Toward a genetic transformation system for the marine microalga *Emiliania huxleyi*

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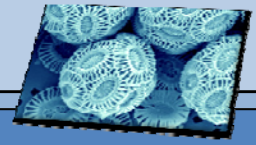

A start

The aim of this work was to establish a transformation system for the cosmopolitan coccolithophorid *Emiliania huxleyi* by accomplishing the following mandatory methods:

- Cultivation on agar plates.
- Development of a selection method for transformants.
- Construction of an *E.huxleyi*-specific expression vector.
- Cloning of a strong promoter to drive gene expression.
- Development of a transformation protocol.

Result

- *E.huxleyi* can grow on agar plates.
- Cells are sensitive to the antibiotics chloramphenicol, cycloheximide, G418 and puromycin.
- The modified diatom vector using zeocin resistance as selection marker gene was inoperative.

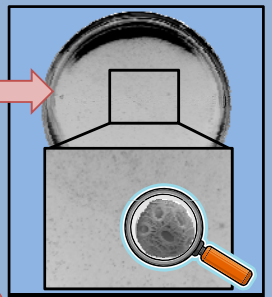

Fig.1: *E.hux* grows on agar plate.

Tab. 1: How antibiotics effect of *E.huxleyi*.

antibiotic	growth	concentration
Kanamycin	+	1 mg/mL
Streptomycin	+	1 mg/mL
Zeocin	+	1 mg/mL
Hygromycin B	+	1 mg/mL
Phleomycin	+	500 µg/mL
Blasticidin	+	200 µg/mL
G418	-	500 µg/mL
Choramphenicol	-	100 µg/mL
Puromycin	-	50 µg/mL
Cycloheximide	-	1 µg/mL

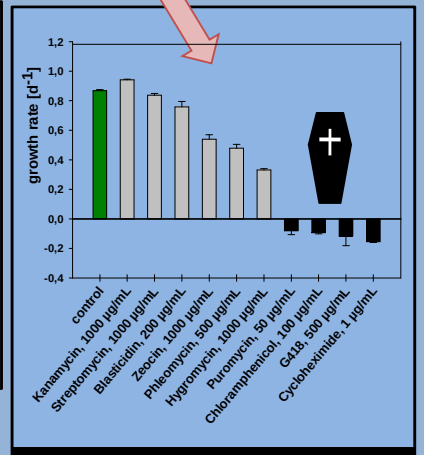

Fig. 2: Growth or motality rates of *E.huxleyi* treated with antibiotics.

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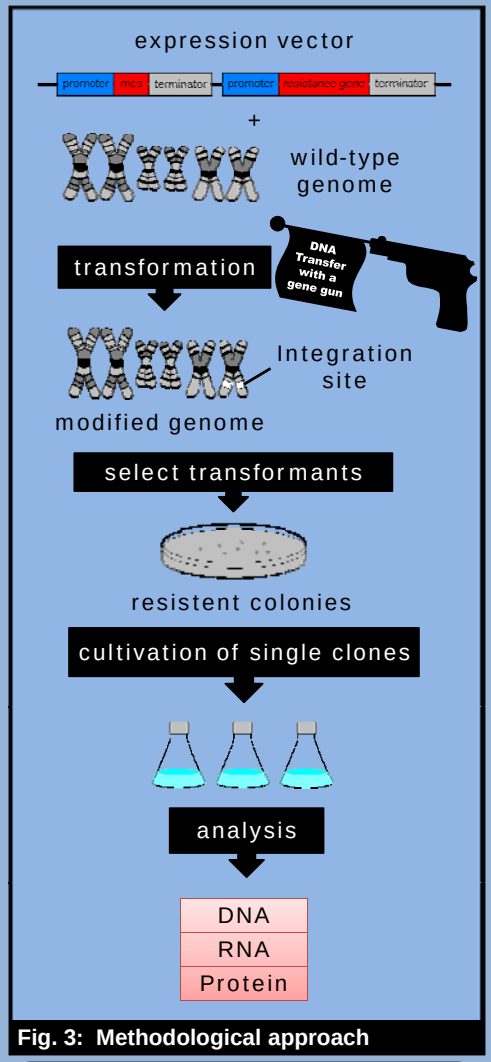

Fig. 3: Methodological approach

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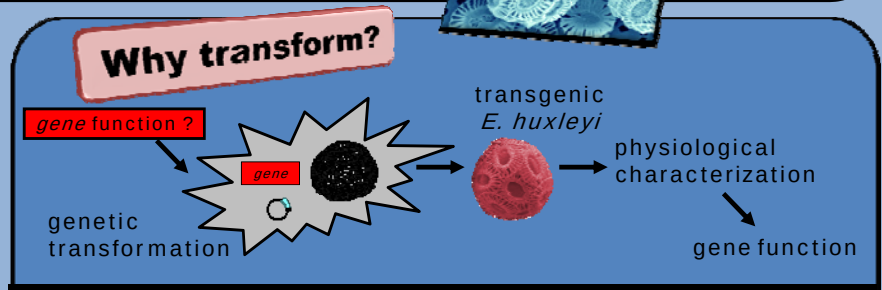

Establishing a transformation system will provide a powerful tool for functional genomics.

Problems

- To keep *E.huxleyi* cultures axenic.
- To choose the right selection marker gene and reporter gene.
- Develop a feasible methodological approach to select for transformants.

Conclusion

Providing a methodological approach to select transformants is a key towards the establishment of an *E.huxleyi*-specific expression vector and transformation system:

- Chloramphenicol, cycloheximide, G418, and puromycin and its respective resistance genes are of high potential use.
- G418 and its respective resistance gene is proposed to be most promising as it is already used in microalgae transformation systems[1,2].
- Feasibility of the biolistic approach[3] to transform *E.huxleyi* remains to be shown.

Future Perspective

- Map regulatory sequences (promoters).
- Design functional expression vector cassette.
- Develop a new transformation vector.
- Do further transformation experiments with a gene gun.

References

[1]Dunahay et al. (1995) *J Phycol* 31:1004-12; [2]Zaslavskaia et al. (2000) *J Phycol* 36:379-86; [3]Sanford et al. (1993) *Method Enzymol* 217:483-509