

AKINETES FORMATION AND GERMINATION IN *CYLINDROSPERMUM MAJUS* KUTZ.

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ABSTRACT

The isolation and purification of *Cylindrospermum majus* Kutz. Were made from different water and soil samples. Akinetes formation was observed during the incubation of samples (cultures) at 4°C for six months period. Germination of akinetes took place only after transferring the isolate to a new culture media at 26°C. The gradual steps of akinetes germination were followed and illustrated for the first time in this species. The results showed that the outer most layer may expand to the available references do refer to such calyptra formation and such growth and development.

INTRODUCTION

Cylindrospermum majus Kutz. is widely distributed in Iraq and was first recorded by Al-Kaisi (1970), along the Tigris river. Maulood *et al.* (1986) reported its presence in and around Baghdad. Like other members of Cyanobacteria no detailed study yet exist in Iraq on this genus, which is represented by five species in different habitats throughout the country (Hinton *et al.* 1983). Akinetes formation in this species have been dealt by many authors (Miller *et al.* 1968, Fay 1969, 1970, 1973, Fay *et al.* 1962, 1964, 1968, Wolk 1965, Gentile *et al.* 1969, Fogg 1969, Stewart 1973). It appears that little is known about the factors which induced germination of akinetes. The morphological and structural changes after the akinetes formation may be summarized by decrease of the number of polyglucan granules, increase the size of cells, having characteristic shape, modified pigmentation and many large cytoplasmic granules (Fogg 1969, Fritsch 1945, Stewart 1973). In contrast with heterocysts the akinetes envelop

completely surround the cell and thus separate it from the adjacent cells. This has been shown before by Fritsch (1945). There are quite a lot of work on physiology of the akinete related to respiration, pigment analysis and nitrogen content in many isolated akinetes from different members of hormogonales (Stewart 1973, Carr *et al.* 1973, Fogg *et al.*, 1973). Whereas, the growth of akinetes as a method of reproduction and survival still remain vague. Many authors (Fritsch 1945, Carr *et al.* 1973, Fogg *et al.* 1973) related the development to light intensity, sodium acetate, phosphate ratio in the media and sucrose supply (Fogg *et al.* 1973). Temperature as a factor had dealt with, in detail by Dring (1970) on all members of algae but he did not refer to akinetes at all. In Iraq more than forty species of hormogonales have been recorded (Hinton *et al.* 1983) but no detailed biological studies exist on any member of cyanobacteria. The present work is the first biological experimental study in Iraq with respect to the stimulation, formation

and germination steps of akinetes in this species of cyanobacteria.

MATERIALS AND METHODS

Methods described by Wiedeman *et al.* (1964) and Al-Mousawi *et al.* (1983) were followed to collect samples from different localities in Baghdad district and isolate the investigated species from soil and water samples.

Allen's (1968) media with and without combined nitrogen was made, and the pH adjusted to 7.8 to grow the isolated cyanobacteria species. The liquid and solid cultures were incubated aerobically at 26°+1°C under continuous illumination of approximately 65UE/ M²/ Sec. Naystatine (0.02g/l) was added to the culture medium to suppress the growth of competitive microorganisms during the isolation and purification. Purifications were made by transferring the single filaments into new medium which aided by either growing the parent cultures in unidirectional light or by adding an extra agar layer on top of the growing plate surface, so, that individual filaments would grow through away of other contaminated micro organisms. Identification of *Cylindrospermum majus* was performed by using the classical phycological texts of Desikachery (1959). Preservation of samples were made by storing the cultures at 4°C. Semi-permanent preparations of filaments were made by amounting drops of the cultures in a mixture of glycerine jelly and crystal violet and allowing them to dry for 8-12 h. with cover slip in place. Observations were made using a Research micro scope type Olympus Vanox with camera attachment type C35A and photographs were taken.

RESULTS

During the storage of samples at 4°C, huge formation of akinetes were observed unexpectedly. Almost all vegetative cells were changed to the thick walled akinetes (Fig.1), during the six months storage.

This phenomena was the scope of our study.

In this investigation the sheath remain outside the dense amorphous layer of which further layers are deposited, with innermost layer being formed last (Fig.2). Further more in contrast, the cytoplasmic granule decreased in vegetation period and increase in resting period (Fig.3). In this investigation, the whole vegetative culture turned to a cluster of akinetes and the only variable factor was the reduction of temperature from 26° C to 4° C beside shortage of nutrient in the medium (Fig.1). The whole culture developed and akinetes germination was almost 100% (Fig.4).

The division begins in akinetes within the envelope but sometime start only after the germination has emerged after they transferred to new culture media at 26° C (Fig.4,5). In addition, the remaining envelope (Calyptra) may stay surrounding the mother cell and may expand to the daughter germinated cells as well. The presence of the calyptra were randomly observed between different akinetes within the culture (Fig.3,4,5). The development and germination of akinetes in such detail illustrated steps have not yet been shown by any author in *Cylindrospermum majus*.

DISCUSSION

A lot is known about the formation of heterocysts and its development under the light microscope (Carr *et al.* 1973). Whereas the growth and development of akinete is still obscure. Clarke *et al.* (1969) published the ultra structure of akinete development in *Cylindrospermum* species. He showed that the vegetative cells enlarged a localized disposition take place and a dense amorphous material gradually spread and developed into a thick layer surrounding the whole cell. It protruded out word as the akinete mature. Milles *et al.* (1968) showed the presence of polyglucan granules in *Cylindrospermum* cells, but this may decrease in resting period. Many

other authors confirmed that little is known about the factors which induce germination. Harder(1917) observed that akinete germination in *Nostoc*, *Anabaena* and *Cylindrospermum* species took place only in light except where sucrose is supplied in the dark. Concerning the akinetes formation or germination Fay (1969) and

fagg *et al.*(1970)showed that the akinetes formation is coincided with termination of exponential phase of the growth and spore production increased during the post exponential phase. Whereas, Wolk (1965)and Gentile *et al* .(1969) described the sporulation to be related to phosphate level, buffer agent, Calisium ions and Sodium acetate. None

of these authors related the spore formation and akinete production to temperature in cyanobacteria. Germination of akinetes have been observed and 16% of them were germinated during the post exponential phase in *Anabaena cylindrica* (Fay 1969 and Fritsch 1945). Fogg (1969) showed that cell division suddenly begins in akinete within the envelop but sometimes start only after the germination has emerged. In conclusion reducing the temperature may be applied, comparative study and will overcome the isolating difficulties of strains. This finding will undoubtedly ease the following up growing steps of akinetes germination in many other species.



Fig. (1)Thick walled akinetes for *C. majus*

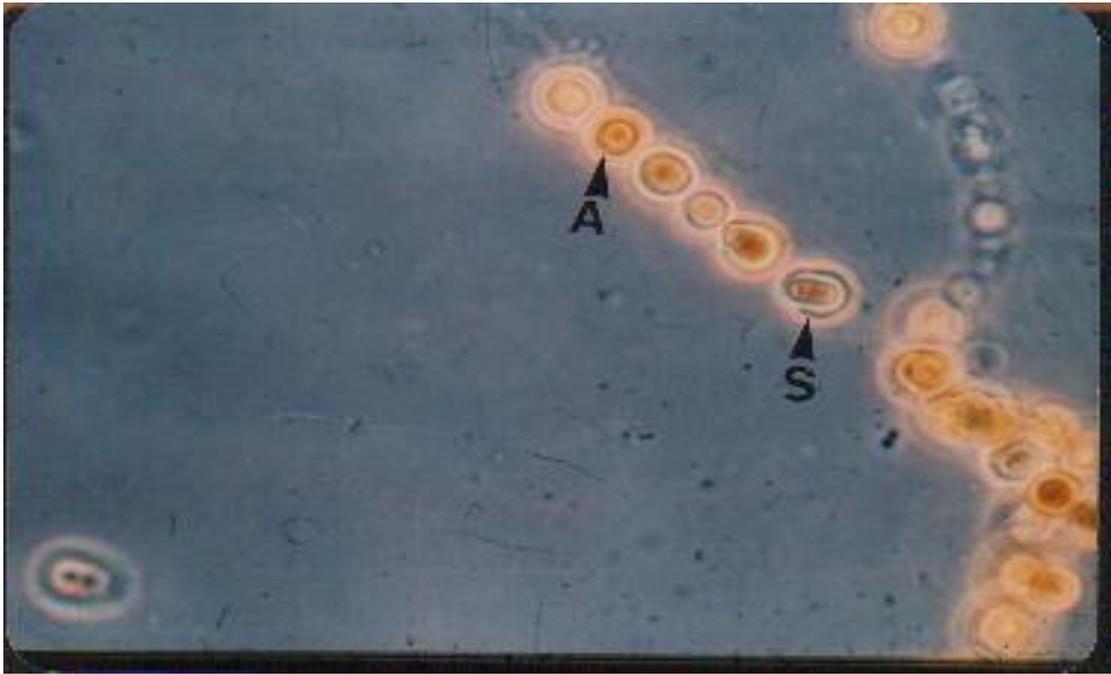
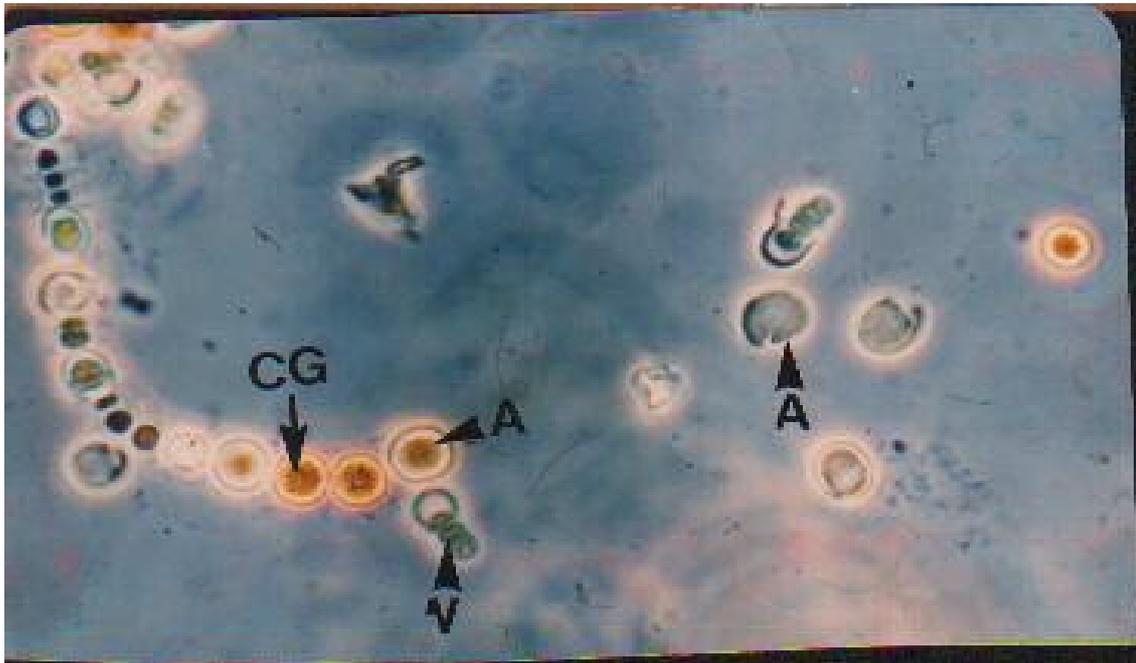


Fig.(2) Formation of the sheath (S) around the cells before maturation of akinete(A)



Fig(3)Cytoplasmic granules (CG)in vegetative (V)and akinete cells (A).



Fig(4) Full (100%) germination of akinetes in *C. majus*



Fig.(5) Germination of akinetes showing calyptra (K),mother cells(M)and daughter germinating cells (D).

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تكوين وانبات الخلايا الساكنة في *Cylindrospermum majus* Kutz.

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الخلاصة

تم عزل وتنقية *Cylindrospermum majus* Kutz. والتي تعود إلى صنف الطحالب الخضراء المزرققة من نماذج مختلفة من التربة والمياه وملاحظة تكوين الخلايا الساكنة (Akinetes) عند حضانة العزلة في درجة حرارة 4 م° وخلال ستة أشهر أما نمو الخلايا الساكنة فقد لوحظ بعد نقل العزلة إلى وسط غذائي جديد ورفع درجة الحرارة إلى 26 م°. المراحل المتعاقبة لنمو الخلايا الساكنة إلى الخلية الخضريّة تم الإشارة إليه لأول مرة في هذه الدراسة وقد وضحت بالصور ولجميع المراحل بضمنها نشوء الـ Calyptra والخلايا النامية الجديدة.