Influence of $^{12}C^{6+}$ ion irradiation on mutant avermitilis*

WANG Shu-Yang(王曙阳)[1,3] CHEN Ji-Hong(陈积红)[1;1)] LI Wen-Jian(李文建)[1;2)]
LIANG Jian-Ping(梁剑平)[1] BO Yong-Heng(薄永恒)[2] MA Xiao-Qi(马晓琪)[1] LIU Jing(刘敬)[1]

[1] Department of Biology Physics, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, China
[2] Gansu Agricultural University, Lanzhou 730070, China
[3] Lanzhou University, Lanzhou 730000, China

Abstract: The effects of $^{12}C^{+6}$ion irradiation on colony morphology and mycelia morphology, as well as on mutation rate have been studied in the B1a high-product strains (ZJAV-Y1-203) mutated by heavy ion irradiation and compared with that in the original strain (ZJAV-A-1). After irradiating the rate of a straw hat colony type having a high ability of producing B1a in ZJAV-Y1-203 strains was higher than that found in ZJAV-A-1 strains. When strains were cultured in a liquid medium for 24 hours, the mycelium becoming thinner could be observed in all of the irradiated ZJAV- Y1-203 groups, but only in the ZJAV-A-1 groups irradiated at the dose of 50 Gy or more. The early growth of mycelium was inhibited in the ZJAV- Y1-203 group irradiated with a high dose. The highest positive mutation rate (23.5%) of ZJAV - Y1 - 203 was reached at the lower dose of 30 Gy while the highest positive mutation rate of 34.2% in ZJAV-A-1 appeared at 50 Gy.

These results indicate that the effects of heavy ion irradiation still exist even in the mutated Streptomyces avermitilis, and only the dose is lower and the effects not so strong compared with the one that is first irradiated with optimized heavy ion doses. This is evidence of the one directional mutation being controlled by many more factors in a organism.

Key words: Streptomyces avermitilis, $^{12}C^{+6}$ ion beam, progress irradiation, influence

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1 Introduction

Heavy ion irradiation induced mutation is an unique method of physical mutation with various parameters, high LET, and RBE characteristics. Heavy ion beams can improve the mutation rate, broaden the mutation spectrum and shorten the breeding cycle. In recent years, great progress has been made in the radiation breeding of gentamicin, alcohol-resistant yeast, and Streptomyces Avermitilis by the Department of Biophysics, IMP, CAS [1–3]. A high B1a producing strain of Avermectin, which is major commercial anti-parasitic agent used in the fields of animal health, agriculture, and human infections, was successfully obtained after the mutagenesis processing by a heavy ion beam and the screening of orthomutation strains at IMP, CAS. The potency of the strains reached 4600 μg/ml and increased by 38.64% than the original strains [4–7]. In most mutation studies, the heavy ions are only used to irradiate the original samples to obtain the expectant results. It has affirmed that the heavy ion beam is a effective microbial breeding method, but does the heavy ion irradiation still affect the mutated specimen, such as the mutant high-producing strains of Avermectin, and induce a further mutation increasing B1a component which is the one of the 8 components included in Avermectins and exhibits the most potent anthelmintic activity [8–13], i. e., further increasing the potency?

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1) E-mail: chjh@impcas.ac.cn

2) E-mail: wjli@impcas.ac.cn

In this paper, the effect of heavy ion irradiation on the mutant high-producing strains (ZJAV-Y1-203) has been investigated compared with the original strain (ZJAV-A-1). The results indicate that the effect of heavy ion irradiation still exists in the mutated Avermectin. In addition, only the dose to reach the highest positive mutation rate is lower and the effects are not as strong as compared with the first optimized heavy ion irradiation of the original strain in addition to the colony morphology and mycelium morphology of the strains being observed. This is evidence of one directional mutation, for instance increasing the B1a component in Avermectins being controlled by much more factors in a organism.

2 Materials and methods

2.1 Strain materials

ZJAV-Y1-203, is a mutant high-producing strain by $^{12}C^{+6}$ ion beam irradiation at IMP, its fermentation titer of B1a has reached 4600 μg/ml in the 5 ton tank trial. For comparison, the strain ZJAV-A-1 with a B1a fermentation titer of 3800 μg/ml was used. ZJAV-A-1 is the original strain of the ZJAV-Y1-203.

2.2 Medium

Seed medium (g/L): starch 25; peanut cake powder 10; soybean powder 10; yeast extract 5; yeast meal 6; $CoCl_2$ 0.01 in tap water at pH 7.2 before autoclaving. Fermentation medium (g/L): starch 130; peanut cake powder 10; soybean powder 10; yeast meal 10; KH_2PO_4 0.02; $MgSO_4$ 0.02; $CoCl_2$ 0.01 in tap water at pH 7.2–7.5 before autoclaving.

2.3 $^{12}C^{+6}$ ion beam irradiation

We took fresh mutant strain (ZJAV-Y1-203) and original (ZJAV-A-1) spores, which were the 7 d slant cultivation at (28±0.6) ℃, and put into the triangular shake flask with glass beads and cultured for 3 h on a rotary shaker (240 rpm) at 28 ℃ (rotary shaker machine, SPH2311D). Then 2.5 mL single spore suspension was transferred into a sterile petri dish (USA，size 35 mm×10 mm) for each sample as an irradiation target.

The spore suspensions were irradiated by 100 MeV/u carbon ions at doses of 0, 10, 30, 40, 45, 50 and 70 Gy with a dose rate of 10 Gy/min. There were three parallel groups for each sample to be irradiated at each dose.

3 The B1a component of directional selection

Each of the irradiated spore suspensions were 10-fold serial diluted, and then we took its dilution of 1:10000 and coated it on the separation plate. After being cultured at (28±0.6) ℃ for 7 d, the colony morphology, size changes, and the number of spores produced of the cultured spores were examined. 60 single colonies were randomly selected and inoculated into the slant medium, and incubated at (28±0.6) ℃ for 7 d, and then inoculated into seed bottles, and left for 24 h. We selected the seeds whose mycelia grew vigorously and inoculated into the fermentation bottle by 4% and examined the mycelium morphology of the non-irradiated original strains and the irradiated ones in liquid medium. The culture broth was withdrawn after fermentation for about 264 h, 3 mL broth was taken to be centrifuged at 4000 rotations /min for 15 min, then we discarded the supernatant and added methanol to 9 ml, which was oscillated in the vortex mixer for 1 h and centrifuged at 4000 rotations /min for 15 min, and then the supernatant was diluted 1:10, and filtered by 0.22 μm membrane for test. The B1a component in the broth was determined by using the HPLC method [18].

If the content of the B1a component in strains is 5% higher after irradiation, it is called positive mutation, otherwise it is called negative mutation. The positive mutation rate is defined as the ratio of colony number of orthomutation to total colony number.

4 Result

4.1 The capacity of B1a component production of irradiated ZJAV-A-1 and ZJAV - Y1 - 203 strains

The content of the B1a component in each irradiated strain by HPLC is shown in Table 1. One can see that more than 54% of the total ZJAV-A-1 strains containing B1a are higher than 3800 μg/mL. The ZJAV-Y1-203 strains having the component of B1a higher than 4600 μg/ml account for 57% of the total strains.

4.2 Colony morphology of the strain irradiated by $^{12}C^{6+}$ ions

Single-spore suspension of the irradiated strains was appropriately diluted and spread on plate agar, and cultured for 6 d, then we observed the colony

morphology, colony size, and spore volume. The results are shown in Table 2.

Table 2 shows that there are some significant changes in colony morphology and the proportion of each type after irradiation [13]. There is still a higher percentage of straw hat and wrinkled colony types in the colonies of the irradiated ZJAV-A-1, and the straw hat type comes to 65.0% of the total number of colonies. The straw hat type in the colonies of irradiated ZJAV-Y1-203 has a much higher proportion, which reaches 80.0%. There are obvious differences in colony diameter between the $^{12}C^{+6}$irradiated group and the non-irradiated group. The colony diameter of the irradiated group is 0.5 mm larger than that of the non-irradiated group. The spore production of the irradiated ZJAV-A-1 is larger than the non-irradiated group. But there is no significant difference in the size of the colony and in the spore production between the irradiated ZJAV-Y1-203 and the unirradiated one.

4.3 Mycelia morphology of the strain irradiated by $^{12}C^{6+}$ ions

After incubation for 24 h in liquid medium, it was found that the majority of the mycelium is thinner in the irradiated ZJAV-Y1-203 (see Fig. 1). While the mycelium thickness in the low dose irradiated ZJAV-A-1 group and in the unirradiated ZJAV-A-1 has no difference, and some thinner myceliums appear only in the higher dose (>50 Gy) group. There is no significant difference observed in the mycelium after being cultured for 48 h.

Table 1. The B1a potency of ZJAV-A-1 and ZJAV-Y1-203 strains after natural separation.

ZJAV-A-1 strain		ZJAV - Y1 - 203 strain	
B1a/(μg/ ml)	number of strains	B1a/(μg/ ml)	number of strains
4200–4500	2	5000–5500	6
3800–4200	30	4600–5000	27
3000–3800	25	4000–4600	18
2000–3000	3	3000–3500	9

Table 2. The comparison of colony morphology between ZJAV-A-1 and ZJAV-Y1-203 irradiated by $^{12}C^{6+}$ ions.

strains	ZJAV-A-1 original Strains	ZJAV-A-1 irradiated strains	ZJAV-Y1-203 irradiated strains
colony morphology	straw hat 75%, smoothing 20%, shrink type 5%	*straw hat 65%, smoothing 20%, shrink type 15%	*straw hat 80%, *smoothing 15%, shrink type 5%
colony diameter	(2.5±0.32) mm	*(3±0.38) mm	*(3±0.38) mm
spore color	gray, yellow at the bottom of colony	gray	gray
spore volume	general	more	more

∗ $p<0.05$ in comparison with the original strains.

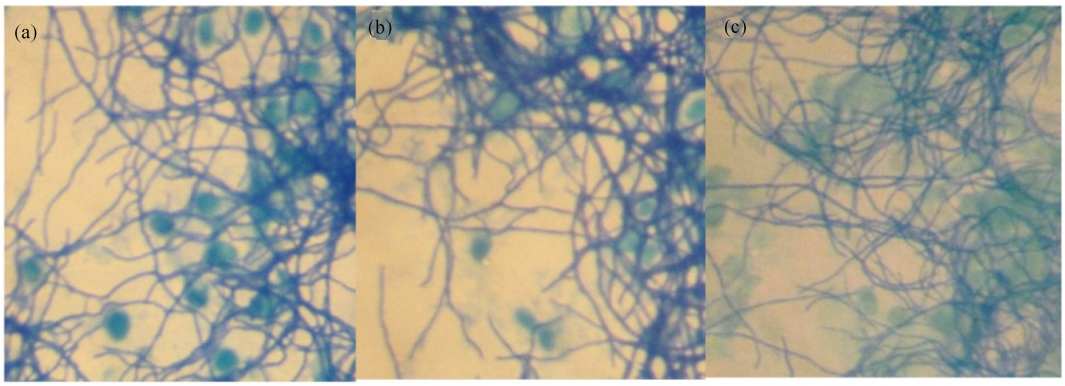

Fig. 1. The mycelia morphology of the strains incubated in liquid medium for 24 h. (a) The original ZJAV-A-1 strains; (b) The irradiated ZJAV-A-1 strains; (c) the irradiated ZJAV-Y1-203 strains.

4.4 Mutation results induced by $^{12}C^{6+}$ ion beam irradiation

After being irradiated by $^{12}C^{6+}$ ions, the survival rate of the colonies and the death rates are shown in Table 3. One can see that, at the dose of 10 Gy, the survival rate of the ZJAV- Y1-203 is lower than the 26.94% which the ZJAV-A-1 reached . When the dose is greater than 30 Gy, the survival rate of the ZJAV- Y1-203 is reduced more slowly than the ZJAV-A-1 (Table 3). This may result from the large quantities of free radicals produced by energy deposition, which causes DNA and membrane damage and other biological macromolecules in the low-dose $^{12}C^{6+}$ ion beam [19–22]. This indicates that at lower doses, the original strain has a strong response to the first irradiation, i.e., the threshold of the body damage repair is low and "repair" is stronger; the mutant strains have a little response to the irradiation, meaning that the threshold of the body damage repair is probably increased. With increasing dose, the "protective barrie repair" of the irradiated mutant strains was enhanced. Because the mutant strains have received $^{12}C^{6+}$ ion stimulation, it has produced a tolerance of radiation stimulation and its capacity of anti-radiation was enhanced. So the survival rate of mutant strains was higher than the original strain, when the radiation dose was greater than 30 Gy. This enhanced repair capacity ensures the repair of most DNA double-strand breaks [23].

Table 3. The mutation results irradiated by a $^{12}C^{6+}$ ion beam^{+}.

irradiation doses/Gy	n	the original strains ZJAV-A-1			the mutant strains ZJAV-Y1-203		
		colonies (number)	survival rate (%)	the lethality (%)	colonies (number)	survival rate (%)	the lethality (%)
0	3	1800±424.26	100	0	1100±284.84	100	0
10	3	485±21.21	26.94	73.06	225±21.21	*20.45	*79.55
30	3	62±6.17	3.44	96.36	55.33±6.31	**5.03	94.97
40	3	45.6±4.43	2.53	97.47	42.50±3.19	*3.86	96.14
45	3	20.33±2.60	1.23	98.77	15.33±1.13	1.39	98.61
50	3	10.33±1.15	0.57	99.43	14.00±1.25	**1.27	98.73
70	3	10.00±1.87	0.56	99.44	7.00±1.02	0.65	99.55

+ The mortality rate=(1−the number of colonies after irradiation / number of colonies without irradiation); the values are the average of triplicated experiments ± standard deviation. $*p<0.05$, $**p<0.001$ in comparison with the original strains.

4.5 Effect of the irradiation on the B1a orthomutation rate

30 single colonies of the typical straw hat type of each sample irradiated at different dose were randomly selected and inoculated in a slant medium and cultured. The cultured slants were fermented in a flask and then the content of the B1a component in each strain was determined by HPLC. The positive mutation rates at different radiation doses are given in Fig. 2. The highest positive mutation rate of 34.2% is reached at 50 Gy for ZJAV-A-1, while the highest positive mutation rate of the ZJAV-Y1-203 is only 23.5% and reached at lower dose of 30 Gy (Fig. 2).

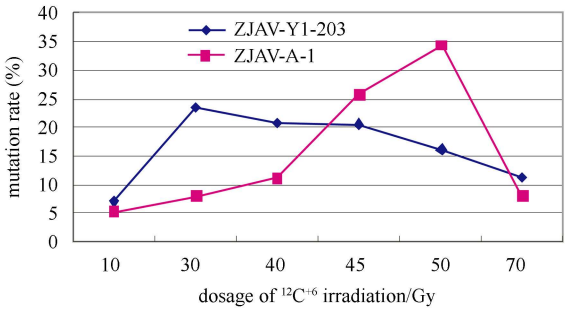

Fig. 2. The relationship of positive mutation rate and radiation dose.

5 Discussion

We witness a significant difference in the rate of survival between the irradiated groups and the progressive irradiation groups at the radiation dose of 10 Gy and 30 Gy or more. This may be due to the differences in the tolerance of radiation stimulation and capacity of anti-radiation. The highest positive mutation rate of the original strain reached 34.2% in the dose of 50 Gy, while the highest positive mutation rate of the irradiated mutant strain was 23.5% in the dose of 30 Gy. The progressive effect reduced the optimizing radiation dose from 50 Gy to 30 Gy, because the irradiated mutant strains have received irradiation stimulation, and they have higher energy than the original strains for energy deposition. There

was a phenomenon that the early growth of mycelium was inhibited in the progressive irradiation groups at high doses of 50 Gy or more.

6 Conclusion

The study shows that heavy ion irradiation has caused obvious changes in the colony morphology, mycelium morphology, and survival rate of Streptomyces avermitilis, which are distinct in the progressive irradiation mutant strains and first irradiated strains. It is possibly that these changes lead to an increase in the content of B1a in Streptomyces avermitilis. The results also show that heavy ion irradiation can still induce mutation in the Streptomyces avermitilis already mutated by heavy ion irradiation, and only dose is lower and the effects are not so stronger compared with the one first irradiated with optimized heavy ion doses. This is evidence for one-directional mutation being controlled by many more factors in a organism.

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