

## FACTORS AFFECTING *AGROBACTERIUM* MEDIATED TRANSFORMATION OF INDIGENOUS *CHLORELLA VULGARIS* BAYERINCK

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### Abstract

*Chlorella vulgaris* Bayerinck widely used as a health food, feed supplement, as well as in the pharmaceutical, biofuel and cosmetics industries. It has been used to determine optimum transformation conditions through *Agrobacterium tumefaciens*. It has been revealed that bacterial density of  $OD_{600} = 1.0$ , 3 days of co-cultivation at 25°C in pH 5.5, and 100  $\mu$ M acetosyringone are the optimum conditions to transform *C. vulgaris*.

Microalgae are sources of dropping fuels, feed, fertilizer, nutritional oils and pharmaceutical products (Borowitzka 2013). It contains proteins, carotenoids, lipids, immune stimulator compounds, polysaccharides, vitamins, antioxidants and minerals products (Chisti 2007, Wang and Zhang 2013). They are used in waste water treatment and other remediation services (Lizzul *et al.* 2014). Potentiality of growth of the alga is the key feature for efficient application at low production cost. Genetic engineering of algae is used to improve the algal strain in biomass productivity as well as potential fuel strength to make it more sustainable and economical. During last several years researchers have improved oil yield and biomass production by gene splicing and modification in lipids synthesis pathways. Stable nuclear transformants of algae have been achieved by electroporation, agitation with glass beads or silicon carbide whisker, particle bombardment and *Agrobacterium tumefaciens* mediated transformation. Major limitations of the transformation methods were overcome by *Agrobacterium*-mediated transformation (Tara *et al.* 2005, Shrawat and Lorz 2006).

To produce high quality biomass, attention must be paid to culture conditions where nutrients in the medium play a major role in the cultivation of algae (Jayaramareddy *et al.* 2014). Large-scale production of *Chlorella* biomass depends on nutrient availability, temperature and light which influence the composition of the biomass by changes in metabolism. The cellular lipid content and polyunsaturated fatty acids decrease with increase in light intensity (Li *et al.* 2011, Widjaja *et al.* 2009). Natural day light and 25 - 30°C temperature are favorable for overall growth of *Chlorella vulgaris* Bayerinck. Higher pH lowers the affinity of *Chlorella* to free CO<sub>2</sub> and under conditions of alkaline pH less unsaturated membrane lipids is produced (Rekha *et al.* 2012).

Although the application of genetic engineering to improve *Chlorella* strains for polysaccharides and fatty acids is in its infancy, significant advances have been achieved in the development of genetic manipulation tools to manipulate central carbon metabolism in microorganisms (Radakovits *et al.* 2010). Advantages of *Agrobacterium*-mediated transformation method over direct gene transfer methods include its feasibility to transfer large DNA fragments, low copy number of transgene integration with little rearrangement, preferential integration into transcriptionally active regions and its simplicity (Cha *et al.* 2012).

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*Chlorella vulgaris* was collected from Jallo Park (Lahore, Pakistan) and observed under light microscope for their morphological features and other cellular details (Graham *et al.* 2009). Optimization of pH and temperature were determined and uniculture was obtained periodically by repeating culturing in liquid and agar media.

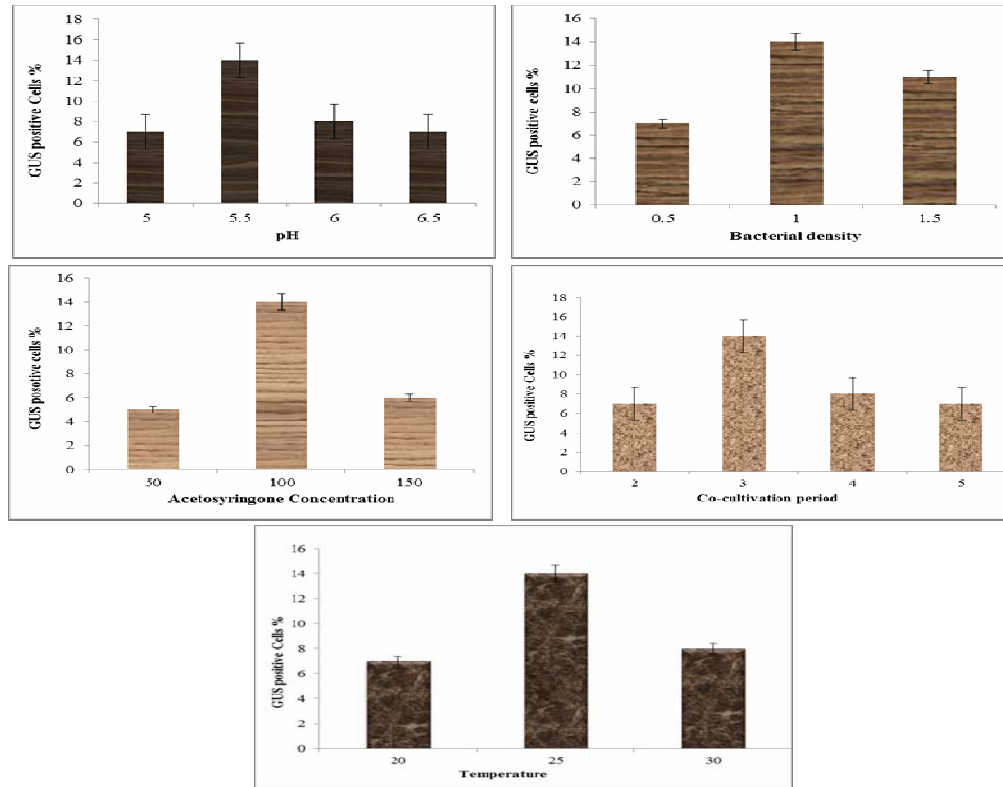


Fig. 1. Influence of different parameters on *Agrobacterium*-mediated transformation of *Chlorella vulgaris*.

To optimize the growth parameters, each parameter (inoculum density, co-cultivation duration, pH of co-cultivation medium, concentration of acetosyringone) was optimized by keeping other parameters constant. For *Agrobacterium* mediated transformation first *C. vulgaris* was cultivated on BB agar media (Bold 1949). For transformation *A. tumefaciens* stock of EHA 101 (pZY 102) was made by streaking on solidified LB medium containing appropriate antibiotics and incubated for 3 days at 28°C. The day before explant inoculation, liquid LB medium containing antibiotics was inoculated with a single colony and kept on shaker until OD<sub>650</sub> reached 1.2. Then the culture was centrifuged to pellet the cells. The pellet was subsequently re-suspended in liquid co-cultivation medium and shaken at 220 rpm for 30 min before inoculation. The bacterial suspension was spread on the thin layer of *C. vulgaris* culture ( $5 \times 10^6$ ) growing on agar plate supplemented with acetosyringone. The plate was incubated for 2 days in the dark at 25°C (Co-cultivation).

The co-cultivation algal cells were recovered by growing in BBM supplemented with cefotaxime and incubated to eliminate *A. tumefaciens*. The harvested cells were washed twice with liquid BB medium via resuspension by mild vortexing and centrifugation. Histochemical GUS

assay was performed for transformed and untransformed cells (Liu *et al.* 2013). In order to study the effects of each parameter on the transformation frequency, one parameter was first varied while other parameters were kept constant based on the findings of the preliminary experiments and general transformation procedure above.

All of the parameters were optimized by screening for transient GUS expression. No expression of reporter genes was detected in non-transformed cells. The effects of the assayed parameters which were known to influence the efficiency of *Agrobacterium*-mediated transformation are given in Fig. 1.

Results indicate that microalgae cells pre-culture duration significantly affects the percentage of GUS expressing cells. Several reports noted that the overgrowth and high density of *Agrobacterium* cause explants mortality (Ghimire *et al.* 2008, Ozawa 2009). A high density of bacteria (OD600 = 1.0) did not affect transformation frequency. The concentration of acetosyringone apparently influences the transformation frequency (Cha *et al.* 2012).

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