<Original article>

EFFECTS OF ULTRAVIOLET LIGHT AND NUCLEIC ACID DERIVATIVES ON THE REPRODUCTIVE RATE OF AZOTOBACTER※

by

LEE, Min Jai and LEE, Young Nock

Dept. of Botany, Seoul National University

李敏載·李永祿 : Azotobacter의 增殖率에 미치는 紫外線 및 nucleic acid derivatives의 影響

ABSTRACT

LEE, Min Jai and LEE Young Nock (Seoul National U. Seoul Korea)— *Effects of ultraviolet light and nucleic acid derivatives on the reproductive rate of Azotobacter* Kor Jour. Bot., 3(2): 1—5. Illus. 1960 - Azotobacter is isolated from soil and its purified species is identified as *A. chroococcum*. The survival rate of Azotobacter irradiated with UV light is measured, and the reproductive rates of the survivals are calculated. In general, not only the survival rate, but also the length of the generation time of the survival progeny is inversely proportional to the irradiated dose of UV light. The reproductive rate of Azotobacter is increased with the exogeneous treatment of nucleic acid derivatives.

INTRODUCTION

Although the mutagenic effects of UV light have been studied by many investigators, these studies were mainly on the gene analysis of the inherent characters, and there have been no extensive studies on the effects of mutagene to the gene population of the individual, in connection with the protective problem.

If it is true that the inherent character reveals as a result of the metabolism, and that "inheritance is characterizable in terms of transmission of control of relative rates of biochemical reactions within complex and interlocked metabolic patterns",[1] it is probable that the long or short of the lifespan of an individual, which can be demonstrated as a result of the extensive and correlative reaction velocity in the metabolism, depends upon the result of the active collaboration of the totality of genes that together make the individual a functioning whole.

In these points of view, to clarify how to reveal the action of the mutagene to the gene populations of an individual under the constant circumstances, this work has been made of the effects of UV light on the reproductive rate of Azotobacter which is isolated from the soil by author himself. And the study has been also made on the variation of the generation time resulted from the exogeneous treatment of nucleic acid derivatives to clarify the phenotypic variation under the different circumstances.

ISOLATION AND IDENTIFICATION OF MATERIAL

The material is isolated from the soil, collected at Kwangnaru, Korea, July, 1959. The procesure of isolation is followed by Beijerink's method,[2] and it is purified by plane agar method.[3] The method for the identifica-

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Table 1: Identification Test of Azotobacter chroococcum

TESTED NETHODS	MAIN CHARACTERISTICS	IDENTIFICATION
Iodine staining	red 3-6u in length	concord with the characteristics of *Azotobacteriaceae*
Gram staining	negative	〃
Mannitol broth culture	white sediment	〃
Gelatin stab	no liqufication	〃
Litmas milk	becoming clear in 10 to 14 days	〃
Mannitol salt agar	no immadiate color change	〃
Mannitol colony	round shape, white color later became brownish	concord with the characteristics of *A. chroococcum*
Nutrient broth	no growth	〃
PH test	no growth in acidic media	〃

tion test of Azotobacter and the main characteristics of it are summaried in table 1. It is distinguished from *A. agilis* in that there appear brownish colour in old culture, and that it does not grow in nutrient broth. And it is not *A. indicum* for it does not grow in acidic media below pH 3.0. As a result of these it is identified as *A. chroococcum*.[4]

METHODS

Azotobacter chroococcum was used as materials in this study. It was suspended to be about 10^8 bacterial numders per cc in 0.85% saline solutions from agar culture. Each 0.3 cc of this suspension was transferred and spread evenly in petri dish and was irradiated with UV light.

The irradiations were performed by means of a Mazuda's germicidal lamp from which above 90 percent of ultraviolet radiation was in the region of 2537 A. The lamp was operated 15 minutes before exposing cells at a distance of 20 cm: the potential was maintained at 100 Volt.

The irradiations were conducted at room temperature and photoreactivation[5],[6] was minimized in this work, not irradiating with visible light during the irradiation period.

The composition of culture media used in this work shows in table 2, and the pH of media was ajusted at 7, 8. Irradiated Azotobacters were inoculated in 30 cc liquid media in 200 ml capacity Erlenmeyer flask, and cultured in light chamber maintained 28° C.

Table 2: Chemical composition of media

Media	Composition	
Basal Medium (Lipman)	Mannitol	15g
	k_2HPO_4	0.2g
	$MgSO_4$	0.2g
	$Cacl_2$	0.02g
	$Fecl_3$ (10%)	1 drop
	Distilled water	1000 cc
DNA Derivatives-enriched Medium	Deoxycytidylic acid	10mg
	Deoxyadenylic acid	10〃
	Thymidine	10〃
	Deoxyguanosine	10〃
	Deoxycytidine	10〃
	Deoxyabenosine	10〃
	Deoxyguanylate-Ca	10〃
	Calcium thymidylate	10〃
	Basal Medium	1000 cc

RNA Derivatives-enriched Medium	Cytidylic acid	10mg
	Adenylic acid	10〃
	Uridine	10〃
	Guanosine	10〃
	Cytidine	10〃
	Adenosine	10〃
	Guanylate-Na	10〃
	Uridylic acid	10〃
	Basal Medium	1000 cc

The calculation of the survival numbers of Azotobacter irradiated with UV light was performed by counting the numbers of the colony formed in agar media of petri dish, in which the irradiated materials were inoculated by dilution method. And the bacterial numbers were calculated by Thoma's Hemacytometer when the culture proceeded above 10^6 bacterial numbers per CC.

If one assumes that the number of Azotobacter (a) in the inoculum become b for any generation thereafter, the formular becomes $b=ax2^n$, where n is the number of generations. When the generation time is constant, one can determine the average generation time during the same interval by substituting for n in the equation $G=t/n$, where G is generation time and t is time elapsed:

$$G=\frac{t.\log 2}{\log b-\log a}$$

To determine the generation time of Azotobacter in logarithmic growth phase, above equation is used.

EXPERIMENTAL RESULTS

Survial rate of Azotobacter by UV light irradiation shows in fig. 1. In general, it seems that the survival rate of Azotobacter decreases as the irradiated dose of UV light increases, and the lethal effect of UV light does not decreases even in the RNA derivatives or DNA derivatives enriched media.

Although the lethal rates of Azotobacter by irradiation increases at the same proportion of irradiated dose, the culturing period for the constant bacterial turbidity varies irregularly with the irradiated dose. These phenomena demonstrate that the reproductive rate varies with the irradiated dose, and the actual result of calculated reproductive rate of Azotobacter shows in fig. 2—4. As the fig. 2 is showing, the reproductive rates of Azotobacter in logarithmic growth phase increase at the same proportion of irradiated dose of UV light.

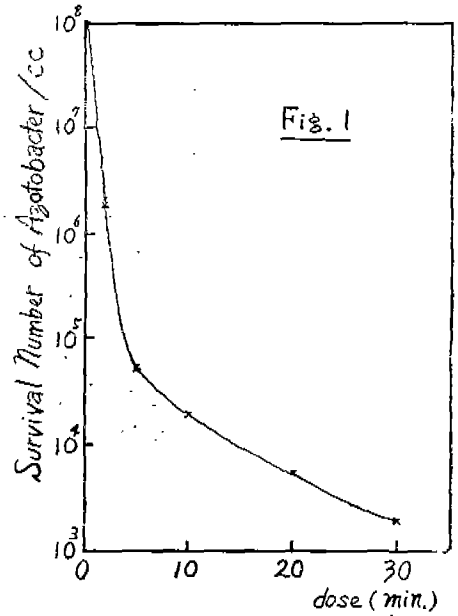
Table 3: The Variation of Generation Time of Azotobacter in the Logarithmic Growth Phase under the Different conditions. (hours)

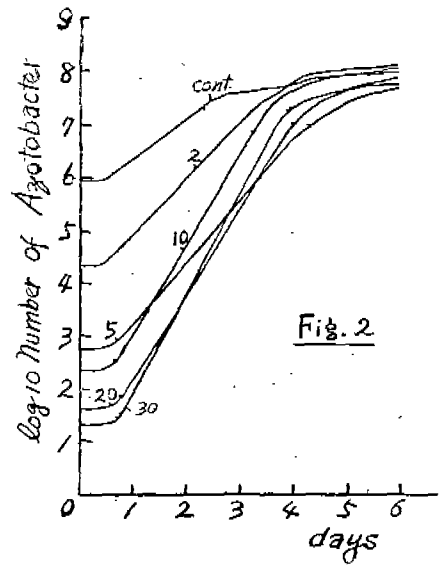
Media \ Dose of U-V Light (min.)	Cont.	2	5	10	20	30
Basal	9.30	7.20	6.19	4.33	4.17	3.90
RNA derivatives-enriched	7.94	5.77	5.41	3.61	4.33	3.41
DNA derivatives-enriched	6.50	5.49	4.49	3.21	3.05	2.62

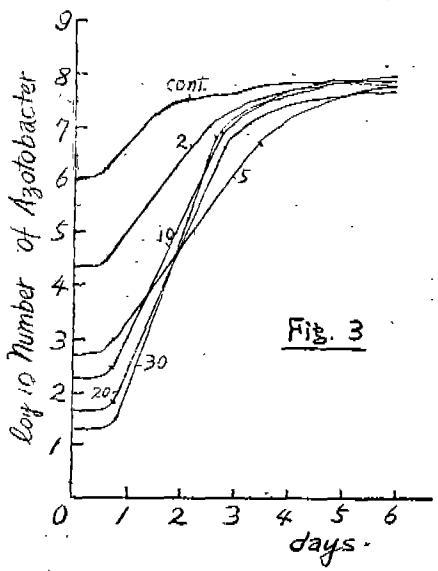
The generation time of Azotobacter in the logarithmic growth phase under the different circumstances is tabulated in table 3. It is shortened as the dose of UV irradiation increases, especially in DNA derivative or RNA derivatives enriched media rather than in basal medium.

DISCUSSION

The result of the survival rate of Azotobacter by the UV light irradiation is somewhat similar with the result of Demerec and Latarjet[7] in *E. coli*. Although the survival rates of Azotobacter do not vary in the DNA derivatives or RNA derivatives enriched media this, of course, does not mean that the survival rates of Azotobacter do not vary, even though any amount of nucleoside are enriched.







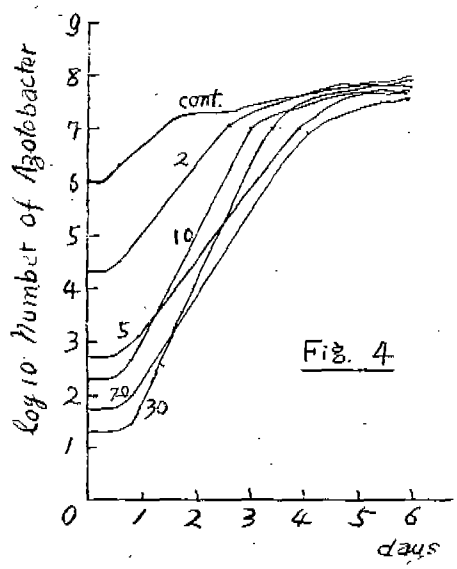


Fig. 1 : Survival rate of Azotobacter irradiated with ultraviolet light.

Fig. 2-4: Reproductive rate of Azotobacter under the different conditions. Number indicates irradiation plot in miniute. (Fig. 2: in basal medium. Fig. 3: in DNA derivatives enriched medium. Fig. 4: in RNA derivatives enriched medium)

It seems mainly due to[8] the increase of external nutrients by autolysis of dead cells that the reproductive rates of Azotobacter in logarithmic growth phase increase at the same proportion of irradiated dose of UV light. But, if this increase of reproductive rate is resulted only from the external environment by autolysis of dead cells, it is not reasonable that the culturing period of 30 min. irradiation plot with the least survivals among the plots which is inoculated same bacterial numbers including dead cells, is shorter than that of 5 min.

or 20 min. irradiation plot, when they reach any stage of the same bacterial population.

In addition to this increase of total population in the plots with the more dose of UV light irradiation, it seems that the more dose of UV light irradiation is the more shortened the generation time of Azotobacter. Therefore, it is clear that variation of reproductve rate must be, mere or less, due to[1] the genetic effects induced by UV light irradiation.

On the other hand, the lag phase or logarithmic growth phase in nonirradiated culture is shortened prominently, and this must be due to[8] the difference in bacterial number of the original inoculm.

The generation time of Azotobacter is shortened by exogeneous treatment of nucleic acid derivatives, and the degree is greater in case of DNA derivatives than RNA derivatives.

W.H. Price[9] reported that the rate of ribose nucleic acid to protein in *Staphylococcus muscae* is proportional to the generation time: that is the faster the cell can form ribose nucleic acid, the more rapid its growth. This explains the shortening of generation time by exogeneous RNA derivatives in this work reasonably.

On the other hand, it is well known that the desoxyribose nuclic acid content per cell is constant and independent of the generation time. A.D. Laren and W.N. Takahashi[10] reported that the infectious RNA from TMV is 6 time as sensitive to inactivation by UV as it is in the form of intact virus, and that inactivation of infectious TMV involves only a local change on RNA chain. But, the effect of exogeneous DNA in this work suggests that irradiated living cell which cotain DNA bring about some change on DNA molecuIs as well as RNA molecules. And if the mutagenic effects of UV take into consideration, it is very reasonable. Therefore, it is clear that the variation of the generation time by UV irradiation is, more or less, due to the genetic effects.

Therefore, it seems that the shortness of the average lifespan of Azotobacter by UV irradiation is resulted not only from the influence of the environmental conditions, but also from the variation of genetic factor of the individual.

摘 要

1. Azotobacter 를 土壤에서 分離하여 檢索하고 紫外線에 對한 그의 生存率과 生存菌의 子孫의 平均壽命을 測定하였다.

2. Azotobacter 의 生存率뿐만 아니라 生存菌의 子孫의 平均壽命도 紫外線의 照射線量에 反比例하였다.

3. Azotobacter 의 增殖率은 exogeneous nucleic acid derivatives 의 影響으로도 增加되었다.

LITERATURE CITED

1) Wagner, R.P., and H.K. Mitchell: Genetics and Metabolism (1955)

2) Beijerinck: Cent. Bakt., 11 (7), 561 (1901)

3) Lipman: N.J. Agr. Exp. Sta. Ann. Rpt., 25, 237 (1904); Lipman-Brown, P.E., 199 (1911)

4) Bergeys Mannual of Determinative Bacteriology (1948)

5) Goucher, C.R., D.A. Waldman and W. Kocholaty: Jour. Bact., 69 (6), 703–750 (1955)
Goucher, C.R., Ichiro, Kamei and W. Kocholaty: Jour. Bact., 72 (2), 184–188 (1956)

6) Newcombe, H.B. and H.A. Whitehead: Jour. Bact., 61, 243 (1951)

7) Demerec and Latarjet: Nat. Acad. Sci., 32, 36–46 (1946)

8) Oginsky and Umbreit: An Introduction to Bacterial Physiology (1955)

9) Price, W.H.: J. Gen. Physiol., 35, 741 (1952)

10) Mclaren A. Douglas and W.N. Takahashi: Radiation Res. 6 (5) 532–542 (1957)