Isolation and characterization of a motility-defective mutant of *Euglena gracilis*

Review

The authors present the results of the isolation and characterization of a novel low-motility strain of *Euglena gracilis.* They test several physiological parameters on it, stating that it has potential use for industrial applications. In general terms is a quite interesting study and the authors "created" the strain and show its potential uses and advantages. This research can have a high applicability and should be published.

There are minor changes required, which are stated below. Specially, there is overlapping between the materials & methods and results sections, with part of the methodology being described in the results, which makes the paper a bit hard to follow. It is recommended to clearly separate both sections, leaving all the methodological work and description of protocols in the material and methods part.

Introduction

Line 64-69.- seem that some references are missing.

Line 80.- "Based on this obstacle, few locomotion defective mutant strains have been identified and characterized." Reference please.

Materials and methods (M & M)

Line 110.- "Strains, culture, and media" More details on the upkeeping of the stock cultures would be useful to understand the physiological state of the algae due to the culture media, for example: initial inoculations, What was the maximum cell density allowed in the cultures? did the cultures reached stationary phase when renewed? etc.

Statistical section is missing, how were the analysis performed? Was necessary to convert any data?

Results

Line 222-. "Screening of motility-defective mutants of *E. gracilis*" That methodological description should be in the M & M section.

Line 259.- Why the shaking of the wild strains in KH medium would cause the loss of the flagella? The difference was significative in relation to the strain in CM medium, however this is mentioned only in the figure, should be also mentioned here and provide the t and p values.

Line 268.- Statistical analysis results are missing here. Is just mentioned significantly lower values but is unknown on what this claim is based on.

Line 271.- The lack of a carbon source in the CM media is not mentioned in the M & M section.

Line 289-299.- In Figure 4 standard error bars are provided and the difference is clear in the figure, however there is no statistical analysis conducted or its results provided. Please provide an analysis.

Line 301-311.- Part of that sentence should be in the material and methods section, this section should focus on the results that are mentioned just at the end of the paragraph.

Line 307-308.- How much weight for each strain-cell?

Line 314.- “acculmulation”

Line 352-354.- “These results suggest that the mutant is defective in the components related to flagellum motion and/or formation that are not critical for survival and proliferation” this statement applies to lab conditions only, it may be quite important in the environment.

Line 374.- “therefore, we surmise that they also show sufficient paramylon accumulation by nitrogen restriction, which enables the subsequent accumulation of wax ester” has been this observed in other species-strains? any reference to backup this statement?

Line 390.- “We hypothesized that the motility-defective mutants would save energy to move the flagella, which in turn would promote carbohydrate accumulation or faster growth. Although the mutant accumulated more carbohydrates than the wild type under autotrophic culture conditions, the results indicate the effect of energy conservation is not significant” The sentences contradict each other, will need clarification.

Figure 2.- The legend of figure 2 must be improved, is hard to understand what the authors are saying. Please be more concise, for example: Growth curves of the wild type (white) and M-3ZFeL (green) strains on heterotrophic KH (A) and autotrophic CM (B) media.

Figure 4.- Y axis is not clear in what units is expressed in the figure itself, please clarify axis.

The y axis legend must be improved, OD680 is not clear at all; also, the nm abbreviation is missing. Please describe better the axis, e.g.: Optical Density measured at λ 680 nm.

Figure 6.- Point out in the figure’s legend that the y axis scale is different in each figure.