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

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Glycerol has emerged as a new biofuel with important properties as a result of the novel Mcneil combustion cycle (Aquafuel Research 2010), which allows standard production compression ignition engines to combust glycerol at high efficiencies and with very low emissions without chemical alteration or the addition of combustion enhancers. It holds the key to developing an entirely new environmentally-sustainable, biofuel industry with enormous commercial potential in all fields of application of engine power production. Halophytic microalgae like Dunaliella show a high glycerol production potential. Our aim is to develop a low-cost system for producing industrial quantities of glycerol in pure streams from halophytic microalgae isolated from marine water of Namibia. In this study NaCl concentration had a strong effect on the growth rate and generation time of six strains belonging to two novel halophilic microalgae species *Dunaliella* (T35, T36 and T37) and *Asteromonas* (T33a, T33b and T33c) as confirmed by Bayesian and Neighbour-Joining analyses. Both species were shown to be capable of growth at different ionic strength of 0.5, to 4.0 M NaCl in a modified Johnson's culture medium; at 12:12 photoperiod, pH 7.5, average of 1500 lux light intensity and 23 ± 0.2^{o}C temperature. On exposure to hyperosmotic shock the amount of glycerol produced as a function of cell density greatly increased and was dependent on the applied NaCl concentration. After 28 days growth, for *Dunaliella* species the highest glycerol accumulation on a cell basis was obtained from T35 (203.2 ± 3.1 pg/cell) at 4.0 M NaCl, from T36 (177.0 ± 2.6 pg/cell) at 3.0 M NaCl and T37 (234.9 ± 10.0 pg/cell) at 4.0 M NaCl concentration. Ranges of 50 to 400 pg/cell glycerol have been reported by Ben-Amotz and Grunwald (1981) for Dunaliella grown at 0.5 to 4.5 M NaCl and 94.26 pg/cell glycerol for Dunaliella species from a hypersaline river of India (Phadwal and Signh (2003)). Similar to *Dunaliella* cells, *Asteromonas* accumulated 53.8 ± 0.8 to 209.5 ± 10.1 pg/cell in modified Johnson's growth media at 1.0, 2.0, 3.0 and 4.0 M NaCl concentrations respectively. Following hyperosmotic shock glycerol accumulated both with and without illumination, indicating that the precursor for glycerol synthesis during hyperosmotic stress is also derived from sources other than photosynthesis, most probably from starch breakdown.