

Are radiopharmaceuticals self-sterilizing? Radiation effect of gallium-68 and lutetium-177 on bacillus pumilus and staphylococcus succinus

Simon Poetzsch

Charite Universitätsmedizin Berlin - Campus Virchow-Klinikum

Winfried Brenner (✉ winfried.brenner@charite.de)

<https://orcid.org/0000-0003-2478-6004>

Sarah Spreckelmeyer

Charite - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität and Berlin Institute of Health, Department of Nuclear Medicine, Augustenburger Platz 1, 13353 Berlin

<https://orcid.org/0000-0003-1348-0309>

Research article

Keywords: Radiopharmaceutical, sterilization, microorganism, aseptic

Posted Date: July 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-38980/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

For radiopharmaceuticals, aseptic preparation in combination with filtration is the most chosen sterilizing method. In general, the production of radiopharmaceuticals needs to fulfil the requirements of good manufacturing practice. In the scope of this work, we focused on the positron emitter gallium-68 and on the therapeutically used beta- and gamma-emitter lutetium-177, as they are routinely used for *in-house* synthesis of radiopharmaceuticals in nuclear medicine departments. Our hypothesis is, that radiopharmaceuticals might be self-sterilizing due to a high radioactivity concentration and high-energy radionuclides in the preparation for intravenous injections.

Results

Incubation with gallium-68 and lutetium-177 for both 30 minutes and 5 hours post-dispensing did not cause any significant effect on bacteria growth. As the theoretical dose is only 0.1–0.6% of the Ph. Eur. recommended dose of 25 kGy, we conclude that the beta and positron energy of lutetium-177 and gallium-68 as used for standard radiopharmaceutical *in-house* production is not sufficient to decrease the number of colony forming units compared to the control values.

Conclusions

Based on these findings, gallium-68 and lutetium-177 labeled radiopharmaceuticals are not self-sterilizing under the tested conditions with respect to bacillus pumilus and staphylococcus succinus. Consequently, strict aseptic preparation conditions in addition to end-sterilization of the radiopharmaceutical e.g. through membrane filtration are strongly advised for *in-house* productions.

Background:

Radiopharmaceuticals are injections or infusions for diagnostic or therapeutic use which effect is caused by the incorporated radionuclide. Radioactivity has, depending on its character, a very high energy that can cause single- or even double-strand breaks of DNA that eventually lead to apoptosis of the respective cells.[1] In nuclear medicine, this feature is used to detect or even treat various diseases. As radiopharmaceuticals are applied parenterally, they must be sterilized before use. In the European Pharmacopoeia, six different methods are described to achieve sterility with a sterility assurance level (SAL) of 10^{-6} – steam sterilization, dry heat, radiation, gas, membrane filtration and working under aseptic conditions (Ph. Eur. Vol 9.0, 5.1.1). For radiopharmaceuticals, aseptic preparation in combination with membrane filtration is the most chosen sterilizing method. Other methods are usually not applicable due to the short half-lives of the radionuclides and/or incompatibility of the radiopharmaceutical with elevated temperatures as they may contain heat-sensitive biomolecules. In general, the production of

radiopharmaceuticals needs to fulfil the requirements of good manufacturing practice (GMP) and sterile (starting) materials are used wherever possible. The (automated) production is performed under aseptic conditions in laminar-air flow hoods class A or in hot cells/ isolators using synthesis modules in GMP clean rooms. The final step of the production is mostly the filtration of the radiopharmaceutical through a 0.22 µm membrane filter for end-sterilization. In order to check for sterility conformity, the radiopharmaceutical is incubated (retrospectively) for two weeks on growth media as described in the European Pharmacopoeia (Ph. Eur. Vol 9.0, 2.6.1).

Our hypothesis is, that radiopharmaceuticals might be self-sterilizing due to the fact that they contain a high radioactivity concentration and high-energy radionuclides. To the best of our knowledge, this hypothesis was thus far only tested for [^{99m}Tc]-radiopharmaceuticals[2, 3] and [¹⁸F]-radiopharmaceuticals[4]. Brown et al. state, that the time lag between preparation and the sterility test of [^{99m}Tc]-radiopharmaceuticals should be as short as possible. Their reasoning is, that longer time lags have a greater chance to obtain negative sterility results although the preparation might have been already contaminated at the time of dispensing. Jörg et al. investigated the “autosterilization” effect of [¹⁸F]-radiopharmaceuticals and concluded, that intrinsic [¹⁸F]-radiation is not sufficient for achieving sterility of the radiopharmaceutical. In our opinion, the main drawbacks, which we will avoid in our experiment set-up of these studies, were the long incubation times of up to 11 hours with the radiation source and the direct inoculation of the radiopharmaceutical of interest with the microorganisms. These long incubation times are not realistic in the daily routine because *in-house* synthesized radiopharmaceuticals in nuclear medicine departments are usually applied within one hour after preparation to the patients. Furthermore, samples taken directly out of the inoculated microorganisms-radiopharmaceutical solution contain remaining radioactivity. Thus, the seeded sample on growth media may be effected by the remaining radioactivity.

In the scope of this work, we will focus on the positron emitter gallium-68 and on the therapeutically used beta- and gamma-emitter lutetium-177, as they are routinely used for *in-house* synthesis of radiopharmaceuticals in nuclear medicine departments. Lutetium-177 is primarily a beta-emitter (490 keV) that decays after 6.7 days to the stable hafnium-177 but also emits gamma rays with an energy of 113 keV (3%) and 210 keV (11%). Gallium-68 is a positron emitter (1899 keV (88%) and 822 keV (1%)) with gamma energies of 511 keV (178%) and 1077 keV (3%) with a half-life of 68 minutes.[5] Both radionuclides can be linked to peptides such as DOTATOC (edotreotide) and PSMA.[6]

The growth behavior of two different germs have been evaluated at two different steps during the preparation – either pre-dispensing or post-dispensing. *Bacillus pumilus* is a known radiation resistant species and can be used to validate ionizing radiation sterilization as used in the European Pharmacopoeia as sterility marker.[7] Additionally, this germ is resistant to environmental stresses. *Staphylococcus succinus* was chosen as a member of the wide-spread genus staphylococcus.[8] Both germs are Gram-positive and categorized in the lowest biological safety class and can be handled in normal laboratories.

As gallium-68 and lutetium-177 labeled radiopharmaceuticals are mostly prepared *in-house*, we assumed that the time of injection will be within 30 minutes post-dispensing. Consequently, we chose 30 minutes as first incubation time point. We also added a 5 hours incubation point post-dispensing, as this might be relevant for lutetium-177 radiopharmaceuticals when they are produced ahead of injection.

Additionally, pre-dispensing measures were chosen. During the synthesis of e.g. [⁶⁸Ga]Ga-DOTATOC, the starting materials are heated to 95 °C for approximately 15 min. Here, we tested the effect of an elevated temperature on the bacteria after 15 minutes. Additionally, gallium-68 radiopharmaceuticals are often post-processed with a maximum of 10% (V/V) ethanol. To take this also into account, we tested the bacterial growth after 30 min incubation with 10% (V/V) ethanol.

Results

Staphylococcus succinus

The post-dispensing results of the 30 minutes and 5 hours incubations are shown in Fig. 1. After 30 minutes incubation, the amount of colony forming units (cfu) in the control fraction increases to approximately double the amount compared to the start value. The gallium-68 and lutetium-177 treated samples show a three-fold increase of cfu after 30 minutes of incubation at room temperature in 0.9% NaCl solution. The increases in cfu are not significant compared to control values ($p = 0.44$ for gallium-68 and $p = 0.42$ for lutetium-177).

After 5 hours of incubation, the control and radiation treated samples show no significant change in cfu ($p = 0.45$ for gallium-68 and $p = 0.77$ for lutetium-177).

Bacillus Pumilus

The results of the 30 minutes and 5 hours incubation experiment post-dispensing are shown in Fig. 3. After 30 minutes, the amount of cfu in the control fraction does not change compared to the start value. The gallium-68 and lutetium-177 treated samples show no significant change compared to the control values ($p = 0.30$ for gallium-68 and $p = 0.83$ for lutetium-177) of cfu after 30 minutes of incubation at room temperature in 0.9% NaCl solution.

After 5 hours of incubation, the gallium-68 treated samples show a trend towards a decrease in growth ($p = 0.17$), although without statistical significance. The lutetium-177 treated samples show no significant change in growth compared to the control value ($p = 0.89$).

Theoretical Dose

The theoretical dose was calculated using standard radiochemical formulas (Supporting Information). The theoretically achievable dose in the radiopharmaceuticals produced *in-house* ranges from 45 Gy to

161 Gy for gallium-68 and 12 Gy to 119 Gy for lutetium-177. Compared to the radiation dose to achieve an SAL of 10^{-6} , we achieve only 0.1–0.6% of the necessary dose of 25 kGy (Table 1).

Table 1
Overview of theoretical dose in radiopharmaceuticals.

Nuclide	Dose concentration [GBq/mL]	Incubation time	Theoretical dose [Gray]	Ratio to 25 kGy Ph. Eur. [%]
Gallium-68	0.2 *	30 min	44.54	0.2
Gallium-68	0.2*	5 h	161.09	0.6
Lutetium-177	0.1**	30 min	11.98	0.1
Lutetium-177	0.1**	5 h	118.59	0.5
*Ga-68 preparations in <i>in-house</i> productions: 2 GBq in 10 mL				
**Lu-177 preparations in <i>in-house</i> productions: 7 GBq in 80 mL				

Discussion

Within 30 minutes and 5 hours of incubation, the gallium-68 and lutetium-177 treated samples show no significant decrease of colony forming units. As the theoretical dose is only 0.1–0.6% of the Ph. Eur. recommended dose of 25 kGy, we conclude that the beta and positron energy of lutetium-177 and gallium-68 are not sufficient to decrease the number of cfu compared to the control value. Sterility can thus not be achieved through self-sterilization by the chosen radionuclides.

The pre-dispensing experiments however show, that elevated temperature at 95 °C for 15 min leads to a complete destruction of the tested viable microorganisms. The addition of 10% (V/V) ethanol decreases the cfu to 27% and 83% for staphylococcus succinus and bacillus pumilus, respectively. Bacillus pumilus is a spore-forming germ. Several bacillus strains e.g. bacillus pumilus were tested towards their hardness to ethanol by P. Thomas. He came to the conclusion, that bacillus pumilus is resistant to ethanol up to a concentration of about aqueous 90% (V/V) ethanol.[9] Elzain et. al describe the antibacterial effect of different concentrations of ethanol on various staphylococcus species. The authors reported, that staphylococcus species are sensitive to ethanol and ethanol can thus be used as an antimicrobial.[10] Our results match those results described in the literature. Although these results may be promising in regards to achieving sterility with elevated temperatures during the preparation step, the radiopharmaceutical could still get contaminated during the subsequent dispensing step.

Conclusions:

Based on these findings, gallium-68 and lutetium-177 labeled radiopharmaceuticals are not self-sterilizing under the tested *in-house* production conditions with respect to bacillus pumilus and staphylococcus succinus. Consequently, strict aseptic preparation conditions in addition to end-sterilization of the radiopharmaceutical e.g. through membrane filtration are strongly advised for *in-house* productions.

Material And Methods

All materials used were sterile single use materials e.g. syringes. Gallium-68 was eluted from the pharmaceutical grade $^{68}\text{Ge}/^{68}\text{Ga}$ generator (GalliaPharm, Eckert & Ziegler Radiopharma GmbH, Germany). Lutetium-177 was obtained from ITG Garching. Bacillus pumilus (DSM 492) and Staphylococcus succinus (DSM 105508) were obtained from DSMZ-German Collection of Microorganisms and Cell Cultures GmbH. Radioactivity counting was performed using a borehole counter (Nuklear-Medizintechnik Dresden GmbH, Germany). Cultivation media tryptone soya broth USP was obtained from OXOID Deutschland GmbH. Bacteria concentration was calculated with plate count agar (146269) bought from Merck KGaA. All other chemicals were obtained from Sigma Aldrich.

Cultivation Of Bacteria

Bacillus pumilus and staphylococcus succinus are stored at $-80\text{ }^{\circ}\text{C}$ in tryptone soya broth USP with 25% (V/V) glycerol as cryoprotectant. For every experiment, an overnight culture was established from the frozen culture. For that, a spatula tip of frozen bacteria culture was transferred with a sterile pipette tip into a sterile 15 mL falcon tube filled with 14 mL NaCl solution. After homogenizing the bacteria solution, 50 μL was transferred into 10 mL cultivation media for overnight culture at $37\text{ }^{\circ}\text{C}$.

Post-dispensing Experiments

The overnight culture was homogenized. The bacteria vials for the control and gallium-68/lutetium-177 treatment were prepared as follows: One mL (approximately 1×10^9 cfu) of the overnight culture was added to 9 mL 0.9% NaCl solution and homogenized. A sample from this solution was taken to estimate the cfu at the beginning of the experiment (start). As control values, samples were taken from the untreated bacteria-solution after 30 minutes and 5 hours (control).

A 1.5 mL glas vial containing either 1 mL gallium-68 (approx. 1–2 GBq) or 1 mL lutetium-177 (approx. 0.8–1.4 GBq) was immersed into the 0.9% NaCl bacteria vial for 30 min or 5 hours. Before taking samples at 30 min or 5 hours, the bacteria vial was homogenized.

100 μL of the bacteria vial was used for further dilutions and seeding on counting agar plates. After 24 h, the cfu were counted and the total cfu calculated as described below.

Pre-dispensing Experiments

The overnight culture was homogenized. The bacteria vials for the ethanol and 95 °C treatment were prepared as follows: One mL (approximately 1×10^9 cfu) of the overnight culture was added to 9 mL 0.9% NaCl solution and homogenized in case of the 95 °C treatment. For the ethanol treatment, 1 mL sterile ethanol was added to 8 mL 0.9% NaCl solution and one mL of bacteria were added.

100 μ l of the bacteria vial was used for further dilutions and seeding on counting agar plates. After 24 h, the cfu were counted and the total cfu calculated as described below.

Calculation Of Total cfu

For calculation of the total cfu, a dilution series of the bacteria vial was taken into account.

Dilution	name	Volume of Solution from bacteria vial [mL]	Volume 0.9% NaCl [mL]
1	A1		
1:100	A2	0.1 mL of A1	9.9
1:1000	A3	1 mL of A2	9

Overall the number of bacteria in the bacteria vial after the incubation time can be calculated by *number of colonies * dilution * 100*.

Abbreviations

SAL

sterility assurance level

cfu

colony forming units

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

SP designed, performed and analyzed the experiments. WB evaluated the results concerning clinical applications. SS designed, performed and analyzed the experiments and repeated the experiments in order to proof its repeatability. All authors contributed to, read and approved the final manuscript.

Acknowledgement

Not applicable.

References

1. Barnard S, Bouffler S, Rothkamm K. The shape of the radiation dose response for DNA double-strand break induction and repair. *Genome Integr.* 2013;4(1):1.
2. Brown S, Baker MH. The sterility testing of dispensed radiopharmaceuticals. *Nucl Med Commun.* 1986;7(5):327–36.
3. Stathis VJ, et al. Effect of technetium Tc 99 m pertechnetate on bacterial survival in solution. *Am J Hosp Pharm.* 1983;40(4):634–7.
4. Jorg G, et al. Inhibition of viability of microorganisms in [(18)F]-labeled radiopharmaceuticals. *Nucl Med Biol.* 2017;44:105–13.
5. [cited 2020 9th June 2020]; Available from: <http://www.nucleide.org/Laraweb/index.php>.
6. Breeman WA, et al. (68)Ga-labeled DOTA-peptides and (68)Ga-labeled radiopharmaceuticals for positron emission tomography: current status of research, clinical applications, and future perspectives. *Semin Nucl Med.* 2011;41(4):314–21.
7. Prince HN. D-values of *Bacillus pumilus* spores on irradiated devices (inoculated product). *Appl Environ Microbiol.* 1978;36(2):392–3.
8. Place RB, et al. *Staphylococcus succinus* subsp. *casei* subsp. nov., a dominant isolate from a surface ripened cheese. *Syst Appl Microbiol.* 2002;25(3):353–9.

9. Thomas P. Long-term survival of *Bacillus* spores in alcohol and identification of 90% ethanol as relatively more spori/bactericidal. *Curr Microbiol.* 2012;64(2):130–9.
10. Abdelrhman M, Elzain SME. Mohammed Elfatih Ahmed Ibrahim, *Effectiveness of ethanol and methanol alcohols on different isolates of staphylococcus species.* *Journal of Bacteriology & Mycology*, 2019. 7(4).

Figures

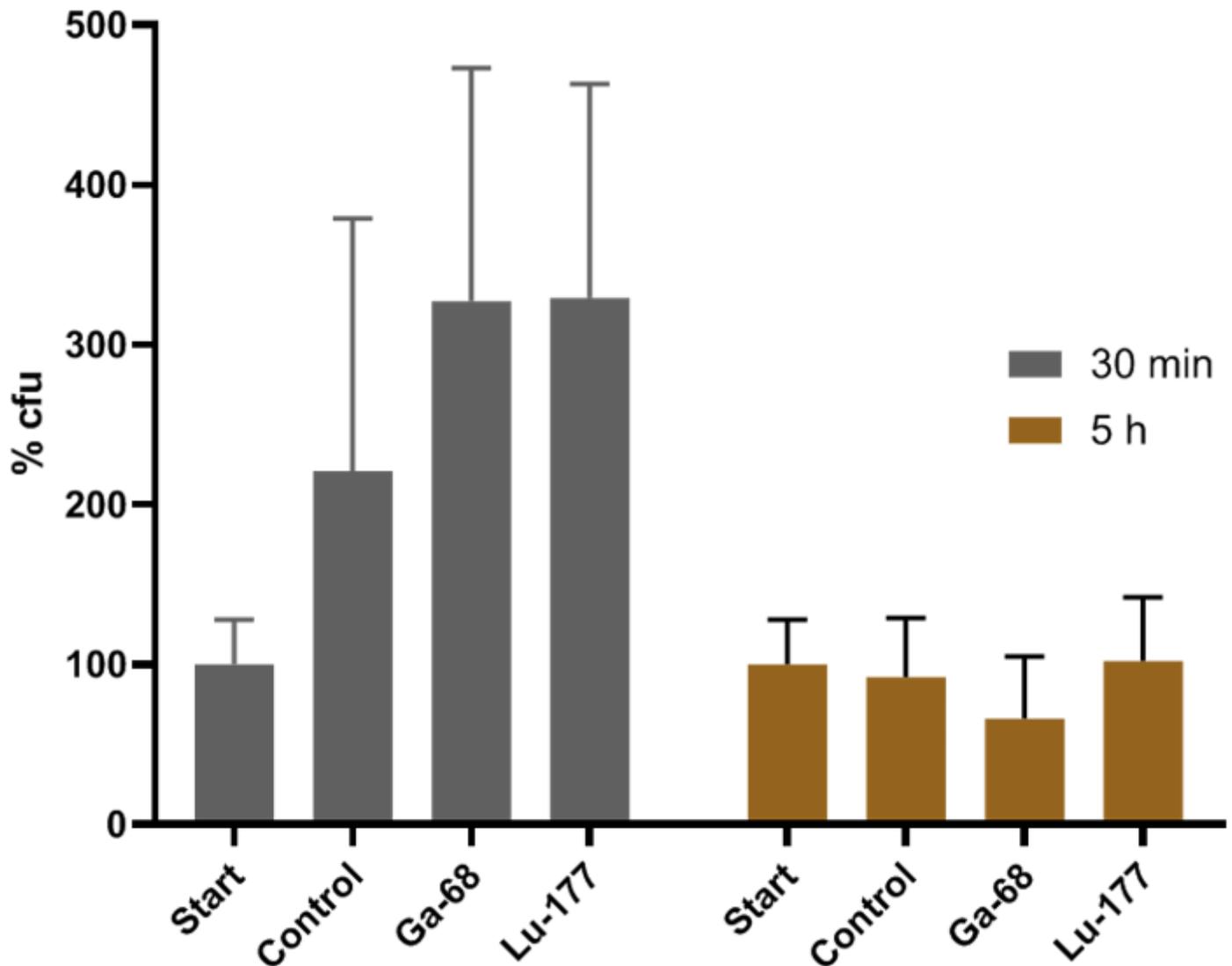


Figure 1

Staphylococcus succinus: Results of 30 min incubation and 5 hours incubation; $n \geq 3$

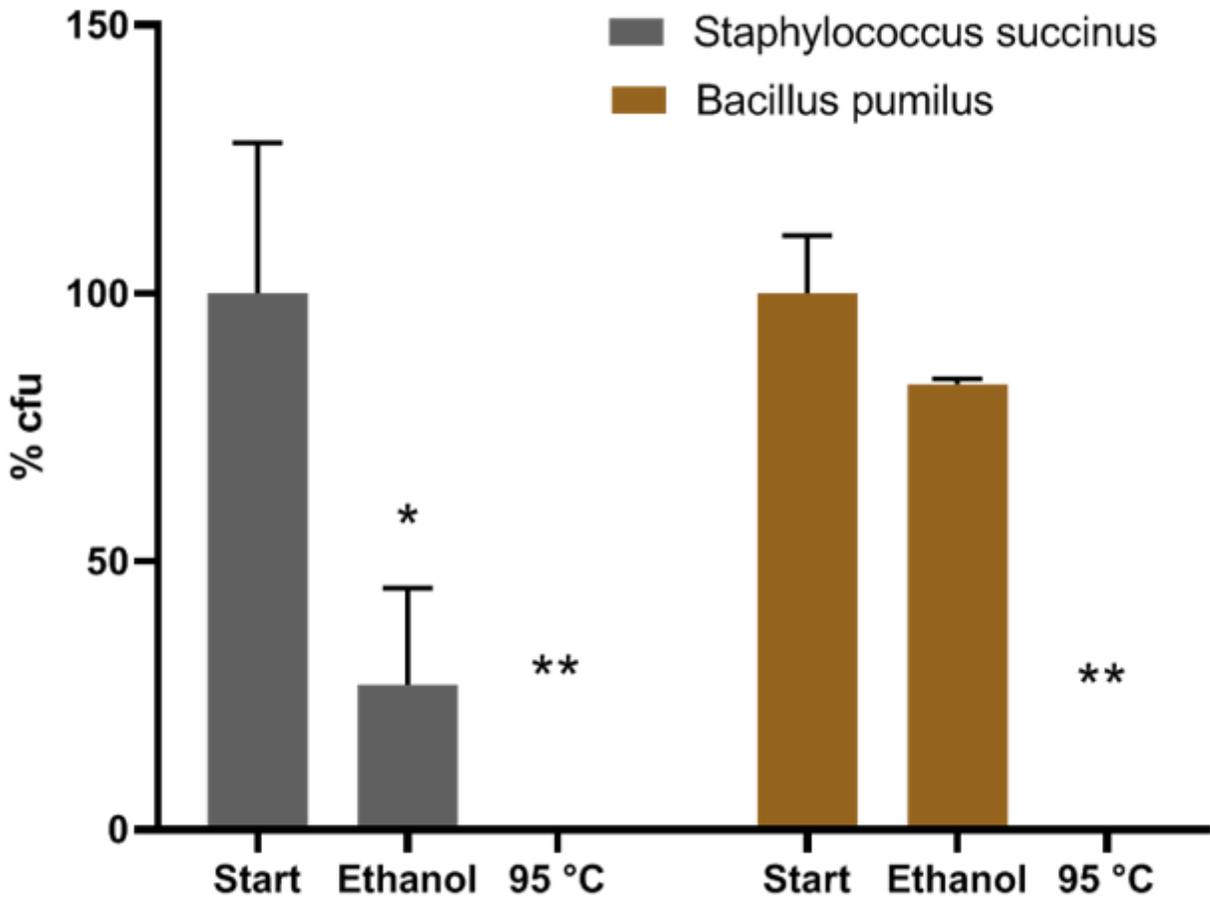


Figure 2

Results of incubation with ethanol or at 95°C of the two different bacteria species; n≥3 (* = p-value <0.05
 ** = p-value <0.005)

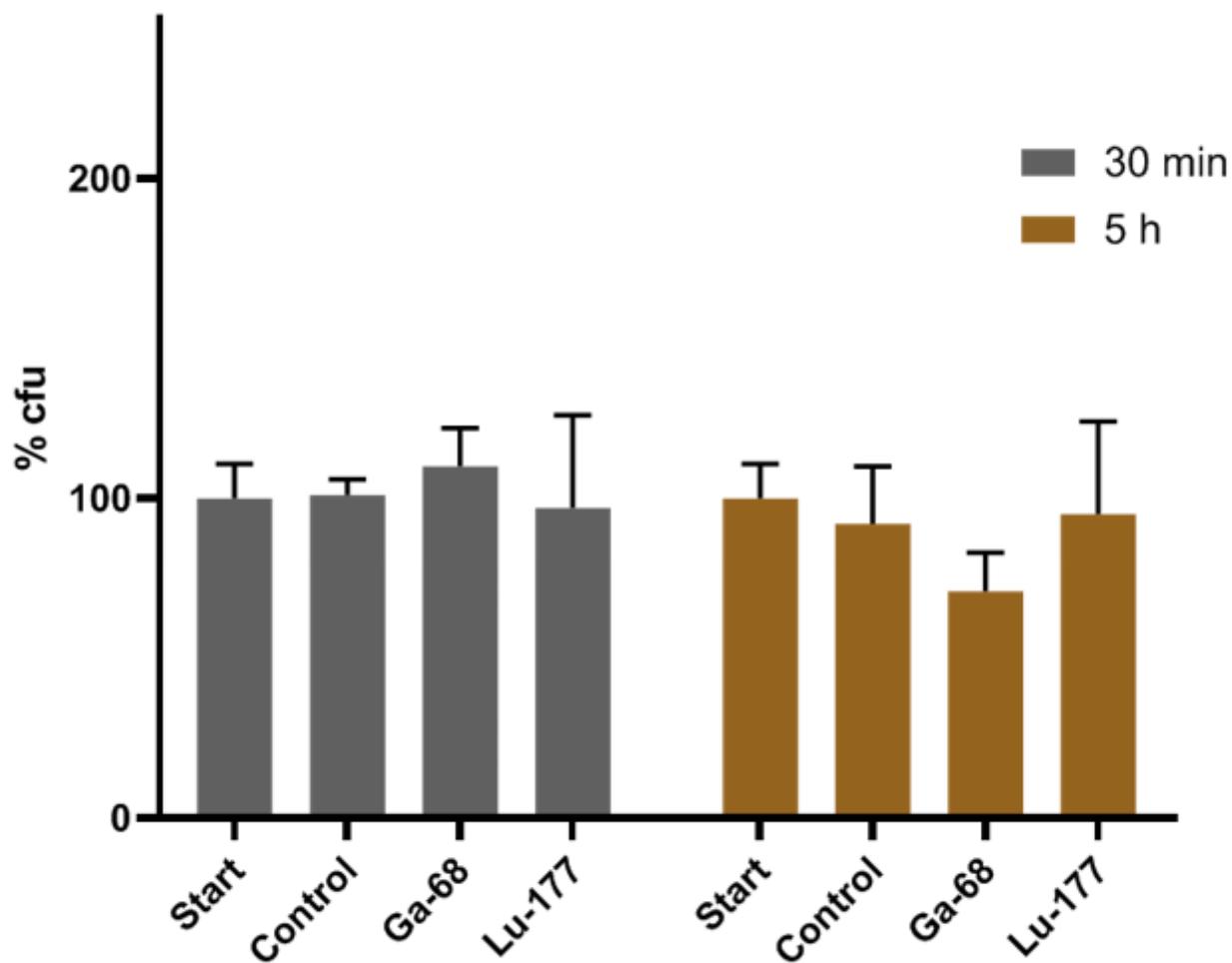


Figure 3

Bacillus pumilus: Results of 30 min incubation and 5 hours incubation; $n \geq 3$

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [PoetzschBrennerSpreckelmeyerSupportingInformation.docx](#)