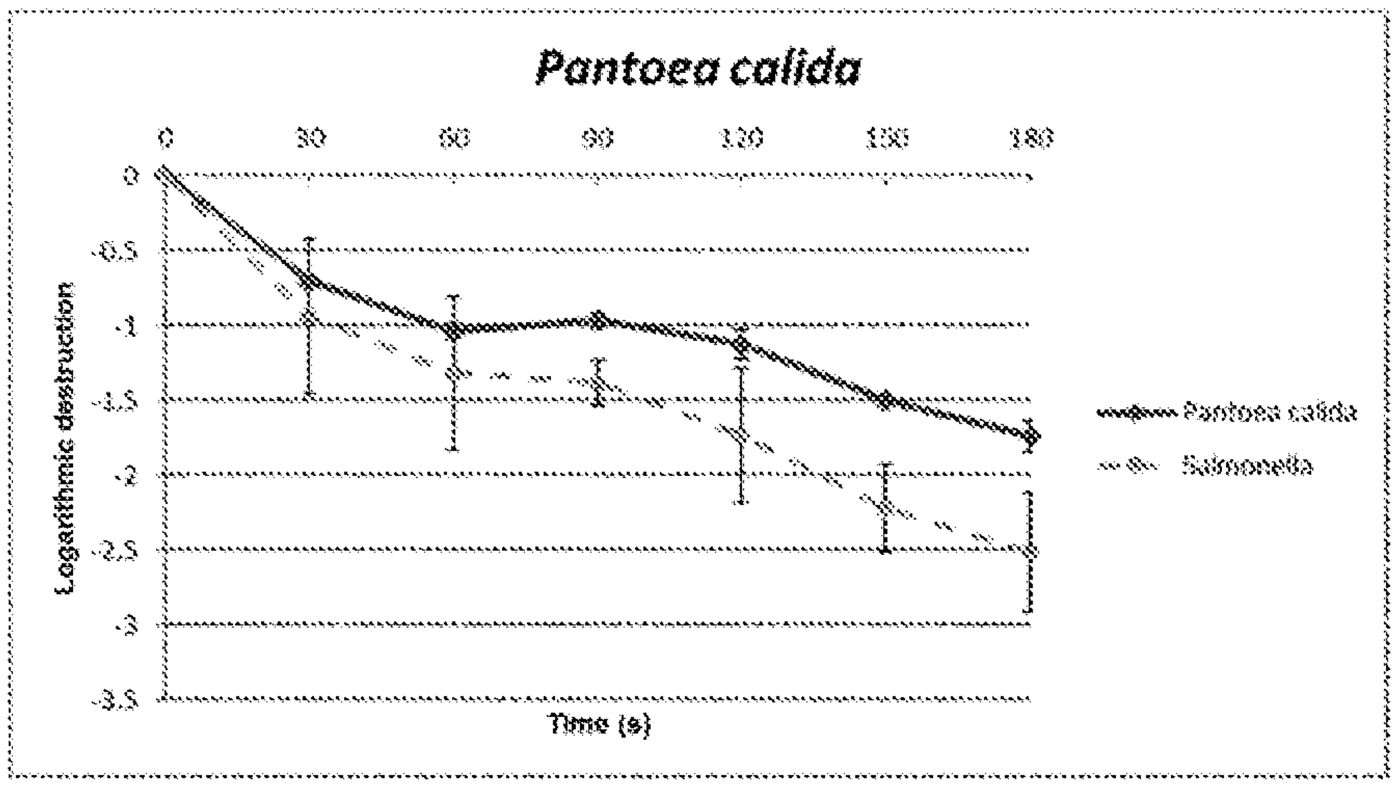
Pantoea calida
0
30
60
90
120
150
180
0
-0,5
-1
-1,5
-2
-2,5
-3
-3,5
Logarithmic destruction
Time (s)
Pantoea calida
Salmonella

Fig. 3

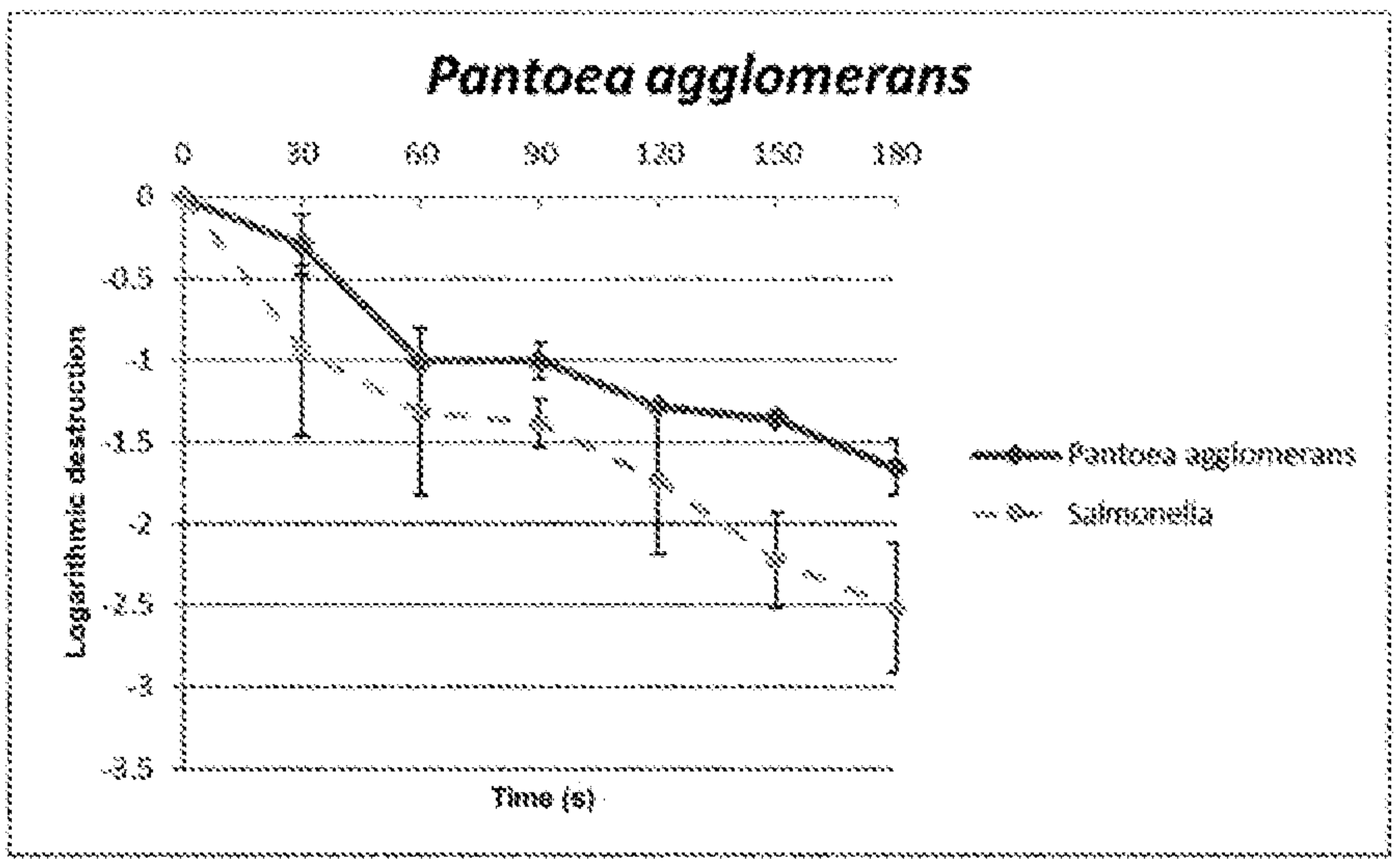
Pantoea agglomerans
0
30
60
90
120
150
180
0
-0,5
-1
-1,5
-2
-2,5
-3
-3,5
Logarithmic destruction
Time (s)
Pantoea agglomerans
Salmonella

Fig. 4

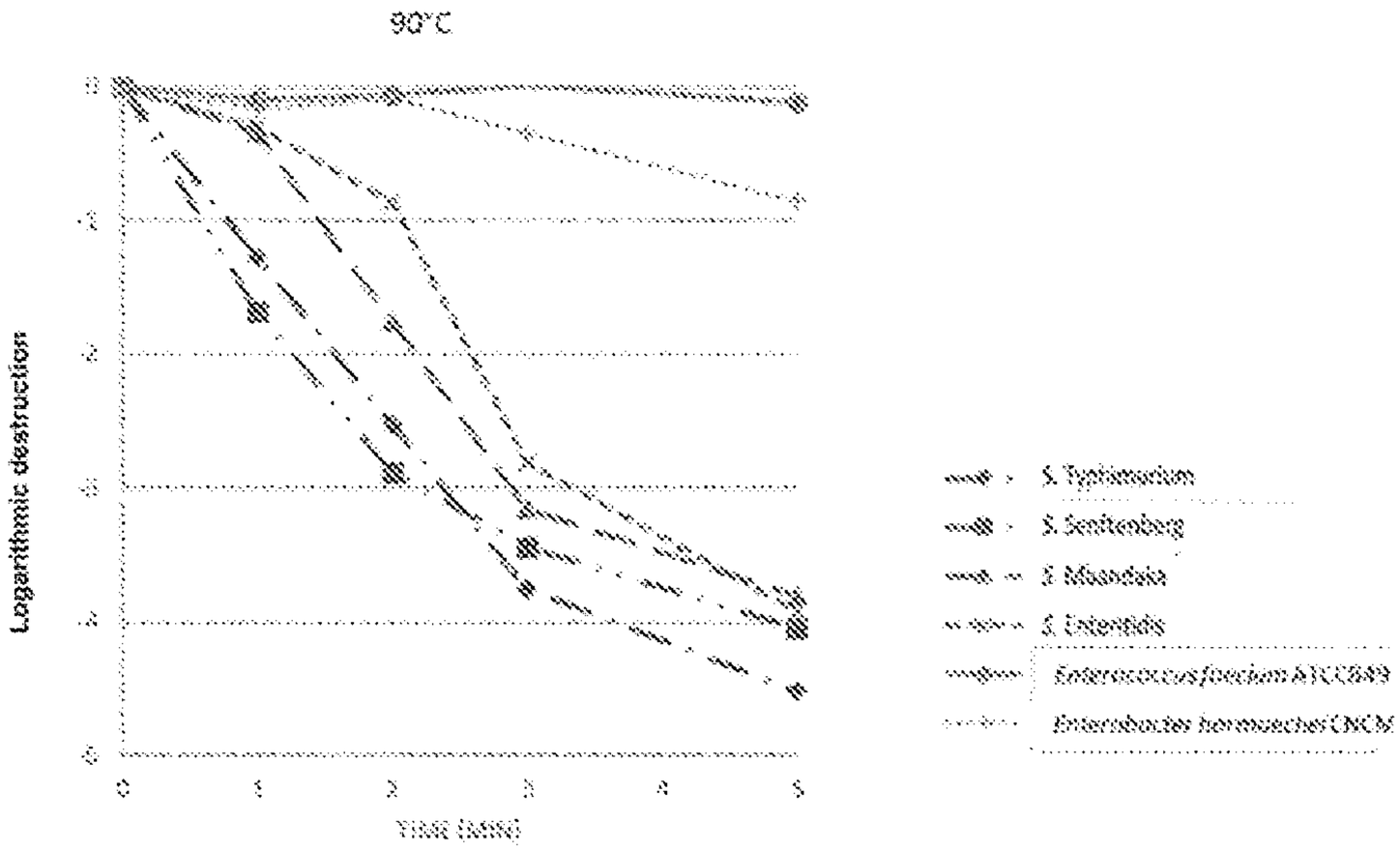
90°C
Logarithmic destruction
0
1
2
3
4
5
TIME (MIN)
S. Typhimurium
S. Senftenberg
S. Mbandaka
S. Enteritidis
Enterococcus faecium ATCC8459
Enterobacter hormaechei CNCM

Fig. 5A

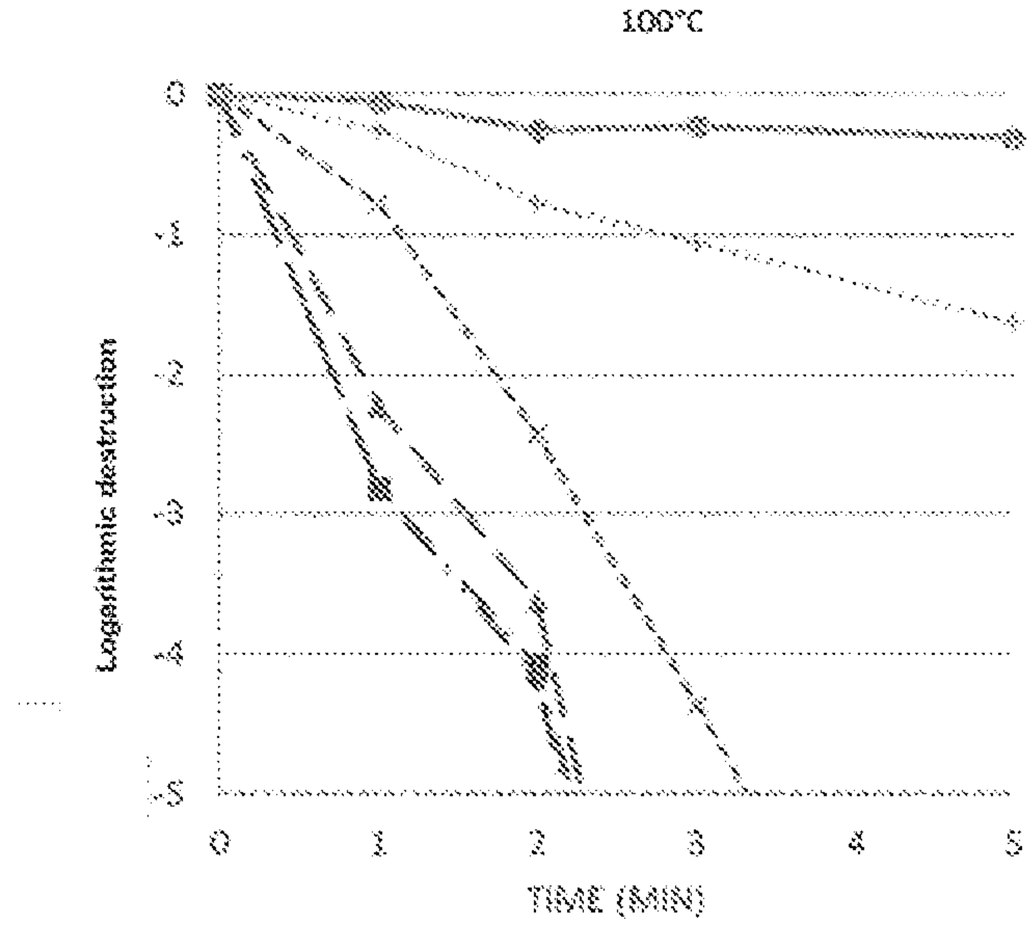
100°C
Logarithmic destruction
0
1
2
3
4
5
TIME (MIN)

Fig. 5B

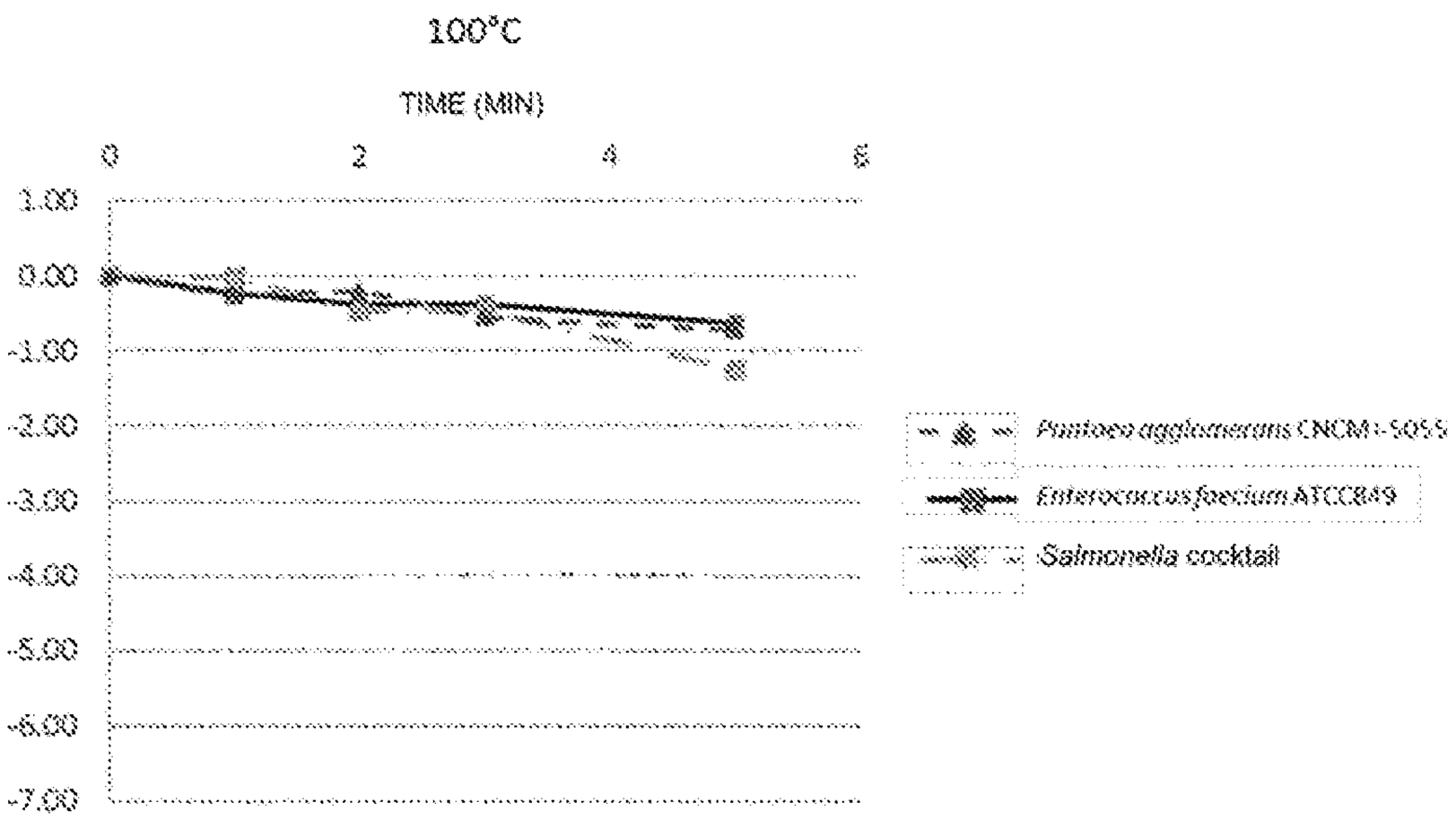
100°C
TIME (MIN)
0
2
4
6
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
-7.00
Pantoea agglomerans CNCM I-5055
Enterococcus faecium ATCC 849
Salmonella cocktail

Fig. 6A

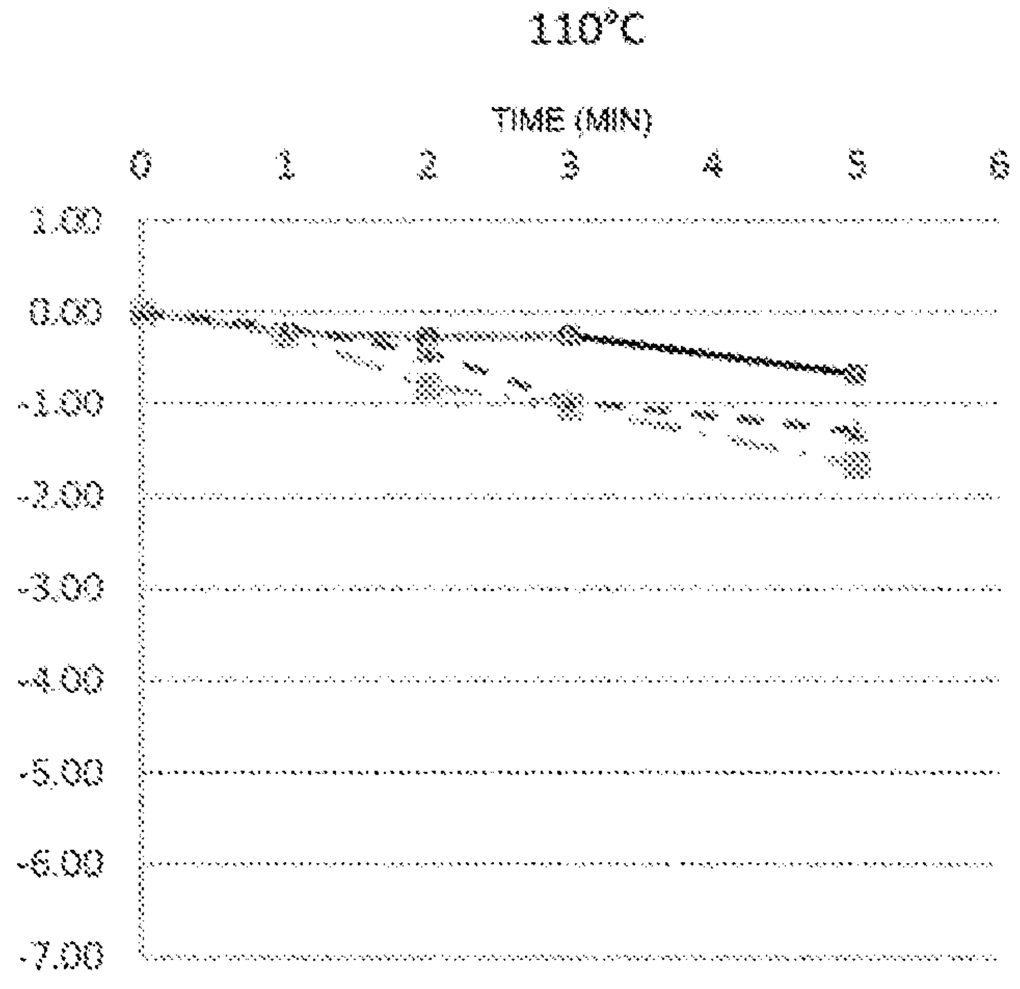
110°C
TIME (MIN)
0
1
2
3
4
5
6
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
-7.00

Fig. 6B

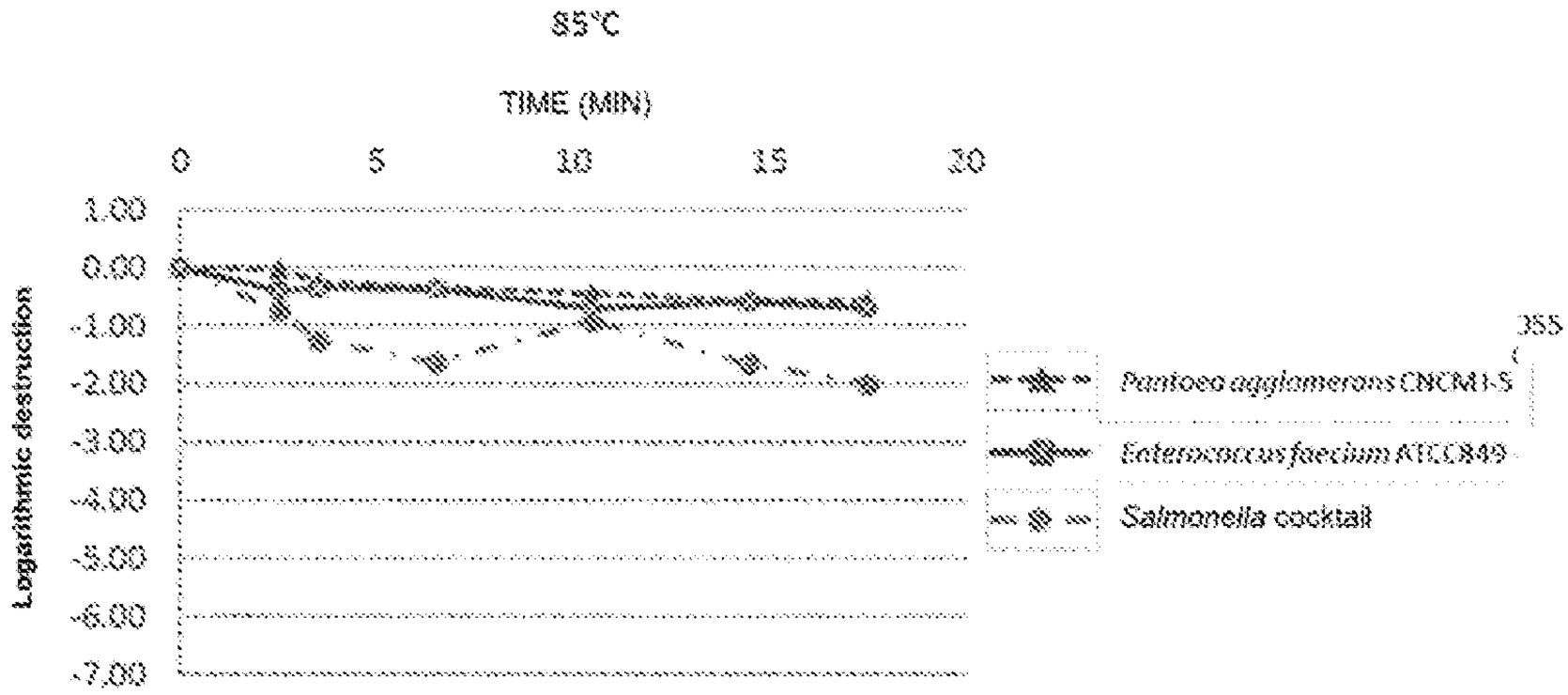
55°C
TIME (MIN)
0
5
10
15
20
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
-7.00
Logarithmic destruction
Pantoea agglomerans CNCM I-5
Enterococcus faecium ATCC 49
Salmonella cocktail

Fig. 7A

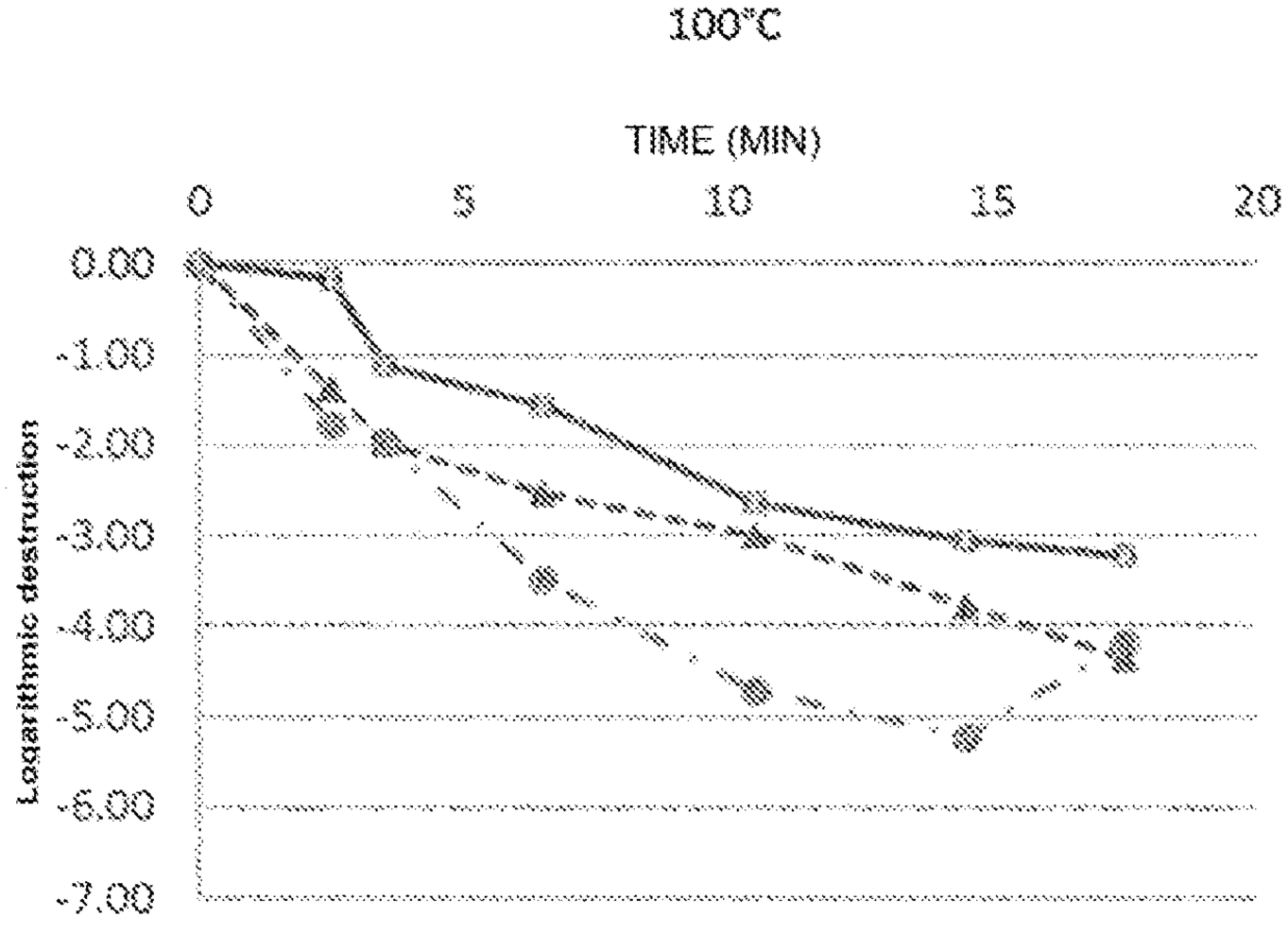
100°C
TIME (MIN)
0
5
10
15
20
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
-7.00
Logarithmic destruction

Fig. 7B