TRANSIENT EXPRESSION OF A LACZ TRANSGENE IN SHRIMP (P. schmitti) USING TWO DIFFERENT GENE TRANSFER METHODS

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Introduction

Microinjection for introducing DNA into one cell embryos have been widely used to obtain transgenic animals for different purposes. However, this technique requires complicated procedures to carefully manipulate a low number of embryos. Therefore, alternative methods have been used to introduce foreign DNA into animal embryos. Beakonization is a method for efficient transfer of molecules into living cells (1). Gene transfer methods have not been reported for crustacean. Here, we report on the transient transformation of the economically important shrimp (*P.schmitti*) by using microinjection and baekonization and extend previously reported results of our group (2).

Materials and Methods

Eggs collection

Experiments were performed in a production facility (Santa Cruz del Sur, Camagüey, Cuba). Ready to spawn females were placed in 30 L independent spawning tanks with filtered water. Fertilized eggs were collected and the jelly coat was removed by incubating the eggs in 0,3 % Urea in sea water at 25 °C for 3-5 min. Then, embryos were transferred to PBS 1X for manipulation.

DNA preparation

A circular plasmid (pCH110, 7,2 kb), which contains an $SV40_{\text{early promoter}}$ > lacZ reporter gene, was used. DNA was resuspended in NT buffer (10 mM Tris HCl, pH 7,5, 88 mM NaCl) and 0,25 % phenol red was added to visualize cytoplasmic microinjection.

Microinjection

Microinjection of unicellular embryos was performed in PBS 1X employing needles with tips ≈5 µm (outside diameter) pulled on a PN-Narishige micropipette puller. Holding pipettes with an internal diameter of ≈180 µm were pulled manually. Microinjection (≈9 nl, 10 ngDNA/µL) was performed at room temperature for 1 h, until the first cell division occurred. Under these conditions, about 25 to 30 eggs were injected. After microinjection, the embryos were incubated in a glass beaker with sea water.

Baekonization

Embryos were put into 1,5 mL Eppendorf tubes which were used as baekonization vials. Each vial was placed in the reactor where baekonization occurred. Fixed parameters included DNA concentration (50 ng/µL), volume (50 µL, 150 - 230 embryos), number of pulses (30/sec), pulse duration (5 - 8 sec) and distance between the anode and sample surface (d=1 mm). We varied amplitude (7,3 - 14,5 kV), burst time (0,5 - 4,0 sec) and cycles (1 - 4). After baekonization, the embryos were put in sea water until they reached the naupliu stage.

β-galactosidase (β-gal) assay

Late embryos and nauplius were fixed and stained as described before (3).

Results and Discussion

In microinjection experiments both non-injected and injected embryos reached the nauplius stage with 25-30 % survival. At this stage, 1-3 (out of about 30 injected embryos) showed β-gal activity. Baekonization experiments were conducted in various conditions. However, only those described here gave positive results.

Exp.	t(sec)	T(kV)	cycles	N	% survival	β-gal (%)
1	4	7,3	1(120 pulses)	154	15,6	4,2
2	control			140	21,4	—

Non-manipulated embryos, taken as negative controls, revealed no β-gal activity. In baekonization experiments, values between 6 to 71 % of survival were obtained with respect to the control group. The best survival rate was obtained with t = 0,5 sec in four cycles. However, under these conditions, no β-gal expression was observed.

Further studies will have to be conducted to find the optimal conditions for baekonization-mediated gene transfer in shrimp embryos, including the DNA concentration that seems to play a key role during this process (4). Nevertheless, these results strongly suggest that gene transfer is feasible in shrimps employing these methods.

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