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Transgenic cyanobacteria constructed as photosynthetic factories producing combined medicines

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Introduction

Cyanobacteria are a group of photosynthetic prokaryotes which carry on oxygenic photosynthetic similar to chloroplasts in eukaryotic algae and plants. Comparing with *Escherichia coli* gene engineering cyanobacteria used as hosts of medicine genes have several advantages as follows: no endotoxins mostly, no inclusion bodies, cheap photoautotrophy, rich nutrients, oral-taking, less problems of intellectual property rights (Shi, et al. 2000).

In the past eight years, five medical genes have been transferred to cyanobacteria and expressed successfully (Shi, et al. 2000); superoxide dismutase (SOD) for anti-oxidation (Takeshima, et al. 1994); mettallpthionein-I (MT-I) for reducing heavy metal toxicity (Sun, et al. 1994); tumor necrosis factor alpha (TNF-α) killing cancer cells (Lui, et al. 1999); and prourokinase (Pro-UK) as a thrombolyic agent (Luo, et al. 2000). Four of them have been completed at our group. Here the expression of TNF-α in *Ananbaena* sp PCC 7120 and its effects on the photosynthesis of the host cells is reported.

Materials and methods

Organisms and growth conditions

Anabaena sp.PCC7120, a filamentous, heterocystous cyanobacterium, was obtained from Pasteur Institute and cultivated in BG-11 medium. The plasmids pRL-489, RP4+pRL542 in *Escherichia coli* HB101 were kindly provided by Prof. C. P. Wolk (MSU-DOE).

Molecular studies

Changing SD sequence was by PCR method(Li, et al. 2000).The construction of the vectors was described as Zhang et al(2001).The transformation of the cyanobacterium was carried on with triparental conjugative transfer(Liu, et al 1999).. The detection radioimmunoassay(RIA) (Li, et al. 2000).

Photosynthesis and respiration measurements

Oxygen exchange was measured with oxygen electrode (Shi, 1987).

Results

The construction of two shuttle-expression vectors pMD-489-TNF_1 and pMD-489-TNF_2 with different SD sequences (Li, et al. 2000) and the transformation of *Anabaena* sp. PCC7120 were described as Zhang et al. (2001)

The expressions of TNF-α in two transgenic *Anabaena* were showed in Fig.1. The data indicated that the expressions of TNF-αchanged with cell growth and the maxima in two transgenic strains were appeared around 21th day. The expression of the vector pMD-489-$TNF_1$was 3.0% of soluble protein and 0.13% only for the vector pMD-489-TNF_2.

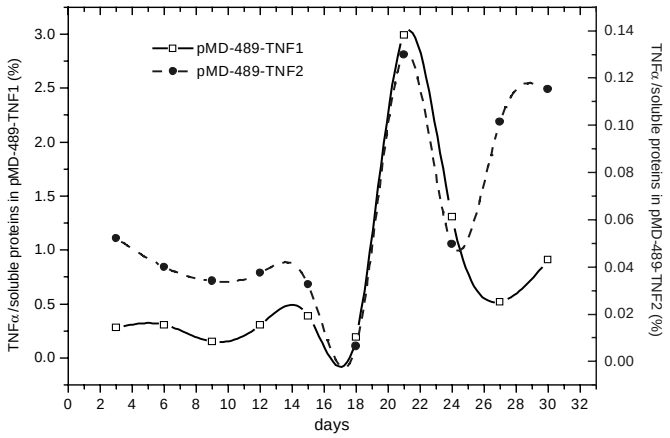

Fig.1. The expression of TNF-α in transgenic *Anabaena*

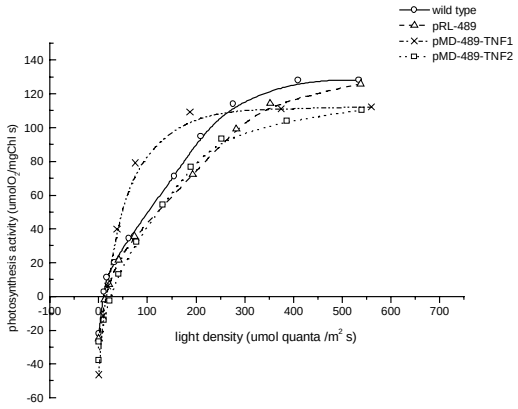

Fig.2. Light curves of photosynthesis in transgenic and wild *Anabaena* cells

The relationship between TNF-α expression and the responsibility for light was indicated in Fig.2 and Table 1. From these data when the expression of TNF-α was getting higher, the light saturation point was getting lower, and photosynthesis activities before the point were getting higher. This means TNF-α increases the responsibility for light. Meantime the respiration in transgenic cells was stronger than in wild type cells, and light complementary point was higher.

Table1. The responsibility for light in transgenic and wild *Anabaena*

	Wild type	Transformed by plasmid pRL-489	Transformed by vector pMD-489-TNF_1	Transformed by vector pMD-489-TNF_2
Saturation points (μmol/m^2.s)	290	350	250	75
Complement points (μmol/m^2.s)	9.35	14.517	24.87	15.52
Net photosynthesis (μmol/mgchl/h)	128	126	112	110
Respiration (μmol/mgchl/h)	22	25	39	38
True photosynthesis (μmol/mgchl/h)	150	151	151	149

The relationship between the expression of TNF-α and activities of two photosystems was showed in Fig. 3. It seems that there was no obvious relationship between TNF-α expression and true photosynthesis. For PSI, when the TNF-α expression maxima increased the maxima of PSI activity decreased. For PSII, the maxima of the activities appeared after the maxima of TNF-α expression.

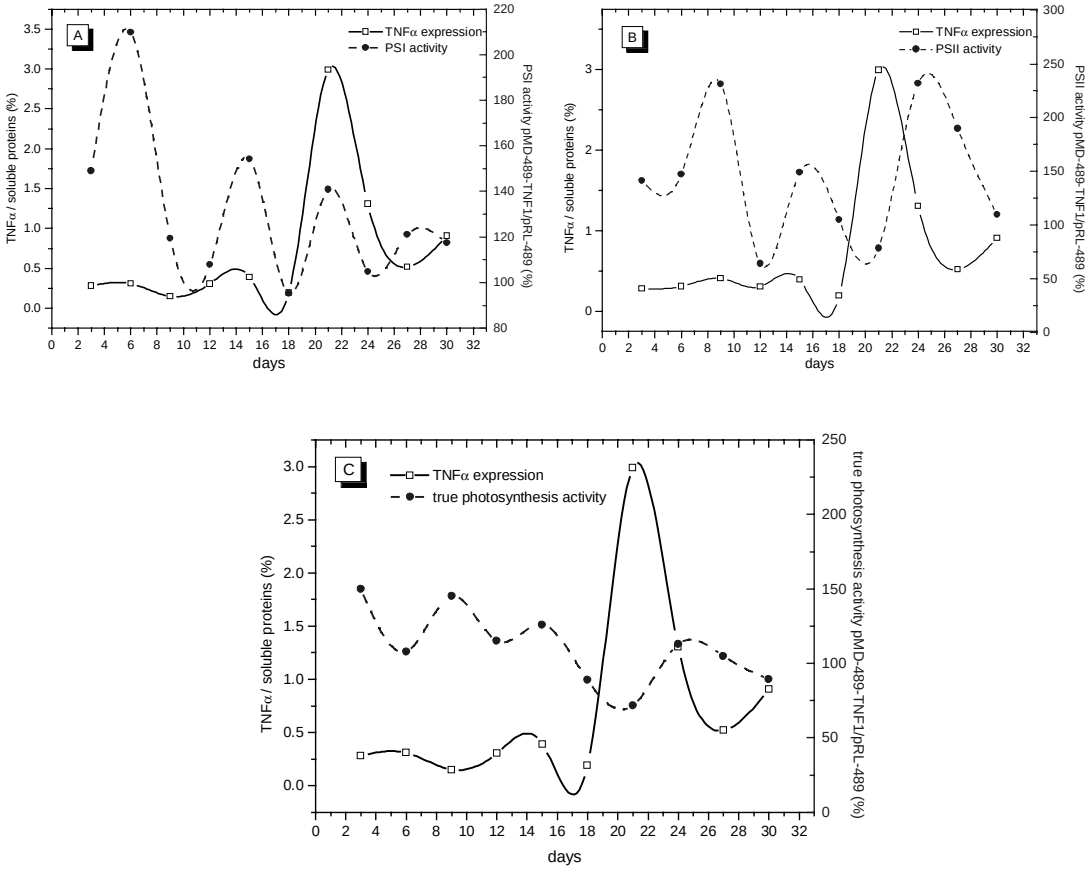

Fig.3. the relationship between TNF-αexpression and two photosystems activities

A. between TNF-α expression and PSI
B. between TNF-α expression and PSII
C. between TNF-α expression and true photosynthesis

Discussion

Using transgenic cyanobacteria as photosynthetic factories to produce combined medicines has been a novel way. Following understanding their molecular biology and photosynthesis physiology the factors will be constructed more and more in the future.

From this work it has been suggested that the expression of foreign genes of medicines was usually after cyanobacterial cell growth and regulated each other. Between expression and two photosystems the effects seemed positive, however between TNF-αexpression and true photosynthesis was not clear. It means that although TNF-αstimulated the photosynthetic electron transfer in two photosystems the photosynthesic oxygen evolution did not increase. Our data also showed the growth of transgenic cells lower than the wild cells. It needs to search where the electrons have been going to. The stimulation of light responsibility in transgenic cyanobacteria is an interesting event. In our group ,transformed *Anabaena* sp. PCC 7120 with other vector pIB-01 and pIB-02 or transformed *Synchococcus* sp. PCC 7002 with vector pKT-TNF possessed the same characteristics even after the saturation point exhibited the photoinhibition. All of the cells contained less phycobiliproteins. This can be suggested that TNF-α enhanced the light transfer between phcobilisomes and two photosystems. TNF-α has been proved that it can play a role of signal transductor in human cells(Newton, et al. 1999) and is it possible to become a light signal transductor in transgenic cyanobacterial cells?

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