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ROLE OF L-CYSTEINE ON AZIDE MUTAGENECITY IN AZOTOBACTER

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AZOTOBACTER is a free living nitrogen-fixing bacterium. Successful induced mutagenesis is not of common occurrence in certain strains of this organism[1]. Sodium azide which acts as a substrate[2] for enzyme nitrogenase involved in the process of biological nitrogen fixation, also acts as a potent mutagen[3]. L-cysteine is reported to inhibit azide mutagenicity in *Salmonella typhimurium*[4]. Certain mutations in cysteine biosynthetic pathway are also reported to reduce or abolish the mutagenic property of azide[5,6]. In the present communication we report the effect of L-cysteine on azide mutagenicity in a cysteine requiring mutant of *A. chroococcum.*

A cysteine requiring mutant[7] of *A. chroococcum* (N-4) was used in the present study. Burk's modified nitrogen-free medium[8] supplemented with 20 μg/ml ammonium sulphate (NA) was used for scoring cys^+ revertants. Complete medium was constituted by supplementing Burk's basal medium with 0.3% yeast extract (YE).

Cells were grown in YE medium to late log phase at 30°C in a shaking water bath. The cells (10 ml) were harvested by centrifugation and suspended in 1 ml of 0.01 M $MgSo_4$; 0.5 ml of this suspension was added to a tube containing 100 μg/ml azide in YE medium. Cell suspension (0.5 ml) was separately added to another tube containing azide (100 μg/ml) and L-cysteine (200 μg/ml) in YE medium. A zero sampling was done immediately after adding the cell suspension from both the tubes and the rest of the culture was allowed to incubate for 2 hr at 30°C under shaking condition.

The viable count was scored on complete medium and the cys^+ revertants were scored on NA medium. Cys^+ revertants from NA plates were further confirmed by spotting them individually on NA and complete medium.

The results (table 1) show that sodium azide alone is mutagenic to the cells of *A. chroococcum*. When azide treatment is given in the presence of L-cysteine, its mutagenicity is increased manyfold. This increase is however related with a high degree of killing. Azide mutagenicity is known to be exerted through the formation of a compound "azide metabolite"[9]. This metabolite is formed only in the growing cells[10] and L-cysteine stops this conversion making azide ineffective[5]. Our findings indicate that L-cysteine does not repress/feed back inhibit its biosynthetic enzymes as reported in *S. typhimurium*[11]. It seems that cysteine auxotrophy in the present case has not originated from a mutation in either cys E or cys K genes since this mutation does not inhibit the azide mutagenicity. These genes are reported to synthesize enzymes participating in cysteine biosynthesis required for conversion of azide into azide metabolite[5,6]. It is important to mention here that the mutant N-4 has been derived from a nitrogenase constitutive mutant and has the ability to grow in the presence of azide and ammonium

Table 1 *Cysteine interaction with azide on induced cys^+ reversion*

	Azide			Azide + L Cysteine		
Treatment time (hr)	Cell density	Survival (%)	Cys^+ revertants per 10^7 cells	Cell density	Survival (%)	Revertants per 10^7 cells
0	1.1×10^8	100	9.3	1.2×10^8	100	7.5
2	6.6×10^7	60	19.0	2.4×10^6	2.0	1150.0

acetate. One of the reasons for higher cys$^+$ revertants obtained in combination treatment of L-cysteine and azide could be the favourable condition available due to the presence of L-cisteine, a requirement of N-4 mutant and the cysteine may allow better conversion of azide into azide metabolite.

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IMPACT OF LACK OF CALLOSE ON POLLEN DEVELOPMENT IN *NAJAS MARINA* L.

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IN *Najas marina* L., the study of microsporogenesis reveals the absence of callose wall around the meiocytes and microspore tetrads, yet the microsporogenesis proceeds normally except that the mature pollen lacks exine. The present investigation suggests a correlation between the presence of callose and the development of pollen wall.

The pollen mother cells (PMCs) in the great majority of angiosperm taxa, are surrounded by deposition of callose which is a β-1,3 polyglucan composed of β-D gluco pyranose residues[1]. Callose is first deposited on the inner side of the PMC walls at the stage when the massive cytoplasmic connections between the PMCs are formed. But later, these connections are broken due to the additional deposition of callose. The following functions are attributed to callose: (i) It protects the differentiating sporogenous cells from harmful hormonal and nutritional influences of the surrounding vegetative cells[2], (ii) It functions as a molecular sieve to enable the autonomous development of the haploid pollen nuclei, independently segregated to their own cytoplasm[3], (iii) Callose supplies carbon compounds like glucose which furnish a basic framework of the future exine[4] and (iv) carries a template for the future exine establishments[5].

The present investigation was undertaken to study the development of pollen in the absence of callose around the meiocytes and their derivatives, *i.e.* dyads and tetrads. Anthers containing various developmental stages of pollen were collected from the greenhouse grown specimens of *Najas marina* L and were fixed in ethanol acetic acid (3:1 v/v). The material was sectioned on a rotary microtome and the sections were stained with periodic acid Schiff's and aniline blue reagents as suggested by Jensen[6]. The dehydrated pollen grains were examined under S4–10 Cambridge stereoscan electron microscope for their wall nature.

In *N. marina* the meiocytes (figure 1A), dyads and tetrads do not show the presence of callose deposition when stained with aniline blue and periodic acid Schiff's reagents.

Scanning electron microscopic examination of mature pollen reveals the absence of exine around them. However, evaginations on the pollen wall (intine) often give false impression of exine (figure 1C). These evaginations are formed due to the pressure exerted by the densely accumulated starch grains on the pollen wall from the inside (figure 1B). Furthermore, earlier reports also stress the fact that the pollen of *Najas* lacks a definite exine[7,8].

These observations appear to rule out the possibility of callose in protecting the developing sporogenous cells from harmful hormonal and nutritional influence of the surrounding vegetative cells and acting as a sort of blanket to enable the autonomous development of the haploid pollen nuclei in many plants as suggested by De Halac and Harte[2] and Heslop *et al*[3]. However, in *N. marina* callose is not necessary to meet these two