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Summary of Experiments

Sample: SUPER-LUBE® MULTIPURPOSE GREASE WITH SYNCOLON® (PTFE)
Lot number 9923

PROCEDURE 1

Material and Methods.

Preparation of Microorganisms. E. coli ATCC 8739 was grown on a Trypticase Soy Agar (TSA) plate and was passed one time. Organisms from this second plate were used to prepare a 0.5 McFarland (approximately 1 x 10^8 CFU*/ml) suspension.

Enumeration of Microorganisms. Serial ten-fold dilutions of the 0.5 McFarland suspension were prepared using sterile saline. Duplicate 1.0 ml samples of the 1 x 10^{-6} and the 1 x 10^{-7} dilution were added to sterile Petri plates. Approximately 10 ml of Trypticase Soy Agar were added to each of the plates and allowed to solidify. Plates were incubated at 37^{o}C for 24-72 hours and read.

Sample Preparation. 10 ul of the 0.5 McFarland suspension were added to 9.99 gm of the sample. The sample was vigorously mixed in order to produce an emulsion. This emulsion was incubated at room temperature for the duration of the study.

Enumeration of Sample. Two dilutions of the emulsion were prepared, a 1/50 and a 1/1000. The 1/50 dilution was prepared by mixing 0.2 gm of the sample with 9.8 ml of sterile saline. The 1/1000 dilution was prepared by mixing 0.2 gm of the 1/50 dilution with 3.8 ml of sterile saline. Duplicate 1.0 ml samples of the 1/50 and the 1/1000 dilution were added to sterile Petri plates. Approximately 10 ml of Trypticase Soy Agar were added to each of the plates and allowed to solidify. Plates were incubated at 37^{o}C for 24-72 hours and read.

Procedure. Duplicate 1 ml samples of the 1/50 and 1/1000 dilutions were assayed immediately after preparation, and then after 24 hours, as described above. Based upon the results obtained, duplicate samples of 0.5 gm of the spiked emulsion were assayed at 7 days, and duplicate samples of the spiked emulsion and the 1/50 dilution were assayed after 14 days of incubation.

**Colony Forming Unit*

Results.

McFarland 0.5 Suspension: 1.5 x 10^8 CFU/ml

Time of Incubation	CFU/ml	Log Inhibition	Percent Inhibition
Initial Sample	7.1 x 10^4	0	0
1 Day	1.8 x 10^2	2.60	99.7
7 Day	0	>4.85	100
14 Day	0	>4.85	100

Discussion and Conclusions.

This experiment was based on USP 51 Antimicrobial Preservative Effectiveness Test, but was substantially modified. One part of a suspension containing 1.5 x 10^8 CFU/ml of E. coli ATCC 8739 was mixed with 999 parts of the sample. This resulted in a 1000 fold dilution of the microorganism with the test sample (Approximately 1.5 x 10^5 CFU/ml). This emulsion was immediately tested and found to contain 7.1 x 10^4 CFU/ml of the microorganism. This was a recovery of approximately 50%, and considering that this was an emulsion, and not a true mixture, was well within the range of expectations. Within 24 hours, this number had dropped to 1.8 x 10^2 CFU/ml indicating a reduction of over 99%.

In order to accurately ascertain the microbial count at the 7 day and 14 day test point, instead of assaying the 1/50 dilution and the 1/1000 dilution, duplicate 0.5 gm samples of the test emulsion were put into sterile Petri dishes and assayed. No organisms were observed. On day 14 a 1/50 dilution was tested and also showed no microorganisms.

The sample of grease does not support the growth of E. coli ATCC 8739. In addition it appears to be greater than 99% bacteriostatic or bacteriocidal after 1 day, and 100% bacteriostatic or bacteriocidal after 7 days.

PROCEDURE 2

Material and Methods.

Preparation of Microorganisms. E. coli ATCC 8739 was grown on a Trypticase Soy Agar (TSA) plate and was passed one time. Organisms from this second plate were used to prepare a 0.5 McFarland (approximately 1 x 10^8 CFU*/ml) suspension.

Enumeration of Microorganisms. Serial ten-fold dilutions of the 0.5 McFarland suspension were prepared using sterile saline. Duplicate 1.0 ml samples of the 1 x 10^{-6} and the 1 x 10^{-7} dilution were added to sterile Petri plates. Approximately 10 ml of Trypticase Soy Agar were added to each of the plates and allowed to solidify. Plates were incubated at 37^{o}C for 24-72 hours and read.

Sample Preparation. 0.5 gm of the sample were spread over the surface of a sterile Petri plate. 10 ul of the 0.5 McFarland suspension were placed on the surface of the grease. The sample was incubated for 5 days at room temperature. Approximately 10 ml of Trypticase Soy Agar were added to each of the plates and allowed to solidify.

10 ul of the 0.5 McFarland suspension were placed on the surface of a Trypticase Soy Agar plate to serve as a control.

Plates were incubated at 37^{o}C for 24-72 hours and read.

**Colony Forming Unit*

Results.

McFarland 0.5 Suspension: 1.5 x 10^8 CFU/ml

Based upon the assay above, the 10 ul sample that was spread over the surface of the Trypticase Soy Agar plate should have produced approximately 1.5 x 10^6 actual colonies. The result that was obtained

is shown in Figure 1. Although a few colonies can be seen, most of the plate is completely overgrown. This is consistent with a plate that had been inoculated with over 1 million CFU.

In contrast, as shown in Figure 2, approximately 30 well formed colonies can be observed. The large white area in the center is the grease. Although not shown well in the figure a few colonies were observed growing in the white area that the grease did not cover.

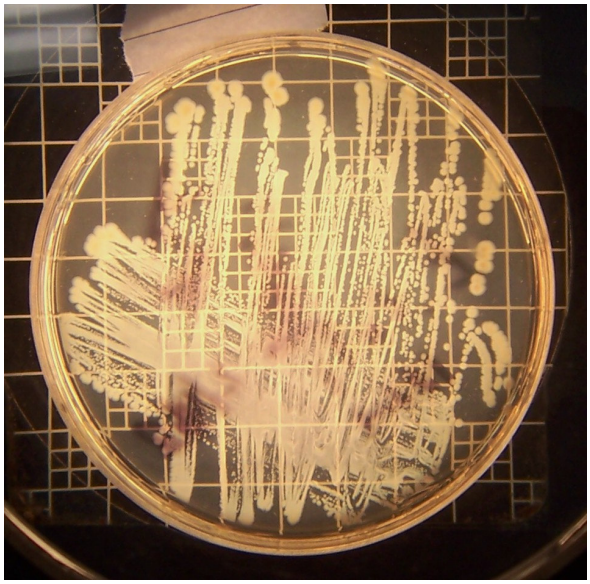

Figure 1. 10 ul of 0.5 McFarland Suspension

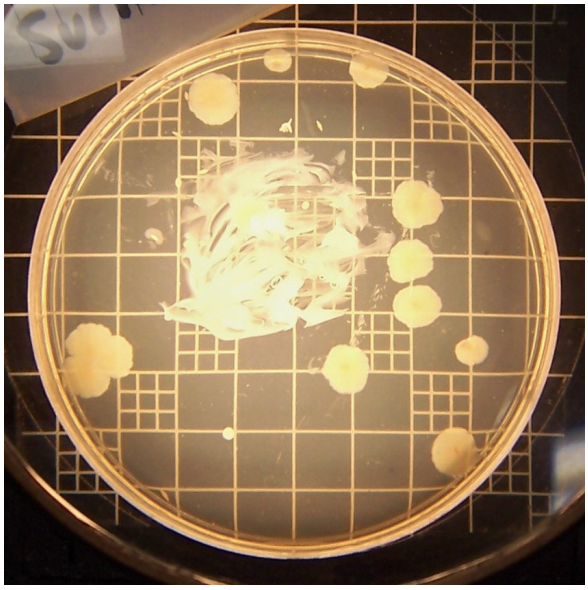

Figure 2 Grease Sample

Discussion and Conclusions.

This experiment was conducted to determine if microorganisms placed on the surface of a sample of grease could survive. As shown above after five days of incubation the number was reduced from approximately 1.5 million down to 30. This is a reduction of more than 99.99%. This result supports the findings in the first set of experiments.

Based on these observations SUPER-LUBE® MULTIPURPOSE GREASE WITH SYNCOLON® (PTFE) does not support the growth of E. coli. The results also indicate that in addition to not supporting the growth of these organisms, following contamination the grease substantially reduces the viability of the microorganisms within 24 hours and eliminates their viability within seven days.

Reviewed by: Lorrence H Green

Lorrence H. Green, Ph.D.

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