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Effect of excessive cadmium chloride on the plasmids of *E. coli* HB101 in vivo

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Abstract—After *Escherichia coli* HB101 with plasmid pWH58, pWH98, or $pTBa_5$ were cultered respectively in amp LB broth which contained 50 mg/L $CdCl_2$ constantly for 24h, these plasmids were isolated from *E. coli*, and the effect of excessive $CdCl_2$ on the *E. coli* HB101 and plasmid DNA was studied by surveying the growth of *E. coli* HB101 and plasmid, argarose gel electrophoresis and analysis of restriction fragment length polymorphism (RFLP) of plasmids, and plasmid transformation. The results showed that 50 mg/L $CdCl_2$ treatment lagged the growth of *E. coli* HB101 for at least 4h, but after grown for 24h there were not significant differences in the growths of *E. coli* HB101s and the productions of plasmids between the treatment and control. These results implified that *E. coli* HB101 have induced adaptability to cadmium stress and excessive $CdCl_2$ did not inhibit the replication and amp^r gene's expression of plasmid DNA in vivo of *E. coli* significantly. 50 mg/L $CdCl_2$ treatment for 24 hours might cause the sequence's change of plasmid DNA, but could not lead to the random breakage of plasmid DNA strands. Moreover, after 50 mg/L of $CdCl_2$ treatment in vivo the transformation activities of plasmid did not altered, implied excessive $CdCl_2$ could not affect the superhelical structure of plasmid and also not break the loop of plasmid DNA evidently.

Keywords: $CdCl_2$, plasmid DNA, restriction fragment length polymorphism(RFLP).

1 Introduction

Cadmium is one of important environmental pollutants. It is very toxic to biology (Barber, 1994; Collard, 1990; Goyer, 1995; Nassiri, 1996, Pandey, 1994; Willuhn, 1996). The carcinicity and mutagenicity of cadmium is the researche's focus of cadmium's toxicity all along (Antila, 1996; Frank, 1993; Oberdoerster, 1989; 1995; Sunderman, 1990). However, we still have very less understanding on the mechanism of cadmium's genotoxicity. DNA is genetic material of biology, so research direct on the effect of cadmium on DNA in vivo should be very important to understanding genotoxicity of cadmium.

plasmid is one of extrachromosomal genetic elements. It is double-stranded, closed circular DNA molecules, and can replicate independently. Especially to the relaxed plasmid there are 10—200 copies of the plasmid in a host cell, and *E. coli* HB101 grow fast, so taking plasmid as the experimental material could gain the information of the long-term stress of cadmium on DNA in a relatively short term. Moreover, plasmid exist in cytoplasmic, it have not the protection of histone, so it is more easy to plasmid be attacked by pollutants. Plasmid is an ideal molecular model for the study on genotoxicity of pollutants.

2 Materials and methods

Escherichia coli HB101 was supplied by Prof. Sun Zhiling in West China Medical University, China.

Plasmids: In this research three plasmids were applied. They are pWH58, pWH98 (the two plasmids were cloned by the authors from the wheat DNA), and $pTBa_5$ (the plasmid was presented by Prof. Hu Zhong and Hu Yunqian in Kunming Institute of Botany, Chinese Academy of Sciences). All of the three plasmids are recombinant ones with pBR322 as vector.

Treatment of $CdCl_2$: After transformation the three plasmids were transfered respectively into *E. coli* HB101 by the $CdCl_2$ procedure (Meng, 1996). 200μl activated, single-cloned *E. coli* HB101s with different plasmid in amp LB broth was inoculated separately into 200 ml the amp LB broth which contain 50mg/l. $CdCl_2$ and the corresponding control amp LB which do not contain any pollutant(per liter amp LB broth contain: bacto-tryptone 10 g, bacto-yeast extract 5 g, NaCl 10g, amp 50 mg/L pH 7.2—7.5; Sambrook, 1989). Then they were cultured in shacking of 100 r/min at 37℃ constantly for 24h. During culture surveyed the growth of *E. coli* HB101 by measure its D_{600nm} using 721A spectrophotometer at 4h, 8h and 24h.

Isolating of plasmid: After the plasmids had grown in *E. coli* HB101 for 24h they were isolated by lysis alkali procedure(Sambrook, 1989) at the same time and were dissolved in 400μl 1×TE buffer, then were determined by U. V. spectrophotometer. Finally, these plasmids were stored at 4℃.

Agarose gels electrophoresis of plasmids: The isolated plasmids in the above step were run through 0.7% agarose gel immediately. The amount of plasmids which were put in electrophoresis was 2μl per lane. The voltage was 5 V/cm. The concrete manipulation see Sambrook(Sambrook, 1989).

Plasmid DNA's RFLP: The isolated plasmids were digested respectively, with excessive restriction enzyme (10u/μg DNA), TaqI (at 65℃), HindIII or BamHI(at 37℃) overnight. The results were measured through 2% or 0.7% argarose gel electrophoresis. Recorded the results by ultraviolet illumination.

Plasmids transformation: The above isolated plasmids were separately introduced into *E. coli* HB101 by the $CaCl_2$ procedure (Meng, 1996) at the same time with the same competent *E. coli* HB101. All transformation condition kept constant. In a transformation experiment the amount of competent *E. coli* was 200 μl, plasmid was 1 μg. Finally, counted up the transformation activities of the plasmids.

All the above experiments were repeated twice.

3 Results and discussion

3.1 The effect of $CdCl_2$ on the growth of *E. coli* HB101 and plasmid

Table 1 shows that the stress of $CdCl_2$ lagged the growth of *E. coli* HB101. Comparing with the corresponding control the lag phase is about 4h, but after growing for 24h there was not significant difference in the growth of *E. coli* HB101 and the production of plasmid between $CdCl_2$ treatment and the corresponding control.

Table 1 The effect of $CdCl_2$ on the growth of *E. coli* HB101 and plasmid*

t, h	pWH58(D_{600nm})		pWH98(D_{600nm})		pTBa$_5$(D_{600nm})	
	CK	Cd	CK	Cd	CK	Cd
4	0.493	0.084	0.512	0.076	0.478	0.094
8	1.090	0.305	1.150	0.314	1.120	0.291
24	1.620	1.470	1.641	1.508	1.590	1.514
The production of plasmid, mg plasmid/L bacteria						
	1.205	1.193	1.184	1.168	0.983	1.076

* The average of two experiment results

3.2 The argarose gel electrophoresis of the plasmids

Fig. 1 shows that (1) after $CdCl_2$ treatment in vivo for 24h the electrophoresis migration rate of plasmid $pTBa_5$ increased evidently, but those of plasmid pWH98 and pH58 did not change significantly in comparison with the corresponding control; (2) after $CdCl_2$ treatment all the electrophoresis migration sites of the three plasmids were concentrative and no one of the migration binds of the plasmids had an obvious tail.

3.3 The RFLP analysis of the plasmids

Fig.2 shows that after $CdCl_2$ treatment all the restriction fragments of TaqI, HindIII and BamHI of plasmid $pTBa_5$ altered obviously, but those of plasmids pWH58 and pWH98 did not change significantly in comparing with the corresponding control. Moreover, the results also demonstrated $CdCl_2$ treatment did not increase the noise of the restriction fragment in argarose gel electrophoresis.

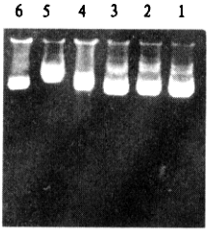

Fig. 1 The argarose gel electrophoresis of plasmid (0.7% agarose gel)
Lane 1, 3, 5: CK; 2, 4, 6: $CdCl_2$; 1, 2: plasmid pWH58; 3, 4: plasmid pWH98; 5, 6: plasmid $pTBa_5$)

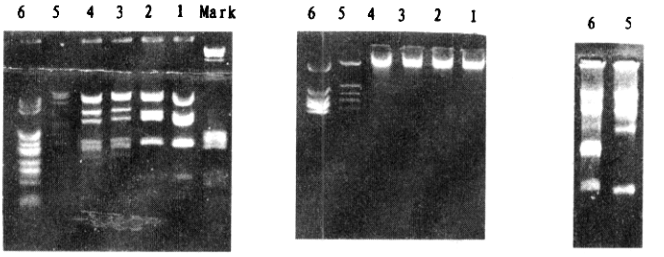

Fig. 2 The electrophoresis pattern of the plasmids by the digestion of restriction enzyme(2A, 2B: 2%, 2C: 0.7% argarose gel)
Lane 1, 3, 5: CK; 2, 4, 6: $CdCl_2$; 1, 2: plasmid pWH58; 3, 4: plasmid pWH98; 5, 6: plasmid $pTBa_5$

3.4 The transformation activities of the plasmids

The results are shown in Table 2.

Table 2 The effect of $CdCl_2$ on the transformation activities of plasmid*

	pWH58	pWH98	$pTBa_5$, clone/μg DNA
CK	1.6×10^4	1.7×10^4	1.45×10^4
$CdCl_2$	1.35×10^4	1.55×10^4	1.35×10^4

* The average of two experiment results

The results in Table 2 demonstrate that $CdCl_2$ treatment did not affect the transformation activities of the three plasmids evidently.

The results of *E. coli* HB101's growth displayed that cadmium inhibited the initial growth of *E. coli* HB101 but *E. coli* HB101 almost could grow as well as the control after grown under the cadmium stress for a long term. It implied that *E. coli* HB101 have induced adaptability to cadmium stress. Under cadmium stress *E. coli* HB101 with plasmid could grow in amp LB broth demonstrated that the amp + gene of plasmid could express in the existence of excessive $CdCl_2$. The results of plasmids' productions also showed the excessive $CdCl_2$ in vivo did not hold back the plasmids' replications significantly. Moreover, the above results showed the excessive $CdCl_2$ in vivo could result in the alteration of the cutting site of restriction enzyme. It demonstrate the cadmium has mutagenicity to plasmid DNA, but cadmium do not break DNA strand randomly. The

transformation activity of plasmid is relative to its conformation, and the superhelical plasmid has the highest transformation activity. $CdCl_2$ treatment in vivo did not affect the transformation activities of plasmids obviously implied that $CdCl_2$ did not affect the superhelical structure of plasmid and also did not damage the loop of plasmid DNA.

This research demonstrate the mutagenicity of cadmium at DNA molecular level, but the mechanism of the effect of cadmium on DNA still need further research.

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