[BIO08] Development of a transformation system for *Gracilaria changii* (Gracilariales, Rhodophyta), a Malaysian red alga via microparticle bombardment

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Introduction

Of the seaweed phycocolloids, agar has the higher price in the world market and is mostly extracted from red seaweeds. Critchley (1993) reported the world market value of agar as US $200 million. Hurtado-Ponce and Umezaki (1988) reported that the world's commercial agar comes mostly from gracilaroids. Agar extracted from Gracilariaceae became the first phycocolloid to be used in the human food industry (Armisen, 1995). *Gracilaria* has become an important agar source yielding 60-70% of the world's agar (Schramm, 1991). Chile and China contribute 10,000 tonnes of agar annually. However, this cannot meet the demands for high quality agarose used extensively in biotechnology research.

Malaysia has been importing agar from Korea, Japan, Hong Kong and China. In 1987, Malaysia imported 280 tonnes of agar worth more than RM 6.55 million (Jahara and Phang, 1990). In order for Malaysia to cope with the increasing demand for bacteriological agar in the coming years, there is a need to look into its own resources. In Malaysia, *Gracilaria changii* is one of the most abundant agarophytic seaweeds. Phang et. al. (1996) reported that Malaysian *G. changii* has potential commercial application in the agar industry due to its good quality agar and adaptability to the harsh mangrove conditions.

Genetic engineering and manipulation will have an important role in the advancement of algal biotechnology based industries (Tseng, 1984; Van der Meer, 1988). The development of an efficient genetic transformation system will contribute to the genetic improvement of this commercially important seaweed. The basis for establishing a seaweed transformation system, is development of an efficient transformation method and selection of suitable reporter genes and promoters (Qin et al. 1998).

Micro-particle bombardment and electroporation are commonly used in the transformation of marine microalgae and cyanobacteria (Matsunaga and Takeyama 1995; Toyomizu et al. 2001). However, microparticle bombardment is an effective and convenient method for transforming intact seaweed cells with cell walls (Qin et al. 1997, 1998, 1999). So far only a limited range of promoters have been employed in red seaweed transformation and only transient expression has been accomplished (Kubler et al. 1994). The most commonly used reporter gene is GUS gene (*uid A*). Transient expression of the *lacZ* reporter gene has only been achieved in *Laminaria japonica* (Qin et al. 1997), *Undaria pinnatifida* (Yu et al. 2002) and *Haematococcus pluvialis* (Teng et al. 2002) using SV40 promoter. Several studies on the application of SV40 promoter in obtaining stable transformation of macroalgae have been reported with *L. japonica* (Qin et al. 1997, 1998, 1999). Transgenic *Laminaria* with the hepatitis B surface antigen (HBsAg) gene has been obtained using SV40 promoter via particle bombardment (Jiang et al. 2002). In China, genetic and plant breeding has been applied in the cultivation industry of *Laminaria japonica*. The new varieties obtained yield 8-40% more biomass with 20-58% higher iodine content than the control plants (Wu and Lin, 1987).

With the genetic transformation system established for *Gracilaria*, genetic improvement can be carried out to optimise the exploitation of this commercial seaweed such as increase of biomass, improve agar/agarose quality, disease resistance, salt tolerance and other desire traits can be added to the seaweeds in culture. In future, there may be a possibility to use *Gracilaria* as bioreactors to produce vaccines, biochemicals and pharmaceuticals products.

Materials and methods

Microparticle bombardment

Gracilaria (Gracilariaceae, Rhodophyta) species were obtained from Morib, Malaysia.

The freshly collected samples were cleaned and growth in tank of Guillard enriched seawater. *Gracilaria* maintained under laboratory condition was cleaned and sterile with seawater containing 1.5% Potassium Chloride for 15 minutes. The plasmid construct of pSV40-*lacZ* (6821 bp, Promega, USA) was propagated in *Escheriachia coli* DH 5α, isolated by the standard alkaline lysis method (Ish-Horowicz and Burke, 1981) and purified using the QIAGEN-tip 100 column (Qiagen, Germany). Plasmid coated gold particles (Heiser, 1992) were bombarded into the thalli using the Biolistic PDS-1000/He system (Bio-Rad Laboratories, USA).

One µg DNA was used in each bombardment. For optimization, four different rupture-disc pressures of 4482, 6206, 7584 and 8963 KPa each at distances of 6cm was applied to the thalli. All experiments were conducted in duplicates. Thalli bombarded with uncoated gold particles were used as controls. 10 thalli were bombarded for each treatment.

The bombarded thalli of *Gracilaria* and controls were kept in the dark for two days. The *lacZ* expression was detected *in situ* by histochemical staining. Thalli were washed several times with autoclaved phosphate buffer saline solution (PBS, 0.1 mol L^{-1}, pH 7.0). Fixation was carried out using PBS containing $MgCl_2$ (1 mmol L^{-1}) and 1.25%(v/v) glutaraldehyde for 30 minutes and the thalli were then incubated in the staining solution [0.25w/v X-gal, 10 mmol L^{-1} PBS at pH 7.0, 1 mmol L^{-1} $MgCl_2$, 150 mmol L^{-1} NaCl, 3.3 mmol L^{-1} $K_4Fe(CN)_6$, 3.3 mmol L^{-1} $K_3Fe(CN)_6$] at 37°C for 4 h. The material was transferred to petri dishes and immersed in a layer of 50% (v/v) glycerol for microscopic observation.

Bombarded *Gracilaria* were cultured under laboratory conditions for one to two months. DNA was extracted from the bombarded thalli of *Gracilaria* and PCR verification was carried out using the specific primers detecting the *lac Z* gene.

Results and Discussion

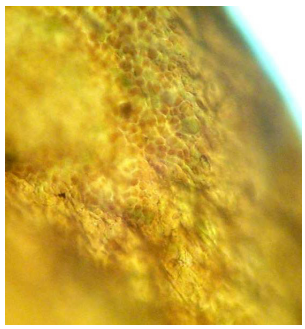
Histochemical staining was carried out to detect the expression of the *lacZ* gene. Transient expression of *lacZ* gene as indicated by a blue colour, was observed in thalli of *G. changii* bombarded under all four rupture-disc pressures applied (Figure 1a). Most of the blue spots were found on the surface area. However, very faint blue background was observed in the control thalli (Figure 1b). This raises the possibility that *Gracilaria* cells may exhibit some background colour. These faint greenish-blue spots can be differentiated from those expressed by the *lacZ* gene, which give more localised and larger spots (20 times more) with deeper blue colour.

The transformation efficiency (the percentage of the number of thalli with blue spots versus the total number of thalli bombarded) of the four rupture disc pressures of 4482, 6206, 7584 and 8963 KPa had mean values of 90%, 80%, 94% and 93%, respectively. Of the four rupture-disc pressures, 7548 and 8963 KPa gave the highest efficiency in transformation. However, the choice of which pressure to use depends on the thickness of the thalli being bombarded.

PCR verification for the integration of *lac Z* gene into the genomic DNA(s) of transformed *G. changii* via microparticle bombardment showed positive amplification of the *lac Z* (Figure 2).

The detection of *lacZ* gene in transformed *G. changii* shows the potential use of SV40 promoter in the genetic engineering of this red seaweed.

(a)



(b)

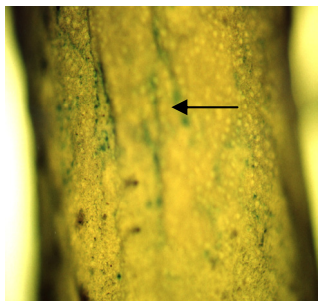


FIGURE 1 (a) Histochemical staining for detection of *lacZ* gene expression on untreated sample of *G. changii* (control); (b) the transient expression of *lacZ* gene on bombarded thalli of *G. changii*

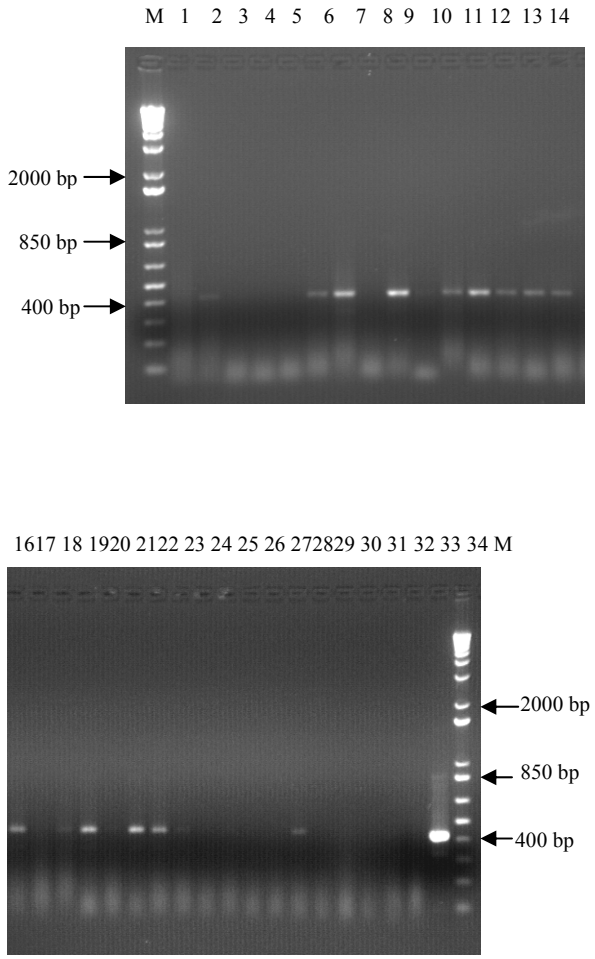


FIGURE 2 PCR analysis of bombarded thalli of *G. changii* via microparticcle bombardment. Lanes 1-33 contained PCR product from genomic DNA extracted from each respective thallus of bombarded *Gracilaria*; lane 34 contained PCR product from plasmid pSV40-*lac Z* (positive control). M: 1 k plus ladder DNA marker.

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