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PRESENCE OF NUCLEASE ACTIVITY IN THE CHICKEN EGG WHITE COULD ATTENUATE CHICKEN TRANSGENESIS EFFICIENCY THROUGH SMGT

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Introduction

In the recent years, making the genetically modified organisms has been one of the most endeavor research fields. Among animal models, transgenic birds are one of the major candidates for production of biopharmaceutical proteins mainly because of having simple protein profile in their egg. So far, several methods have been used to generate transgenic birds including artificial insemination or *in ovo* injection. However, the results have not been satisfactory due to various technically and physiologically obstacles that remain to be overcome. Therefore, the purpose of current study was to survey the chicken egg white nuclease activity as a possible physiological hurdle that heretofore has been ignored.

Materials & Methods

Three experiments were conducted in this study. First, DNA from various sources including prokaryotic and eukaryotic genomic, linear DNA (1300 bp), three types of plasmids (3µg each) with different size (6-15 kbp) and eukaryotic RNA were incubated with or without 100µl egg albumen at 37.5 C° for 1 hour. Second, various incubation times, 1 to 24 h, for the above-mentioned DNA sources and albumen, were evaluated. Finally, the egg albumen (100µl) was treated with heat shock (65 C° for 10 min), EDTA (25 mM), or 20 ul *Proteinase K* (20 mg/ul for 12 hours at 37.5 C°), followed by incubation with 3µg plasmids at 37.5 C° for 1 h. The isolation of nucleic acids from the protein component of egg white was carried out by using phenol-chloroform method.

Results and Discussions

In the first experiment and just after one hour incubation, RNA completely removed and other various sources of DNA was digested and produced DNA fragments between 50 to 1000 bp. After 24 hours incubation time, fragments >50 bp was also digested to a single 50 bp sharp band. Thus, these results indicated that egg albumen has specific nuclease activities which could use both circular and linear DNA as the substrate. The results of the last experiment demonstrated that either heat treatment or EDTA inclusion completely inactivated the nuclease activities, whereas no significant inactivation was observed for *Proteinase K* treatment. In conclusion, detection of albumen nuclease activities could be a clue as an effective degradation factor for DNA molecules which may transiently bound to live sperm membrane through SMGT approach or freely available in the egg following in-ovo injection and ultimately leads to reduced transgenesis efficiency.

Keywords: Nuclease activity, Chicken egg white, SMGT, AI.