# Chemical Mutagenesis of *Ulva pertusa* Kjellman by Ethylmethanesulphonate*

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# Ethylmethanesulphonate에 依한 구멍갈파래 (*Ulva pertusa* Kjellman)의 突然變異誘起*

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## ABSTRACT

Sensitivity to ethylmethanesulphonate(EMS) on *Ulva pertusa* Kjellman thalli was investigated as preliminary studies aimed at mutation breeding of seaweeds. When the thalli were grown in medium containing the mutagen no growth and about 20% growth inhibition occurred at 0.05% and 0.025% EMS-mixed medium respectively. In the case the thalli were treated short time at high concentration mutagen solution followed by culture in control medium no growth and about 10% growth inhibition occurred at 40 min. in 1.0% and 80 min. in 0.5% EMS solution respectively. Among the mutagenized tissues, some showed variation in growth from and cell color contrast to the mother tissue. The mutagenized tissue polypeptide pattern using sodium dodecyl sulphate-polyacrylamide gel electrophoresis was similar that of the mother tissue except about 6.6 kD polypeptides.

## 要 約

突然變異誘起를 通한 有用遺傳形質의 海藻類品種을 開發하기 爲해 化學的突然變異誘發源인 Ethylmethanesulphonate(EMS)에 對한 구멍갈파래(*Ulva pertusa* Kjellman) 葉體의 感受性을 檢討하여 效果的인 突然變異誘起條件을 찾고자 하였다. EMS를 處理한 培地에 葉體를 生長시키며 變異를 誘發시키고자 하였을 때, 0.05%와 0.025%의 EMS 處理區에서 各各 100%와 約 20%의 生長抑制가 일어났다. 한편 高濃度의 EMS液에 葉體를 一定時間 沈積한 後 正常培養함으로서 變異를 誘發코자 했을 때, 1.0% EMS液에 40分과 0.5% EMS液에 80分 處理한 境遇 各各 100%와 약 10%의 生長抑制가 일어났다. EMS 處理된 葉體는 生長形과 體色이 母體와 전혀 다른 變異體로 나타나기도 하였는데 이들의 polypeptide 一部에 變化가 있음이 SDS-PAGE로 調査되었다.

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## INTRODUCTION

In recent years seaweeds are becoming more important as a source of food as well as phycocolloids. It is therefore now being seriously considered to develop basic techniques for breeding them economically feasible sea-crop plants with new properties(Saga et al. 1986). Generation of genetic variability is the first phase in plant breeding program. In this case induced mutations can be used to generate useful variation in inherited characters and such mutants can provide a new source of germplasm for improvement of different traits(Won and Chung, 1988). Even though most mutations make the organism less efficient and are thus disadvantageous, the possibility of developing new desirable traits through induced mutations has intrigued many plant breeders. The main advantage of mutation is the increase of the genetic variability within a relatively short period, including genetically controlled traits not existing in the non-mutated meterial. Furthermore, in contrast with higher plants, the monoploid condition of many seaweed species is invaluable to mutation research and greatly simplifies the experimental design.

Appropriate methods of mutation induction are of high relevance to meet the requirement efficiently(Brunner, 1983). Up to the present, the researches on mutagen application in seaweed has been reported only by Katayama(1985, 1986) who isolated various *Porphyra* mutants by using colchicine and ethlymethanesulphonate. However the effect of the mutagenic treatment on the mutation frequency was not clear in the cases. This might be explained from the fact that little information is available on the concentration of mutagen that is most effective for the isolation of mutants from the materials.

The main purpose of the present study was to investigated sensitivity to ethylmethanesulphonate of *Ulva pertusa* thalli by determining the correlation between mutagen concentration, treating time and survival of the plant tissue as preliminary studies aimed at mutation breeding of seaweeds.

## MATERIALS AND METHODS

Thalli of *U. pertusa* sterile mutant(Migita, 1985) were grown in our laboratory were used for plant materials(Fig. 1). They were dissected into 1 cm diameter each round tissue, then mutagenized with ethylmethanesulphonate(EMS, Kodak Co.) by two protocols. In the first experiments

Fig. 1. *Ulva pertusa* sterile mutant cultures used in experimental plant material.

tissues were cultured in the media containing 0.001~0.05% EMS for two weeks and thereafter transferred to EMS-free ES medium. In the second experiments tissues were treated in 0.5% or 1.0% EMS solution for 10~80 min. followed by thoroughly rinsed in filtered medium, and then cultured in EMS-free ES medium. The 500 ml culture flasks contained 500 ml of liquid medium and 20 pieces of tissue each. The cultures were aerated and subcultured every two weeks. They were maintained under white fluorescent light (ca. 1000 lux) with 12/12h photoperiods at 19± 1 ℃. Mutagenic effects were scored by the growth rate and the phenosafranine dye staining of the cultures at every week. Electrophoretical investigations were conducted by SDS-polyacrylamide gel electrophoresis using a discontinuous system on 12.5% acrylamide gels as described Laemmli (1970) and Conassie blue staining gels.

## RESULTS AND DISCUSSION

Ethylmethanesulphonate is one of alkylating agents which have reactive alkyl groups capable of being transferred to other molecules at positions of high electron density. In several effects on DNA. one major mechanism of mutagenesis by EMS involves the transfer of ethyl groups to the bases such that their base-pairing potentials are altered and GC-AT transitions result. These effects are determined by the dose of EMS, and most important parameters of the dose are concentration and duration of treatment(Brunner, 1983).

In order to investigate the dose effect of EMS on the thalli of *Ulva pertusa,* we want to determine the correlation between mutagen concentration, treating time and survival of the plant tissues by two experiments.

Fig. 2 and Fig. 3 summarize the growth of *U. pertusa* thalli after the treatment with EMS. The results of thalli growth in EMS-mixed medium are shown in Fig. 2a and Fig. 3a. No growth occurred at 0.05% EMS-mixed medium and about 20% growth inhibition was in 0.025% EMS-

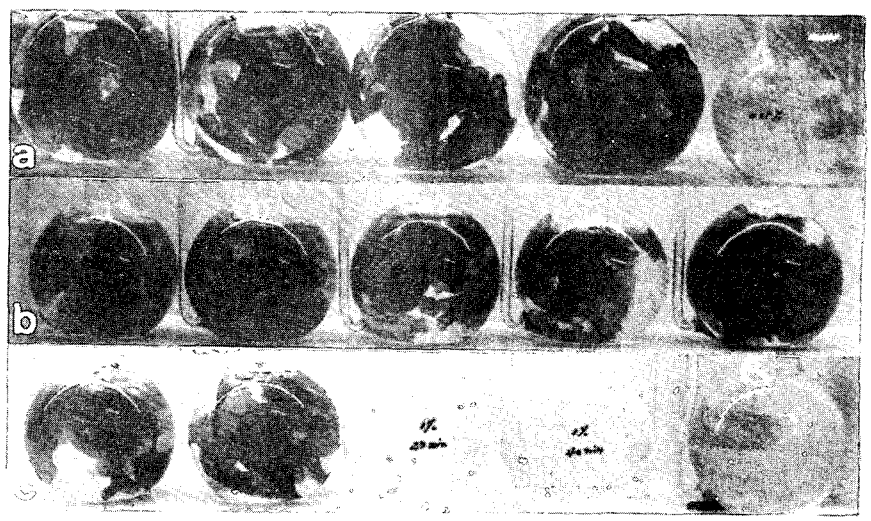


Fig. 2. Three-week-old *U. pertusa* thalli mutagenized by growing in the media containing. 0.001~0.05% EMS(a) and treating in 0.5%(b) or 1.0%(c) EMS solution for 10~80 min.

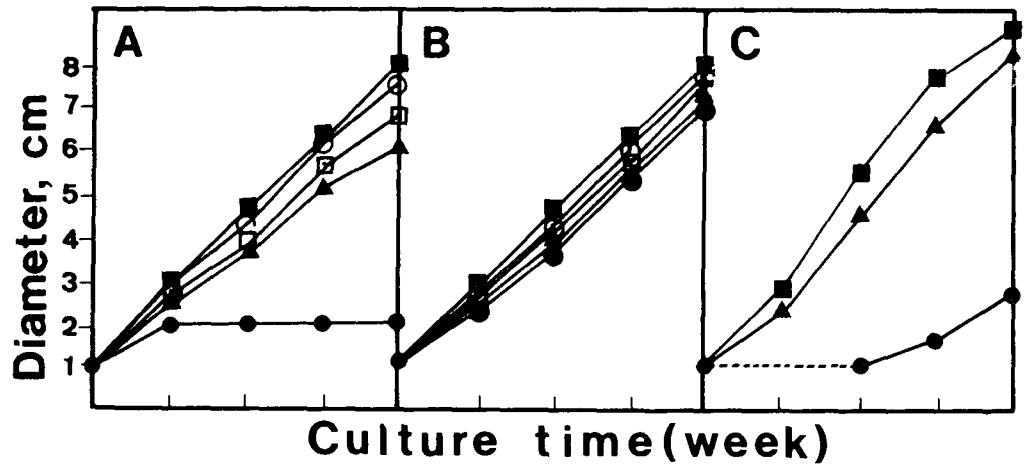


Fig. 3. Growth of *U. pertusa* thalli after the treatment with EMS as described in Fig. 2.

mixed medium respectively.

Therefore, the suitable concentration of EMS demanding a half growth to the control by this method was expected to be 0.025% to 0.05%. Fig. 2b, 3b and Fig. 2c, 3c show the growth of thalli after the treatment for short time with 0.5% and 1% EMS solution. Following the application of 80 min. in 0.5% EMS solution, about 10% inhibition was observed and gave complete growth inhibition in the thalli soak-treated for 40 min. in 1.0% EMS solution. Such a trend indicates that, for obtaining 50% growth rate as compared to the control using this method, soaking the plant tissue over 80 min. in 0.5% EMS solution or 10~20 min. in 1.0% EMS solution are recommended. These data imply that increasing the concentration and time of EMS treatment reduced the growth rate of the treated plant tissue. In general the present results are coincide well with the data by Katayama(1986) who could isolate various biochemical mutants of *Porphyra* using 0.005%~0.01% concentration of EMS. Fig. 4 shows a representative mutagenized tissues obtai-

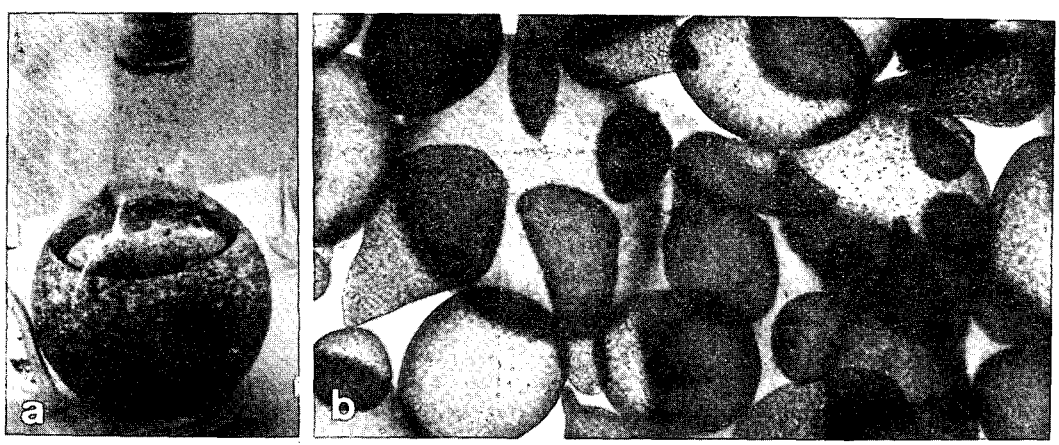


Fig. 4. Morphology of a mutagenized plants in culture flask(a) and under microscope(b) obtained from the survived tissue after treatment the thalli in 1.0% EMS solution for 20 min utes.

ned form the survived tissue after treatment the thalli for 20 min in 1.0% EMS solution. As shown in Fig 3c, their growth were nearly all inhibited. By the phenosafranine staining, they contained less than about 5% living cells of total tissue. The survived tissues were completely changed in growth from to the mother tissue, grew slowly with globular form and showed red or green color. In order to determine whether the mutagenized tissues show expressional changes, electrophoretical investigations were conducted by SDS-PAGE(polyacrylamide gel ecectrophoresis). Majority of the polypeptide banding patterens were all similar, but apparently about 6.6kD polypeptides of the red color varients were lacking contrast to the control.

Determining the sensitivity of seaweed tissue to mutagen will be essential for effective mutagenesis. While, it was difficult to summarize the data reported so far on mutagenesis techniques toward the plants because the results are different depending on explants, culture condition, and mutagen treatment method applied (Maliga et al. 1982). In conclusion we have shown that the various EMS-mutagenesis can be applicable for the induction of mutation from *U. pertusa* thalli. That is, culturing the thalli in 0.05～0.025% EMS-mixed medium or treating the tissue with 1.0% EMS solution for 10～20 min are recommended. A similar approach, as presented in this apper, should be equally applicable for the mutation induction from thalli of other seaweeds.

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