Why *Chlorella* is important - Properties of *Chlorella Vulgaris* RE3921 Having Tolerance against Alkaline Condition -

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1. INTRODUCTION

Organisms which have tolerance against unusual conditions are important for utilization of their enzymes and biological remediation, *etc.* We recently found out an alkali-tolerance green algae in a pooled fresh-water at Ryujin-mura, Wakayama Prefecture, Japan. This algae can grow well under alkali conditions higher than pH 10.0. In this report, we tried to develop a cultivation method *in vitro*, identify its species name and analyze its cell-wall chemical composition in a direction to characterize its mechanism of alkali tolerance.

2. RESULS AND DISCUSSION

In vitro cultivation of the green algae was succeeded the best in VT medium, followed by C, p35 and MG media. The algae was single round micro cells ($\phi \fallingdotseq 4\,\mu$m in a mature state) and did not grow in MBM, MDM, SOT and MA media. Based on partial base sequences of rbcL (891bp) and rRNA 18S (1,756bp) the algae was identified to belong to *Chlorella vulgaris* and named as *C. vulgaris* RE3921. In the VT medium the algae can grow up to pH 11.8. Relationship between thickness of extra-cellular matrix (cell wall) and pH in the VT medium is shown in Figure 1. Thickness of cell wall increased from 0.40 μm at pH7.0 to 1.40 μm at pH11.8. Contents of protein and starch of the whole cells were 42.0% and 4.4% at pH 7.0 and 36.0% and 6.2% at

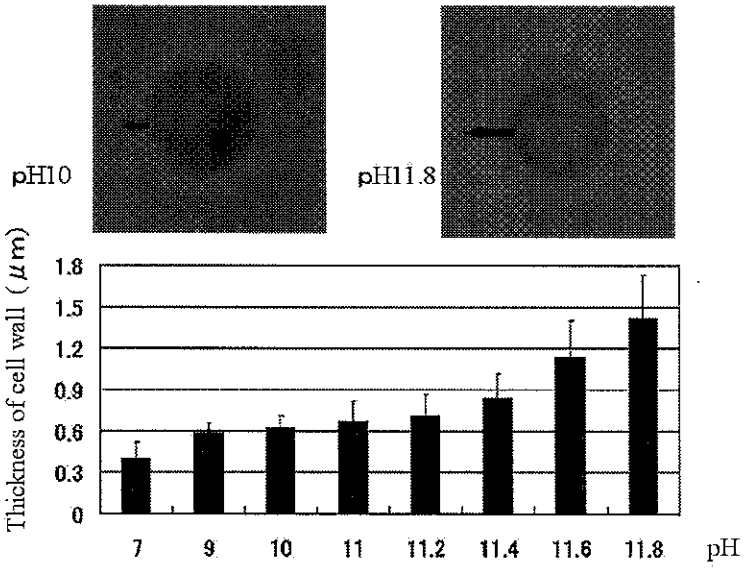

Figure 1. Dependence of thickness of cell wall of *C. vulgaris* RE3921 on pH of the growth media

pH11.0, respectively. Carbohydrate and X-ray diffraction analyses of the residue given after extraction of the whole cells with 80% aqueous acetone and treatment with glucoamylase indicate the lack of cellulose in the cell wall. In addition, FT-IR spectroscopic and solid state ^{13}C-NMR spectroscopic analyses of the same residue indicate higher protein content in comparison with carbohydrate in the cell wall. Therefore we checked if there are any differences in the cell wall protein profiles depending upon growth pH by SDS-PAGE. The result shown in Figure 2 indicates the presence of four proteins having molecular mass values 90kDa, 41kDa, 35kDa and 28kDa whose concentration increased at pH11.0.

In summary, the present results indicate that some proteins in the cell wall of *C. vulgaris* RE3921 were induced at high pH to participate alkaline tolerance. We are analyzing properties of these proteins.

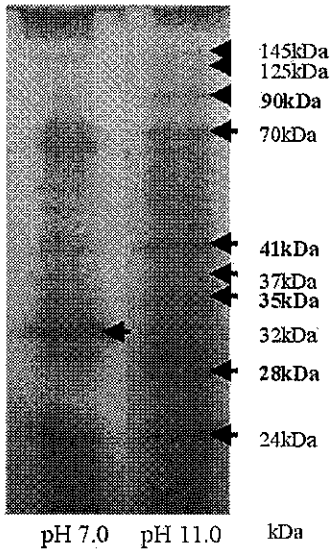

Figure 2. SDS-PAGE analysis of the cell wall proteins of *C. vulgaris* RE3921

(Left: profile grown at pH7.0,
and Right: profile grown at pH11.0)

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