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The difficulty in applying the gene-for-gene hypothesis to more complex host:parasite systems does not preclude its application to such systems. This hypothesis contributed greatly to understanding the genetics of interactions in the less complex systems (such as flax and flax rust) and, as additional data accumulate on the genetics and physiology of complex host:parasite systems, it will undoubtedly facilitate understanding of these systems also.

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Media Sterilization With Propylene Oxide

ROBERT C. GOSS AND JACK L. MARR[1]

Abstract: Sterilization of potato dextrose agar against bacteria air contaminants occurs during the 6th hour of exposure and against fungi in the 4th hour. The fungicidal activity was broader than the bactericidal activity of propylene oxide. Direct application of propylene oxide to Petri plates containing PDA was ineffective. With plastic plates a chemical reaction took place between the chemical and the plastic. In a closed system sterilization of the plates and medium was accomplished at approximately 1.25 ml of propylene oxide per liter of volume. The addition of propylene oxide directly to nutrient broth effected 90% sterility under certain conditions.

The use of propylene oxide as a sterilizing agent for various types of biological products (1, 2, 3) suggests that it could be used for field sterilization of microbiological media or be useful in high-school and college laboratories where sterilizing equipment is absent or inadequate. According to Hansen (4) there is very little physical-chemical alteration of organic substances which have been exposed to propylene oxide. A disadvantage is that the vapors are highly flammable in low concentrations. This experiment was designed to determine if an inexpensive, effective method of sterilization with propylene oxide could be developed.

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Procedure

Difco dehydrated potato-dextrose agar (PDA), nutrient broth or nutrient agar was used in all experiments. Studies on exposure time versus sterilization were made with PDA. Melted, cooled agar was poured into sterile glass Petri plates and exposed to atmospheric contamination for 30 minutes. The plates along with 6 ml of propylene oxide (Distillation Products Industries) were placed in 4-L cylindrical battery jars with glass plate covers. The covers were sealed with Fisher Cell-o-seal. The plates were removed from the chambers at hourly intervals and incubated at room conditions for 48 and 96 hours. Fifteen plates were used to obtain data for each time interval. Bacteria and fungi colony counts were made at their respective time intervals by the Wolffhuegel method.

During the preliminary phase washed, non-sterilized plastic or glass Petri plates were used. The medium was cooled and poured into the plates and exposed to atmospheric contamination for 30 minutes. Known amounts of propylene oxide were placed directly on the surface of the medium with a 2 ml Luer syringe equipped with a 27 gauge 1½" needle. The syringe was directed so that a random distribution of the chemical was obtained.

A study to compare the efficiency of a closed system versus an open system for sterilization of nonsterile medium and plates was made. Known amounts of propylene oxide were placed in 32 ml glass specimen vials. The chemical and the Petri plates were then handled the same as the procedure outlined for the closed system versus exposure time.

The experiments were conducted at room conditions. The plates were considered exposed for twenty-four hours. The evaporation factor of the particular amount of the chemical would determine the actual length of exposure. Five Petri plates were used for each quantity of propylene oxide in an individual jar and each treatment was duplicated five times. Controls consisted of sterile Petri plates and media exposed to atmospheric contamination for 30 minutes and either incubated in the battery jar or at room conditions without propylene oxide.

A series of experiments were devised to investigate the effectiveness of propylene oxide in sterilizing nutrient broth as influenced by temperature. The temperature variables over a period of 96 hours were refrigeration, room condition, and 37°C. Amounts of 0.3 ml and 0.4 ml of propylene oxide per 5 ml of nutrient broth were added to the tubes using the Luer syringe. Immediately thereafter, the tubes were plugged with cotton stoppers and were shook for 30 seconds before incubation. Sixty tubes were used for each amount and temperature condition.

During the first 48 hours of the experiments of high and low temperature the tubes were incubated at the specified temperatures. For the last 48 hours the tubes were kept at room conditions. Growth data was obtained every 24 hours.

Discussion

PDA is capable of supporting the growth of bacteria and fungi. The fungi colonies appeared between 48 and 96 hours and the bacteria colonies appeared within 24 hours at room conditions. Within 2 hours of exposure there is a drop of 19% in the number of bacteria colonies (Figure 1). After 2 hours exposure there is a straight line correlation until the 6th hour. Sterilization of bacteria air contaminates occurred sometime during the 6th hour of exposure. Sterilization of the medium against fungi was achieved during the 4th hour. The results indicate that the fungicidal activity of propylene oxide is broader than the bactericidal activity.

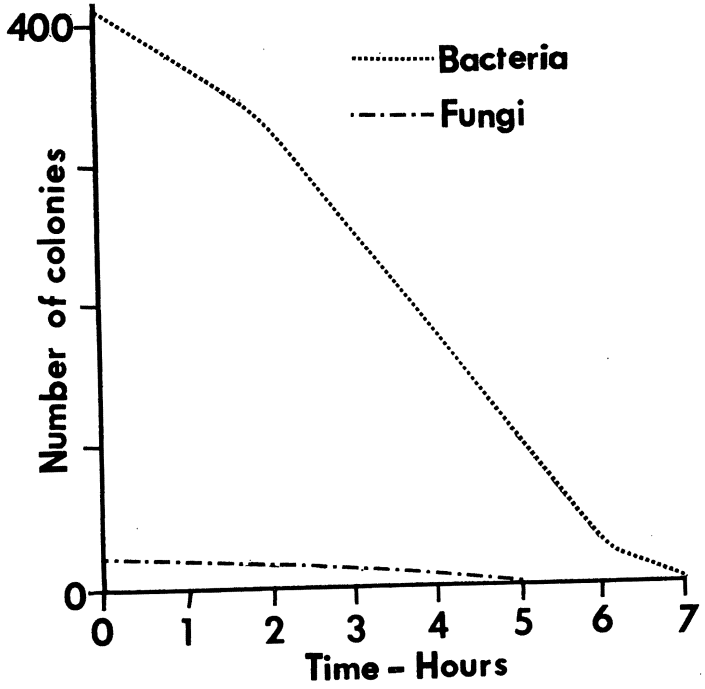

Figure 1. Sterilization of PDA against fungi and bacteria air contaminates versus hours of exposure to propylene oxide.

Qualitative differences in plate sterilization were measured by an inhibition ratio, the formula for which was:

$$\text{Inhibition ratio} = \frac{\text{Number of colonies on treated plates}}{\text{Number of colonies on untreated plates.}}$$

The inhibition ratio is a figure comparing the lethal effect of certain amounts of propylene oxide to the untreated control at a specific time interval. A number greater than 1 would imply either stimulation or heavy contamination with little effect by propylene oxide and a number less than 1 would imply either disinfection or sterilization. A low ratio (less than 1) but with a numerical increase would imply microbialstatic phenomenon.

The analysis of inhibition in relation to the time interval after exposure, the amount of propylene oxide used, and the system used for sterilization indicated that, in general, plate application was ineffective. At 72 hours all treatments used in the plastic plates, except 0.4 ml, had more colonies than the control plates. In addition a chemical reaction took place between the chemical and the plastic plates. The plates were etched on the inside wall above the agar surface.

The analysis indicated that the data obtained with non-sterile glass plates was in opposition to the results of Thompson and Gerdemann (5), who found that the addition of 1 ml of propylene oxide in glass plates was sufficient for sterilization. There were 2 layers of organisms in the washed, non-sterile plates. Numerous colonies of a fast spreading organism appeared on the bottom of the plate within 24 hours. Also, the normal atmospheric contamination appeared on the surface of the medium. Three different methods of washing and 3 different household cleaning agents were used to cut down on contamination. The excessive growth was observable regardless of the method used.

Sterilized plates with non-sterile PDA were used to reduce the growth of the bottom flora. A bacteriostatic effect was noticeable after 24 hours but then a disinfection process was observed. The physical conduct of propylene oxide is such that it lacks penetrating power except in a closed system. In a Petri plate the propylene oxide diffuses upward and out. In a closed system the chemical distributes itself through the volume until an equal density is reached. The chemical properties of propylene oxide are such that water or some other product would be formed when it came into contact with the water of the medium.

Propylene oxide sterilization in a closed system was accomplished when the amount approached 5 ml (Table 1). At the 1 ml level a static trend was noticeable. In the dosage range of 2 ml to 4 ml the level of non-viable organisms approached more than 99% when exposed for 24 hours. Under the conditions of this experiment 12 hours were required for complete evaporation of 6 ml of propylene oxide.

The test run with propylene oxide added directly to nutrient broth indicated that none of the conditions would effect 100%

Table 1. The effectiveness of propylene oxide in a closed system using non-sterile Petri plates and PDA. Sterilization determined by the inhibition ratio.

Amount of propylene oxide	Time (Hours after exposure) 0	24	48	72
1.0 ml	.009	.470	1.030	1.040
2.0 ml	.009	.001	.010	.047
3.0 ml	.000	.000	.002	.016
4.0 ml	.000	.000	.000	.001
5.0 ml	.000	.000	.000	.000

Table 2. Percentage of 5 ml nutrient broth tubes showing growth after direct application of propylene oxide and incubated at refrigerator conditions, room conditions and 37°C.

Temperature condition	Amount of propylene oxide	Time (Hours of incubation) 24	48	72	96
Refrigerator	0.0 ml	13%	13%	100%	100%
	0.3	14	14	24	96
	0.4	14	14	22	69
Room conditions	0.0	98	100	100	100
	0.3	8	22	50	65
	0.4	12	16	25	50
37°C	0.0	100	100	100	100
	0.3	0	20	30	40
	0.4	3	10	10	10

sterility (Table 2). The apparent arithmatic growth increase as incubation time increased regardless of the amount of propylene oxide used was probably due either to the propylene oxide becoming inert or to a diffusion problem. The 90% sterility obtained with 0.4 ml of propylene oxide at 37°C would be attributed to the kinetic energy of propylene oxide at that temperature.

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Influence of Mutable Genes on Induction of Instability in Maize[1]

PETER A. PETERSON

Abstract. Mutable systems possess transposable elements that cause mutability of associated gene loci. The *En* system (composed of *I* and *En*) was used to test the induction of mutability of two selected loci, A_2 and *C* possessing the normal alleles. In a test of over five million gametes one mutable was found at each of the selected sites as well as two others, both dominant and non-allelic to the selected sites.

It appears that this low rate of induction of mutability (as compared to other reports) is a consequence of the non-randomness of site selection; i.e., a certain physical chromo-

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