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EXPERIMENTS FOR TRANSPLANTABLE MULTIPLICATION OF M. LEPRAEMURIUM IN CELL-FREE LIQUID MEDIUM

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Several investigators have confirmed that M. lepraemurium (Mlm) multiplies on primary inoculation in NC-5 medium (1, 2), but that growth ceases after 8 weeks of cultivation at 30C. Quantitative multiplication of bacilli could not be recognized on transfer, i.e., when the bacilli grown in NC-5 medium were passed to freshly prepared NC-5 medium by a routine bacteriologic procedure.

In this paper, three separate experiments concerning the problems related to the incompleteness of NC-5 medium are described.

METHODS

Mlm: The Hawaiian strain (M-59) obtained from a leproma experimentally produced in the subcutaneous tissues of a C_3H mouse was used.
Estimation of the growth of Mlm: The growth of Mlm was estimated by a bacillary counting method and a slide culture method.
Culture medium: The original NC-5 medium, and a newly established culture medium which is referred to as ND-5 medium were used. The composition of the ND-5 medium is given in the experimental results.

RESULTS

1. Subcultivation of Mlm by transfer of a glass slide.

As mentioned previously at the last year's meeting in Kyoto, progressive and continuous multiplication of Mlm can be observed under the limited conditions. When the slide with the bacterial smear was transferred to freshly prepared NC-5 medium at a definite interval, continuous multiplication of Mlm was observed. However, no multiplication was noted when the bacterial mass were scraped off from the slide, resuspended, and inoculated to new medium.

These results indicate that bacterial mass is necessary for further continuous development of Mlm in NC-5 medium. The same fact was noted with Ogawa's system.

2. Elongation and multiplication potential of Mlm in NC-5 medium.

In order to determine the factors influencing potential for elongation and multiplication of Mlm in NC-5 medium, the starting bacterial suspensions were treated with heat, ultraviolet irradiation (u-v) and acid-alkali. The results (Table 1) obtained indicate that the potential for elongation and multiplication in NC-5 medium was completely destroyed by just 2.5 minutes of u-v irradiation. However, no loss of leproma producing ability was observed after subcutaneous inoculation into mice of the irradiated material.

On the other hand, the potential for elongation and multiplication was relatively stable to heat and pH treatment and the loss paralleled the loss of leproma producing ability. These capabilities were lost by heating at 50C for 30 min. and by treating with pH 5 at 37C for 60 min.

Therefore, intactness of DNA molecular structure is the most important factor for elongation and multiplication of Mlm in cell free culture medium. In other words, it is strongly indicated that DNA repair of Mlm cannot take place in the NC-5 medium, whereas it does in vivo.

Table 1.

Potentiality of elongation - multiplication, and leproma production

UV irradiation *	in vitro		in vivo
	Elongation	Multiplication	Leproma production
None	+++	++	+++
2.5 min	-	-	
5.0 "	-	-	
7.5 "	-	-	
10.5 "	-	-	
15.0 "	-	-	+++
30.0 "	-	-	+++
45.0 "	-	-	
60.0 "	-	-	+++

* Distance : 15 cm

Table 2.

Potential for elongation and multiplication and for leproma production.

pH*	In vitro		In vivo
	Elongation	Multiplication	Leproma production
3	++	-	-
4	++	-	-
5	++	-	-
6	++	+	+
7	+++	+++	+++
8	+++	+++	+++
9	+	++	+++

* treated at 37C for 60 min.

3. A new culture system for rapid growth of Mlm.

Recently, I established a new submerged culture system for Mlm. It was named the ND-5 medium (3). The composition of ND-5 medium is indicated in Table 3. As shown in the Table, Dubos medium is employed as the basal medium instead of the Kirchner medium used in the case of NC-5 medium.

Table 3.

Composition of NC-5 and ND-5 medium.

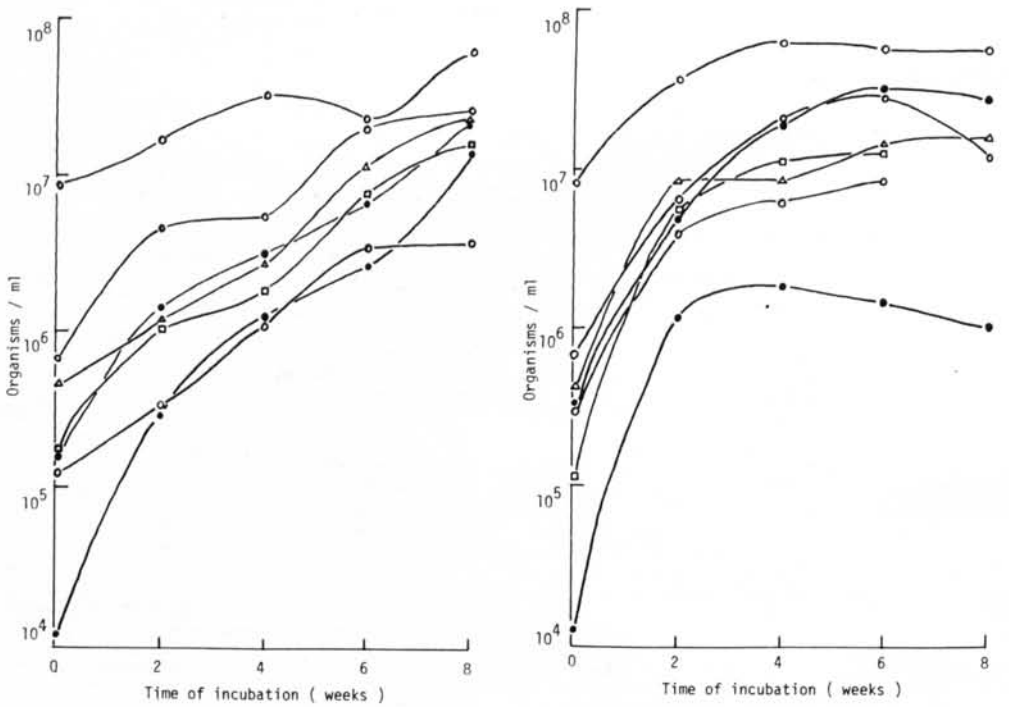
	NC-5	ND-5
Basal medium	Enriched Kirchner medium (0.4% glucose) (0.5% pyruvate) 0.01% Ca. pantothenate pH 7.3 after autoclaving	Dubos medium (11.3 gr/900 ml Aq) pH 7.3 after autoclaving
Additives	Goat serum 2% α-ketoglutaric acid 0.1% cytochrome c 0.08% hemin 0.3% l-cysteine HCl to 5 vol of basal medium	1.0 vol 0.5 vol 1.0 vol 0.5 vol 0.2 vol

When 0.1 ml of a suspension of M1m was inoculated into ND-5 medium, the bacterial cell elongated about two times, and the number of bacilli rapidly increased. The bacterial increase was approximately 10-30 times after one week of cultivation.

The summarized growth curves in ND-5 medium and NC-5 medium obtained in several experiments are illustrated in Fig. 1. The log phase of the growth curve is steeper in ND-5 medium than in NC-5 medium. Possible generation times of M1m in ND-5 and NC-5 medium may be calculated as 1.4-2.6 days and 4-5 days, respectively.

Fig. 1.

Growth curves of M1m cultivated in NC-5 (left) and ND-5 (right) medium.



Furthermore, the mode of growth in ND-5 medium is quite different from that in NC-5 medium, that is, in the case of ND-5 medium, M1m divided in the mode of typical binary fission. On the other hand, in the case of NC-5 medium, as previously shown, the cell elongated extraordinarily and finally divided after budding and branching.

Typical growth patterns in ND-5 and NC-5 medium are illustrated in Fig. 2. The average length of cells in ND-5 and NC-5 calculated from these results was 6.25 and 5.59 μ, respectively. In particular, the range between minimum and maximum length was 2 to 13 μ in ND-5 medium and 1 to 23 μ in NC-5 medium, respectively. It is strongly suggested from these growth patterns and length differences that most of the single cells cultivated in ND-5 medium divided individually and multiplied at the same time.

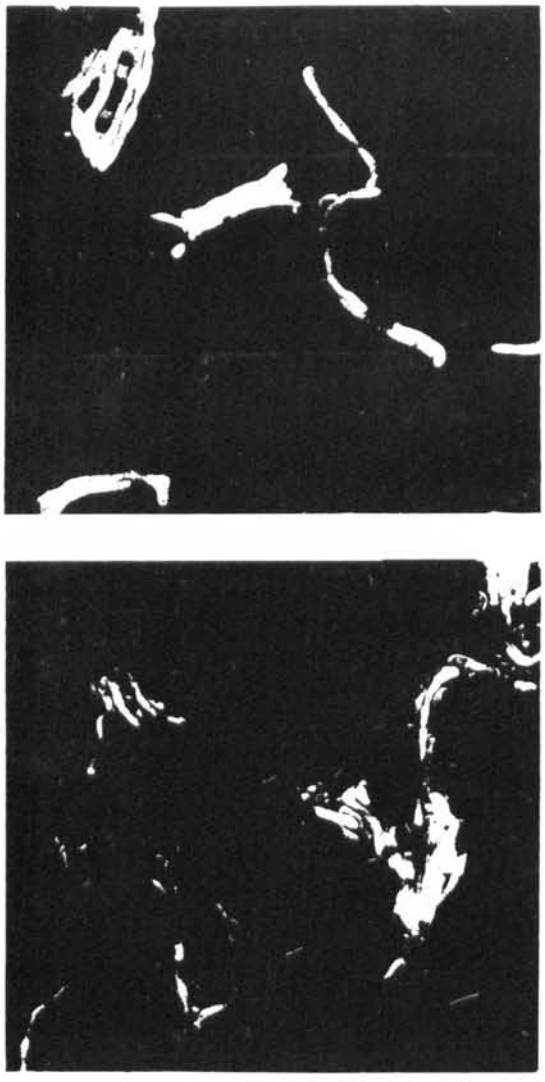
Fig. 2.

Growth patterns of Mlm cultivated in NC-5 (left) and ND-5 (right) medium at 30C for 16 days.

In addition, morphological examination of cells cultivated in ND-5 medium by electron microscopy revealed them to be remarkably more solid than those in NC-5 medium, as shown in Fig. 3.

Fig. 3.

Electron micrographs of Mlm of submerged growth in ND-5 medium.



From the results obtained, the differences between ND-5 medium and NC-5 medium could be summarized as follows.

Table 4.

Differences between NC-5 and ND-5 medium.

Medium	NC-5	ND-5
Basal medium	Kirchner	Dubos
Elongation	Extraordinary	Measurable
Average length* (minimum-maximum)	5.59 μ (1-23 μ)	6.25 μ (2-13 μ)
Generation time	4-5 days	1.4-2.6 days
Growth mode	Budding, branching	Binary fission
Cell morphology	Non-solid	Solid

* Average length of a starting material: 2.3 μ

However, the problem concerning subcultivation of Mlm still remains with ND-5 medium.

DISCUSSION AND CONCLUSION

Subcultivation of Mlm in NC-5 medium can only be performed under limited conditions. Intactness of the DNA molecular structure of the Mlm cell is necessary for elongation and multiplication in NC-5 medium. These findings might indicate that Mlm has a very weak growing ability in vitro, and that NC-5 medium is not sufficient as an artificial culture medium for Mlm. ND-5 medium, which is a modified NC-5 medium, is described here. The ND-5 medium is better than the NC-5 medium on the basis of general bacteriologic knowledge. Therefore, ND-5 medium would be more suitable and profitable than NC-5 medium for studies on quantitative multiplication of Mlm.

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