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Antibiotic sensitivity assay for *Spirulina*: In relation to marker selection for genetic improvement

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SUMMARY

Spirulina is a model organism in mass and outdoor cultivation of algae biomass as a source of protein, chemicals and nutrition. There is urgent need to improve food quality of *Spirulina* for human consumption. It's a need to develop selectable marker system for genetical improvement to introduce new genes. For this purpose the sensitivity of *Spirulina platensis* was tested against 5 different antibiotics (Kanamycin, Streptomycin, Ampicillin, Hygromycin and Chloramphenicol) with varying concentration of 25µg/ml to 1600µg/ml. *S. platensis* showed sensitivity to all antibiotics but maximum inhibition was found with Chloramphenicol. Thus the Chloramphenicol will be best marker for selection for further studies and the chlorophyll *a* concentration for sensitivity assay.

Key words : Spirulina, Transformation, Antibiotic sensitivity, Chloramphenicol

Spirulina is a multicellular, filamentous, unbranched, helical cyanobacterium and belongs to family Oscillatoriaceae with a length of 200- 300µm and a breath of 5-10 µm. *Spirulina's* nutritional qualities are truly "one-of-a-kind", with its structure consisting of nearly 71 per cent of total protein. *Spirulina* represents the highest natural source of protein ever discovered which is superior to all standard plant protein, such as that of legumes (Ciferri, 1983; Babadzhanov *et al*., 2004).

There have not been any approaches available related to the reproducible and stable gene transfer system for *Spirulina* platensis (Vacchhani and Vonshak,1997). This situation blocks the development of new strains and new utilization of this economic species through biotechnology. Introducing a selectable marker gene helps to screen transformants. However, the selectable marker assay for suitable gene transfer in *S.platensis* has not been properly documented. In the present study, the sensitivity of *S.platensis* to 5 antibiotics was examined in order to pick out one or more suitable selectable markers for further gene transfer of this alga with suitable sensitive assay method.

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MATERIALS AND METHODS

Growth and maintenance of strain:

Selected *Spirulina* strain was grown and maintained in Zarrouk medium(Zarrouk,1966), modified by Ogawa and Terui (1970).The cultures were allowed to grow at 25±1°C under continuous illumination by using cool, white fluorescent tubes with approx light intensity of 3000 Lux, above the surface of culture vessel.

Antibiotics treatment:

5 days old actively grown culture was taken and transferred in 50ml fresh medium containing antibiotics (Kanamycin- 200, 400, 800, 1600 µg/ml ; Streptomycin- 100, 200, 300, 500µg/ml ; Ampicillin- 100, 200, 300, 500µg/ml; Hygromycin- 50, 100, 200, 400µg/ml ; Chloramphenicol- 25, 50, 100, 200µg/ml) with 5µg/ml concentration of chlorophyll a. It is then incubated under continuous illumination at 27± 2°C. Samples were used for further studies from 0 to 5 days. Assay run in triplicates.

Estimation of protein :

Protein content was estimated by the method of Lowry *et al.* (1951) with slight modification by Singh and Singh (1997). To 0.5ml of cell suspension, 0.5ml of 1 N sodium hydroxide was added and mixture was placed in boiling water bath (100°C) for 10 min. Mixture was allowed to cool before the addition of 2.5ml of reagent (it contains 50ml of 5% Na_2CO_3, 1ml of 1%Na-K tartarate and 1ml of 0.5% $CuSO_4.7H_2O$). This mixture was thoroughly shaken and left at room temperature for 10-15min. Then 0.5ml of 1N Folin-ciocalteau reagent was added and mixture was centrifuged. Supernatant was taken and after 15 min. of color reaction, color intensity was read