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Radiation Sterilization of Two Commonly Culture Media Used for Bacterial Growth

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ABSTRACT

Radiation sterilization of culture media used for the cultivation of bacteria by Co-60 gamma ray was investigated. Nutrient agar and tryptone glucose yeast extract (TGY) media widely used for the propagation of bacteria were sterilized with 15 kGy dose gamma radiation. Seven different bacterial species were grown as well on the radiation sterilized media as on media sterilized by autoclaving in a conventional way.

Key Words: Culture Media/ Bacterial Species/ Radiation Sterilization

INTRODUCTION

Gamma irradiation is today an accepted method of sterilization for petridishes, test tubes, syringes, dressing and other medical devices that can not easily be made germ free in other ways. The possibility of sterilizing culture media for the growth of microorganisms by gamma radiation were investigated by Becking [(2)], Altmann *et al.* [(1)], Bogokowsky and Altmann [(3)], and Li *et al.* [(4)]

It was reported by Altmann *et al.* [(1)] and Bogokowsky and Altmann [(3)] that gamma radiation offers an attractive and economical way of sterilizing media in sealed plastic containers, eliminating the need to sterilize media and container separately and avoiding the risk of contamination during processing. Such media may be kept for long periods in the laboratory and are always ready for use.

Yang and Xiao [(5)] and Li *et al.* [(4)] found that using Co-60 gamma ray to sterilize the cultural materials of edible fungus is a secure and saving labor and energy new method which could sterilize thoroughly.

In this study gamma radiation from Co-60 was used for sterilization of nutrient agar and tryptone glucose yeast extract agar (TGY) media. The growth of seven bacterial species on these sterilized media was studied in comparison with their growth on the same media sterilized by autoclaving.

MATERIALS AND METHODS

Media

Nutrient agar and tryptone glucose yeast extract agar (TGY) media were prepared according to Oxoid Manual used as a control where they were sterilized by autoclaving. Petridishes and tubes position to get a slant were prepared in a conventional way. Media prepared for sterilization by irradiation were poured into plastic petridishes and plastic tubes, cooled for 30 minutes with covers removed, closed and sealed in polyethylene bags. Irradiation was carried out 3 h later. The glassware and water which were used were not sterile and all the preparations were carried out under unaseptic conditions (Altmann and Bogokowsky [(1)] and Bogokowsky and Altmann [(3)]).

Irradiation

The media were irradiated in batches of five Petridishes and five plastic tubes at 2,4,6,8,10,12,14 and 15 kGy at a dose rate of 0.11 kGy/min.in the research gamma irradiation facility in the National Center for Radiation Research and Technology (NCRRT).

Storage: Irradiated petridishes and test tubes were kept at room temperature and at 35ºC for 14 days.

Inoculation

Seven bacterial species were used to test their growth in the irradiated and autoclaved media. These species were listed in tables (2&3).All bacterial species used were subcultured and incubated at 35ºC for 24 h. Appropriate dilutions were made from each culture and plates were inoculated with 0.1 ml. Size, morphology and number of colonies were recorded for irradiated and control plates after incubation at 35°C for one and two days (Altmann and Bogokowsky [(1)] and Bogokowsky and Altmann [(3)]).

RESULTS AND DISCUSSION

Results tabulated in tables 1, 2 and 3 showed that radiation doses of 2,4,6,8,10 and 12 kGy were not sufficient to sterilize the two used media but the two doses 14 and 15 kGy were found to be sufficient for sterilization of both media nutrient agar and tryptone glucose yeast extract agar. Altmann *et al.* [(1)] and Bogokowsky and Altmann [(3)] showed that a radiation dose of 7.5 kGy achieved the sterilization of triple sugar iron agar and Mueller Hinton medium respectively. The sterilizing dose of 15 kGy which was eventually chosen seems rather high. However, since level of contamination prevailing during the process of preparing media are unknown and bacteria and spores vary considerably in their radioresistance, this dose was chosen in order to achieve sterility under adverse conditions. This in agreement with Altmann and Bogokowsky [(1)] .Li *et al.* [(4)] found that the optimum doses of sterilization for five kinds of cultural materials of edible fungus were 22 kGy for saw dust, 26 kGy for cottonseed shell corncob, sorghum shell and vinegar dross. The dosages of sterilization accords in general with information which stated that the lethal dose of various bacteria ranges from 10-50 kGy. Using high dose of irradiation of Co-60 gamma rays cause degradation of large molecules of living beings to small ones which can be utilized by edible fungus to promote the growth of hypha and hence to increase the biological amount of the product.

The effect of radiation on the PH of the medium was tested a few hours after radiation and after storage at room temperature and at 4°C. Radiation did not cause significant changes in the PH of the medium even after storage at both temperatures. This finding accords with Bogokowsky and Altmann [(3)] for Mueller Hinton medium. But Altmann and Bogokowsky [(1)]) found that the irradiated triple sugar iron agar medium was slightly more acid after irradiation during storage for 4 months at room temperature and at 4°C.

Radiation in this experiment did not cause softening of the agar. Altmann and Bogokowsky [(1)] observed that a change in hardness of triple sugar iron agar after irradiation which became more pronounced as higher doses were used. This softening was probably due to partial degradation of the polymeric structures of the agar but in the range of the sterilizing dose and up to 20 kGy it had no deleterious effect and a durable slant was formed by triple sugar iron agar medium with these doses without any addition of agar. It was found also by Bogokowsky and Altmann [(3)] that the radiation caused softening of Mueller Hinton agar medium, but no difficulties were encountered in the seeding of bacteria.

Table(1): Visual observations of microbial growth on the irradiated and autoclaved media.

Days of storage	Autoclaved media	Irradiated media (Dose in kGy)							
		2	4	6	8	10	12	14	15
2	–	+	+	+	–	–	–	–	–
4	–	+	+	+	–	–	–	–	–
6	–	+	+	+	–	–	–	–	–
8	–	+	+	+	+	+	–	–	–
10	–	+	+	+	+	+	–	–	–
12	–	+	+	+	+	+	+	–	–
14	–	+	+	+	+	+	+	–	–

– = negative bacterial growth + = positive bacterial growth

Table(2): Growth of various bacterial species on irradiated nutrient agar medium compared with autoclaved one of conventional composition

Bacterial species	Dilution	Number of counted colonies		
		Autoclave	Radiation (Dose in kGy)	
			14	15
1. *Bacillus cereus*	2×10^{-7}	143	130	128
2. *Bacillus subtilis*	2×10^{-7}	160	144	141
3. *Micrococcus luteus*	1×10^{-6}	88	72	70
4. *Salmonella typhinurium*	1×10^{-6}	92	77	74
5. *Pseudomonas aeruginosa*	1×10^{-6}	75	60	57
6. *Staphylococcus aureus*	2×10^{-6}	100	82	78
7. *Escherichia coli*	1×10^{-6}	110	91	85

Table(3): Growth of various bacterial species on irradiated tryptone glucose yeast extract agar (TGY) medium compared with autoclaved one of conventional composition

Bacterial species	Dilution	Number of counted colonies		
		Autoclave	Radiation (Dose in kGy)	
			14	15
1. *Bacillus cereus*	2×10^{-7}	140	124	122
2. *Bacillus subtilis*	2×10^{-7}	162	142	140
3. *Micrococcus luteus*	1×10^{-6}	85	65	61
4. *Salmonella typhinurium*	1×10^{-6}	88	71	68
5. *Pseudomonas aeruginosa*	1×10^{-6}	73	55	52
6. *Staphylococcus aureus*	2×10^{-6}	105	88	84
7. *Escherichia coli*	1×10^{-6}	102	77	73

CONCLUSION

It can be concluded t hat media sterilized by gamma irradiation in plastic tubes or petridishes, storable for long periods and always ready for use in the laboratory, seem to be an attractive alternative to autoclaved media.

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