THE ISOLATION OF GROWTH HORMONE GENE FROM EGYPTIAN CATTLE

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Key words: Growth hormone, cattle, Gene Isolation, DNA/DNA hybridization.

Background:

The biotechnological industry depends upon genetic resources including plants, animals, yeasts and bacteria (Sawahel and Peachey, 1993). Because of their relative genetic and biochemical simplicity, ease of manipulation, and rapid growth rates, bacteria have been thoroughly studied. The gut bacterium ***Escherichia coli*** is still the most frequently used host for the recombinant DNA technology (Lukacsovich et al., 1990). The yeast ***S. cerevisiae*** is considered a better host cell for expressing growth hormone cDNA because (a) It is capable of processing post translational modification, (b) the gene product exists in a natural form.(c) it costs less of culturing than tissue culture. and (d) It is a safe organism which has been uses as food additive (Hayami et al, 1989). Growth hormone is one of the major important polypeptide hormones produced in the anterior pituitary cell to regulate growth and metabolism in vertebrates (Tsai et al., 1993).

Objectives:

The future prospect from this study is to inject the isolated growth hormone genes into fertilized ova of rabbits and sheep and to examine the end product after birth for size and the different morphological, physiological and economical characters. This will increase the national income and help to satisfy the shortage of protein in Egypt.

Methods:

For DNA isolation and purification, a high molecular weight DNA of cattle was extracted by Bendich and Bolton 1967 method. The DNA concentration was estimated for the O.D. reading at wave length 260 nm. O.D. ratios, 260 nm/280 nm (1.6-2) and 260nm/230 nm (1.8-2.2) indicate that the DNA solution is well purified (Charles, 1970).

For Recipient preparation. (NBL) and (YCM) media were used for growing ***E.coli B*** and ***S. cerevisiae*** 1 cells. After incubation for 48 hrs. at 37C° in case of ***E. coli B*** and at 30C° in case of ***S.cervisiae 1***, cells were collected by centrifugation.

For transformation techniques. (1) Direct method, pituitary gland DNA of cattle had been transformed to both strains (Souza et al. 1984; Ali and Afez, 1991a). By lithium acetate method,aid lithium to the direct method. The electroporation method were by using 45,60,75 and 90 seconds and for combined effect of electroporation and lithium acetate and electroporation.

For the detection of growth hormone transformants had been carried out by the method of (Brig et al. 1967).
The isolation of recipient and transformants DNA was extracted by bendicha dn Bolton's 1967 method.

DNA/DNA hybridization between each of, original and original strains, original and transformant strains, transformant and transformant strains in both case of ***E. coli B*** and ***S. Cerevisiae 1***. The method includes three steps: 1-the preparation of DNA agar. 2- The preparation of fragmented single-stranded DNA (Charles 1970) 3. Hybridization (El-Sharkawy et al. 1977).

The Retransformation with growth hormone gene was used by two methods: Direct method and Lithium acetate method.

For the utilization of dyes for growth hormone transformants detection by using orange G and α-naphthol dyes (Brigi et al. 1967)

Results and Discussion:

The efficiency of direct transformation indicated that no transformation were obtained without DNA while with direct transformation in the three experiments was slightly different 0.51%, 0.53% and 0.54% for ***E. coli B*** and 0.51% 0.51%, and 0.52% for ***S. cerevisiae 1*** respectively, Table 1. The direct transformation method gave the lowest percentage of transformation than the other described methods (Ali and Hafez, 1991a).

The effect of lithium acetate on the efficiency of transformation, it was noticed that transformation percentages were higher than that of direct transformation as it facilitates the entrance of DNA into the bacterial and yeast cells walls.

Table **1** Lithium cation was used in this research as it is the most effective of all cations tested to enhance transformation precess (Ito et al., 1983) than direct transformation.

The effect of electroporation on the transformation percentages were higher than that of direct transformation especially at the period of exposure of 1 min. (Shigekawa and Dower, 1988) Table **2**, it was clear that transformation ifficiency increases with increasing the time (60 sec.) over which the transformation percentages decreased gradually (Knutson and Yee, 1987, Hashimoto et al. 1985). The interpretation of hole formation mean that the holes induced in the bacterial envelope or yeast membrane during electroporation allow not only introduction of exogenous DNA but also leakage of cytoplasm (Fiedler and wirth, 1988). This supports our view that electroporation is a very promising alternative method for transformation.

The combined effect of lithium acetate and electroporation were higher than that of electroporation or lithium acetate treatment only (Table **2**). This result is in agreement, with the combined effects of temperature, polyethylene glycol (PEG) and heat pulse on ***S.cerevisiae 1*** yeast cells (Ito et al, 1983) and the combination between electric puls and (Li^+).

Conclusions:

The application prospect of this study is the injection of the isolated cattle GH gene from ***E.coli B*** or ***S. cerevisiae 1*** into rabbit or sheep embryos as a trial for producing large animals with good quality to satisfy protein human consumptions.

Literature:

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Table (2) : Number of competents, number of E. coli B and S. cervisia (1) transformants and their percentages and time of electroporation seconds.

Treatment	Time of electrop./ second	No. of competents		% survival		No. of transformants		% transformation	
		E. coli	S. cerevisiae	E. coli	S. cerevisiae	E. coli	S. cerevisiae	E. coli	S. cerevisiae
withjout DNA	0	573	595	100	100	0	0	0.00	0.00
DNA only	0	387	394	67.5	66.2	2	2	0.51	0.50
DNA + Elect	15	299	297	52.1	49.2	3	3	1.003	1.01
DNA + Elect	30	296	282	51.6	47.3	4	3	1.35	1.06
DNA + Elect	45	280	277	48.9	45.7	4	4	1.42	1.47
DNA + Elect	60	274	271	47.8	45.5	5	5	1.82	1.84
DNA + Elect	75	259	256	45.2	43.02	3	3	1.16	1.17
DNA + Elect	90	251	248	43.8	41.6	2	2	0.79	0.81
Without DNA	0	373	595	100	100	0	0	0.00	0.00
DNA only	0	387	394	67.5	66.2	2	2	0.51	0.50
DNA +Elec.	15	304	307	53.0	51.6	5	5	1.60	1.62
DNA+Elec.	30	387	281	50.0	47.2	6	6	2.09	2.14
DNA+Elec.	45	363	266	47.7	44.7	6	7	2.20	2.60
DNA+Elec.	60	244	259	42.5	43.5	8	9	3.30	3.50
DNA+Elec.	75	235	232	41.0	38.9	5	5	2.1	2.16
DNA+Elec.	90	227	221	39.6	37.1	3	3	1.3	1.36

Table (1) : Number of competents, number of ***E. coli B*** and ***S. cerevisaiae 1*** transformants and their percentages.

	Treatment	No. of competents		% survival		No. of trasnformants		% transformation	
		E. coli B	*S. cerevisiae*	*E. coli B*	*S. cerevisiae*	*E. coli B*	*S. cerevisiae*	*E. coli B*	*S. cerevisiae*
Direct	Without DNA	573	588	100	100	0	0	0.00	0.00
	DNA only Ex.1	387	392	67.5	66.6	2	2	0.51	0.51
	DNA only Ex.2	372	389	64.9	66.1	2	2	0.53	0.51
	DNA only Ex.3	369	383	64.3	65.1	2	2	0.54	0.58
DNA + Lithium acetate	without DNA	618	595	100	100	0	0	0.00	0.00
	DNA only	402	394	65	66.2	2	2	0.49	0.50
	DNA + Lith.1	303	293	49	49.2	3	5	0.90	1.70
	DNA + Lith.2	284	276	45.9	46.4	3	5	1.05	1.81
	DNA + Lith.3	246	268	39.8	45.0	2	5	0.81	1.86