Glycerol Production by Novel Strains of *Dunaliella* and *Asteromonas* isolated from Namibian marine water

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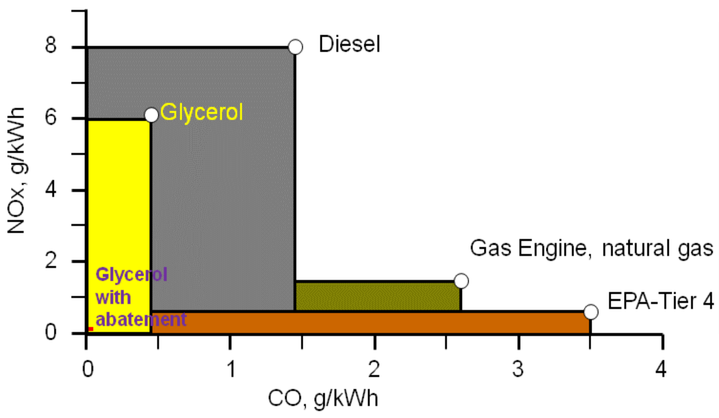
Aim

- To characterise halophytic microalgae isolated from marine water of Namibia as the first step in developing a low-cost system for producing industrial quantities of glycerol in pure streams from halophytic microalgae using process synthesis, modelling, optimization, and process flowsheeting methods.

Background and Innovation

Glycerol is a new biofuel which underpins a commercial CHP technology as a result of the novel McNeil combustion cycle patented by Aquafuel.

- Standard production compression ignition engines
- No addition of combustion enhancers necessary.
- Combustion more energy efficient than any known fossil, bio or synthetic fuel
- Engine performance with glycerol proven.
- No combustion particulate, no SOx, reduced primary NOx, extremely low VOC, aldehyde emissions, no catalyst poisons. Parameters independently verified.



Engine size	Electrical efficiency
10-50 kWe	37%
100-1000 kWe	42%
1000 – 10000 kWe	48%

Emissions	Amount
Particulate	Below detection limits
NOx	<0.1g/kWh possible
SOx	Below detection limits

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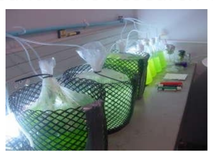
- Glycerol has safe handling properties: water-soluble, biodegradable
- Glycerol could power ships, tankers carrying glycerol as a distributable fuel with minimal risk to the marine environment.
- Glycerol will serve as a feedstock intermediate to replace fossil oil-based bulk chemicals (e.g. ethylene, propylene glycols, 1,4-butanediol, epichlorohydrin, acrolein)

- Glycerol is chemically produced from biodiesel manufacture using plant oil. But demand will outstrip Europe's capacity to produce glycerol from plant oil.
- Halophytic microalgae grow in highly saline environments (salt pans, desalination non-potable waters) and synthesise glycerol as an osmolyte.
- Algal glycerol does not require chemical modification or molecular reformation.
- Cultivation depends on saline, non-potable water

Halophytic microalgae at Sanumarc Research Centre



Methods

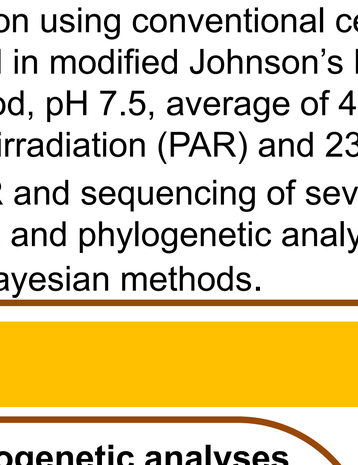
- Algal strains were collected from saline water of Namibia, isolated by the Plymouth Culture collection using conventional cell picking techniques and maintained and cultivated in modified Johnson's Medium (Johnson *et al*., 1968) at 12:12 photoperiod, pH 7.5, average of 4395Wm^2 photosynthetically active irradiation (PAR) and 23 0.2^{o}C temperature.
- Molecular Biology- PCR and sequencing of several genes was undertaken on the strains and phylogenetic analyses were performed using Neighbour-Joining and Bayesian methods.

- For glycerol induction cultures were maintained 14 d in medium with 1M NaCl then transferred to fresh media with varying NaCl concentration.
- Glycerol extraction was according to Ben-Amotz, Avron (1973). Glycerol contents were measured at 410 nm according to Chen *et al*., (2011).

Ben-Amotz, A., Avron, M. (1973) *Plant Physiol* **51**: 875-878
Chen, H., Lao, Y., Jiang, J. (2011) *Science of the Total Environment,* **409**: 1291-1297

Results

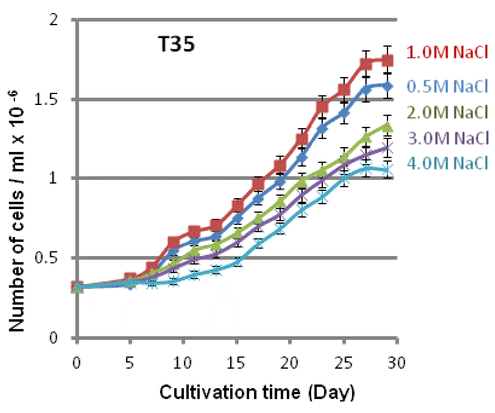
- **Sequencing and phylogenetic analyses of the 28S rRNA gene** identified :
  - T33a, T33b and T33c are most similar to the genus *Asteromonas*
  - T35, T36 and T37 are most similar to the genus *Dunaliella*.



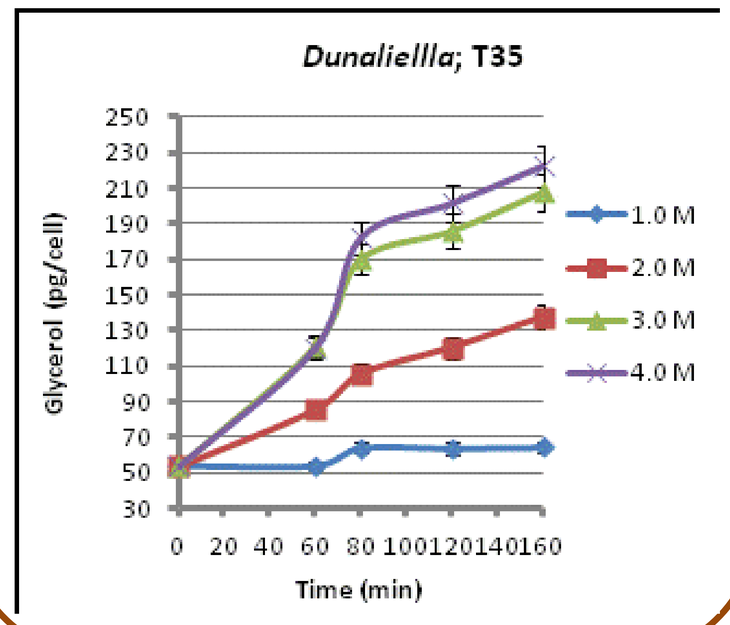
- Analysis of the ITS region showed T35, T36 and T37 were **genetically distinct** from other *Dunaliella* species, forming a unique group.
- Sizing the PCR products of the **18S rRNA gene** for T35, T36 and T37 indicated **no introns** were present for these three strains

- Growth curves for T35, T36 and T37 were similar (see for T35 below)
- Doubling times decreased ~5-fold to 0.7d at 30^{0}C compared to 23^{0}C

Strain	Specific growth rate μ (1M NaCl) (d^{-1})	Doubling time (d) (23C)
T35	0.144	4.8
T36	0.182	3.8
T37	0.164	4.2



- Maximum yield of glycerol was with exposure to 4.0 M salt for T35, T36 and T37



Conclusions

- *Dunaliella* and *Asteromonas* strains isolated from saline marine water in Namibia were genetically distinct from all other known *Dunaliella* strains
- Namibia strains of *Dunaliella* may be better adapted for growth at high temperatures (30^{0}C) than low
- Doubling times and specific growth rate μ provide the basis for modelling production systems to produce industrial quantities of glycerol in Namibia

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