

## Phenotypic Enhancement of *Chlorella vulgaris* for Food Applications

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Due to their rapid growth and low water footprint heterotrophic microalgae have become an attractive source of sustainable plant based protein for the food industry. The green microalga *Chlorella vulgaris* in particular has gained a lot of attention due to the fact that it is one of the few microalgal species that are declared safe for human consumption. However, the green pigmentation of *C. vulgaris* can be detrimental when it comes to the use of its biomass as an ingredient in nutrition related applications where consumer perception of the final product is of importance. Hence, there has been a high demand for the development of *C. vulgaris* strains with reduced chlorophyll content that will not affect the final image or taste of the product.

Random UV-based mutagenesis was used for the creation and isolation of *C. vulgaris* mutant strains that have low chlorophyll content and high doubling times. 3 of the mutant strains were then cultivated in 1.5 L fermenter units using glucose as carbon source and under conditions close to an industrial scale production.

The 3 isolated mutants have low chlorophyll content and have doubling times which are equal or higher to that of the wild type ( $0.103 \text{ h}^{-1}$ ). Moreover, the mutants were able to reach much higher biomass concentration levels (#1: 3.6 g/L; #2: 4.2 g/L; #3: 4.4 g/L) than the wild type (1.5 g/L). In addition to that, the mutants have increased maximum volumetric lutein concentrations (#1: 14.2 mg/L; #2: 10.4 mg/L; #3: 11.7 mg/L) when compared to that of the wild type (3.4 mg/L).

The approach has proven effective for the phenotypic enhancement of *C. vulgaris* and the creation of 3 colorless strains with high biotechnological potential. Furthermore, the strains could also be cultivated as an alternative source of lutein and hence compete with conventional sources such as the marigold flower. The obtained results can be used for the further upscaling of the biomass production process.