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Optimising Light Conditions for Recombinant Protein Production in the Microalgal Chlamydomonas reinhardtii Chloroplast

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Abstract : The green alga C. reinhardtii provides a platform for the cheap, scalable, and safe production of complex proteins. Despite gene expression in photosynthetic organisms being tightly regulated by light, most expression studies have analysed chloroplast recombinant protein production under constant light. Here the influence of illumination time and intensity on GFP and a GFP-PlyGBS (bacterial-lysin) fusion protein expression was investigated. The expression of both proteins was strongly influenced by the light regime (6-24 hr illumination per day), the light intensity (0-450 E $m^{-2}s^{-1}$) and growth condition (photoautotrophic, mixotrophic and heterotrophic). Heterotrophic conditions resulted in relatively low recombinant protein yields per unit volume, despite high protein yields per cell, due to low growth rates. Mixotrophic conditions exhibited the highest yields at 6 hrs illumination at 200µE $m^{-2}s^{-1}$ and under continuous low light illumination (13-16 mg L^{-1} GFP and 1.2-1.6 mg L^{-1} GFP-PlyGBS), as these conditions supported good cell growth and cellular protein yields. A ~23-fold increase in protein accumulation per cell and ~9-fold increase L^{-1} culture was observed compared to standard constant 24 hr illumination for GFP-PlyGBS. The highest yields under photoautotrophic conditions were obtained under 9 hrs illumination (6 mg L^{-1} GFP and 2.1 mg L^{-1} GFP-PlyGBS). This represents a ~4-fold increase in cellular protein accumulation for GFP-PlyGBS. On a volumetric basis the highest yield was at 15 hrs illumination (~2-fold increase L^{-1} over the constant light for GFP-PlyGBS). Optimising illumination conditions to balance growth and protein expression can thus significantly enhance overall recombinant protein production in C. reinhardtii cultures.

Keywords : chlamydomonas reinhardtii, light, mixotrophic, recombinant protein
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