PERIODICO di MINERALOGIA Volume 88, No. 3, 2019

Production of L-Asparaginase II by Recombinant *Escherichia Coli* Cells

C. P. CORNEA*, I. LUPESCU*, I. VĂTAFU*, T. CARAIANI**, V. G. SĂVOIU**, GH. CÂMPEANU*, I. GREBENIŞAN*, GH. P. NEGULESCU*, D. CONSTANTINESCU*

** Faculty of Biotechnology, Bd. Mărăşti No.59, 71331 Bucharest, Romania*
*** National Institute for Chemical-Pharmaceutical Research and Development, Bucharest, Romania, Calea Vitan 112, sector 3, 74373*

Abstract

The current paper presents some studies focused on the development of an experimental system to overproduce L-asparaginase II in two local isolated E. coli strains (E. coli ICCF20 and E. coli IBB13) using recombinant DNA methods in order to transfer a DNA fragment able to improve the level of enzyme activity. Chromosomal DNA from E. coli IBB13 was isolated and cleaved with Bam HI restriction enzyme. The Bam HI restriction fragments were cloned in pUC19 and the recombinant molecules were used for the transformation of E. coli HB101. The recombinant plasmids isolated from E. coli HB101 transformants (24 transformants resistant Ap^r Lac^-) were isolated and used for the transformation of E. coli E13 and E. coli E20. The efficiency of transformation with recombinant plasmids was reduced; all the colonies grown on selective medium were tested for L-asparaginase production, by qualitative and quantitative assays. Six of the transformants have shown a level of L-asparaginase II higher with 63-117% than the parental strains.

Keywords: L-asparaginase, biosynthesis, *E. coli*, recombinant DNA, recombinant plasmids, recombinant strains.

Introduction

L-asparaginase is a relatively wide spread enzyme, found in many animal tissues, bacteria and plant and in the serum of certain rodents but not of man. Increasing interest in L-asparaginase stemmed from its identification as the antilymphoma factor of Kidd (Broome, 1961). Although this enzyme has been isolated from a number of microbial and vegetal sources, only L-asparaginases derived from *E. coli* and *Erwinia carotovora* have anti-tumor activity, particularly in acute lymphoblastic leukemia, being beneficial antineoplastic agents when used in sequential chemotherapy. The fact that not all L-asparaginase species possess anti-tumor properties seems to be related to the affinity of the enzyme for the substrate and to factors affecting the clearance rate from the system. Of the two asparaginases from *E. coli* (EC-1 and EC-2) only one (EC-2) possesses antilymphoma activity, being the most extensively studied. This isoenzyme also differs from EC-1 by its broad pH activity profile and its higher substrate affinity.

A large number of investigations have been performed in order to clarify the genetic determinants involved in EC-2 biosynthesis and regulation, but there are few reports concerning the improvement of enzyme production.

The aim of our experiments was the development of an experimental system to overproduce EC-2 in two locally isolated *E. coli* strains (*E. coli* ICCF 20 and *E. coli* IBB 13). For this purpose, a two-step approach of genetic modification was used: first, selection of *E. coli* strains harboring recombinant vectors (pUC19 containing a BamH1 restriction fragment) and then, reisolation of the recombinant vectors and their use for the genetic transformation of *E. coli* ICCF 20 and *E. coli* IBB 13.

Material and Methods

The bacterial strains used in our experiments are listed in (**Table 1**).

Table 1. Bacterial strains used in experiments

Strain	Characteristics	Source
E. coli ICCF20	L-asparaginase producer	ICCF Bucharest
E. coli E13	L-asparaginase producer	Faculty of Biotechnology Bucharest
E. coli HB101	Lac^-, Ap^S	Faculty of Biotechnology Bucharest
E. coli HB101	PUC19, Ap^R	Faculty of Biotechnology Bucharest

The source of chromosomal DNA used in transformation protocol was *E. coli* E13 strain, a good L-asparaginase producer. *E. coli* chromosomal DNA isolation was performed according to the method presented by Cornea and Barbu (1998). The isolation of pUC19 and the construction of recombinant molecules were done as I. Karcher (1995) indicated.

The genetic transformation procedure of competent cells and the selection of recombinants were performed according to Sambrook et al. (1989).

The purity and the quantity of the DNA were determined spectrophotometrically and electrophoretically.

Chromosomal DNA from *E. coli* IBB 13 was isolated and cleaved with Bam HI restriction enzyme. The Bam HI restriction fragments were cloned in pUC19 and the recombinant molecules were used for the transformation of *E. coli* HB101. The recombinant plasmids isolated from *E. coli* HB101 transformants (24 transformants Ap^R and Lac^-) were isolated and used for the transformation of *E. coli* E13 and *E. coli* E20.

Results and Discussion

The efficiency of transformation with recombinant plasmids was reduced; all the colonies grown on selective medium (24 for each recipient *E. coli* stains) were tested for L-asparaginase production. Both qualitative (phenol red assay) and quantitative (Nessler reagent) tests were performed. Six of the transformants have shown a level of L-asparaginase II higher with 29-150% than the parental strains (**Table 2**).

According to Bonthron (1990), the *ans*B gene in E. coli is located on a Bam HI restriction fragment. The cloning of *ansB* gene from the *E. coli* stain and its nucleotide sequence was also described by Jennings et al. (1990) but an efficient strategy for the improvement of L-asparaginase producing strains was not yet developed.

For this reason, the goal of our experiments was to isolate DNA fragments, to clone them in pUC19, to transfer them into E. coli host and to analyse the effects of this transfer on the L-asparaginase level.

After the transformation of *E. coli* HB101 with recombinant DNA (pUC19) cleaved with Bam HI ligated with Bam HI fragment derived from *E. coli* E13 (chromosomal DNA), 24 Ap^r Lac^- colonies were selected on selective medium containing ampicilline and X-gal and IPTG (**Figure 1**).

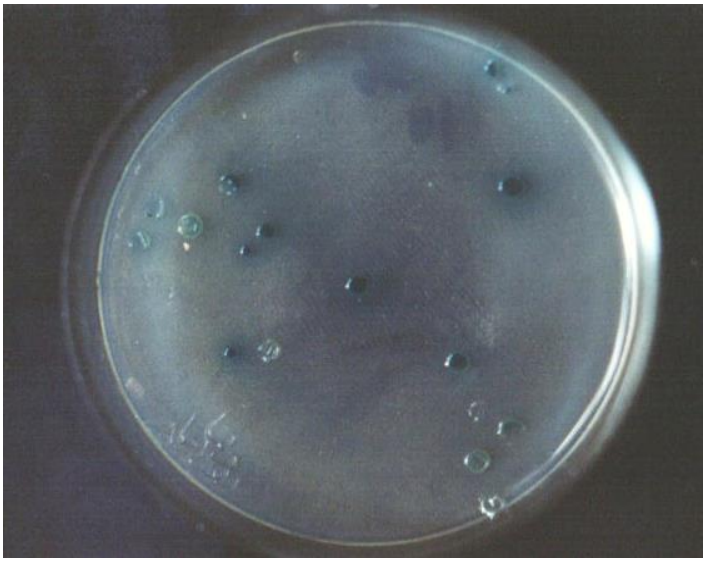

Figure 1. The selection of E. coli HB101 tranformants containing recombinant DNA molecules

The recombinant vectors from these colonies were reisolated and transferred both in *E. coli* ICCF20 and *E. coli* E13. All the 48 types of colonies Ap^r obtained after transformation (24 types for each recipient strain) were tested for the presence of the recombinant plasmid and for the L-asparaginase production.

The plasmid content analysis has shown that the recombinant strains contain a larger plasmid, comparable to the original pUC19 (**Figure 2**). However, the size of Bam HI restriction fragment inserted in pUC19 is smaller than reported in the literature (4-5kb instead of 13-14kb). For this reason, at this moment we are not able to say whether *asnB* gene was inserted in the recombinant plasmids or some regulatory sequences, which improved the level of L-asparaginase.

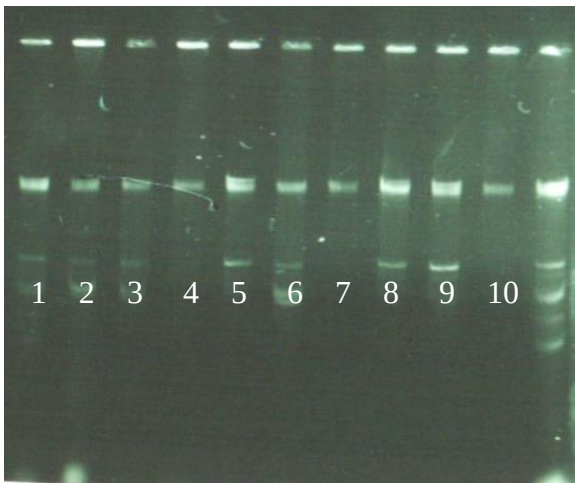

Figure 2. Electrophoresis pattern of recombinant plasmids isolated from different E. coli tranformants: lanes 2, 5 and 8 – E.coli ICCF 20, lanes 3, 6 and 9 – E. coli E13, lanes 1 and 10 – pUC19

Both qualitative (phenol red assay) (fig. 3) and quantitative (Nessler reagent; the value of the pH was 5.0, specific for L-asparaginase II but not for L-asparaginase I) tests were performed.

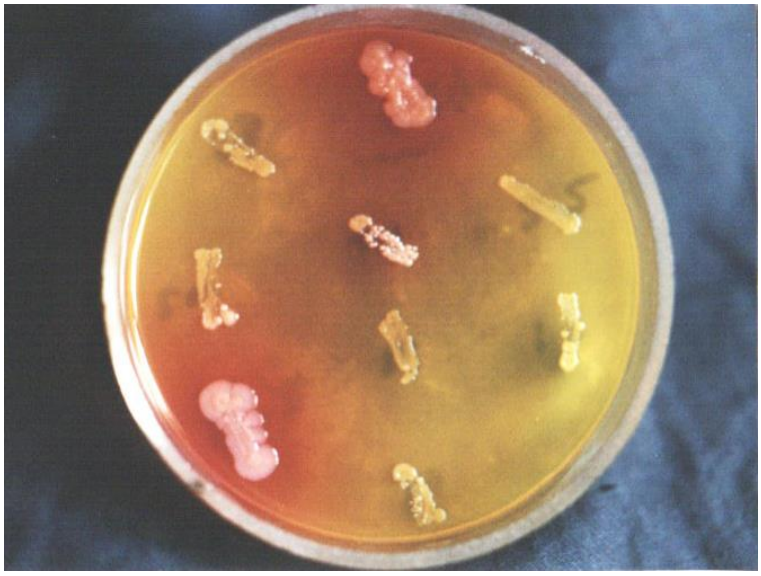

Figure 3. Qualitative assay of L-asparaginase activity recombinant E. coli strains

Six of the transformants have shown a level of L-asparaginase II higher with 63-117% than the parental strains (**Table 2**). The observation that the same recombinant plasmid induced different response in the recipient strains was surprising: the transformants derived from *E.coli* E13 presented a higher activity (**Figure 4**) as compared to those derived from *E. coli* ICCF20 (**Figure 5**) and with parental stains.

Table 2. L asparaginase activity of parental strains and of the transformants

Strain	O.D.$_{550}$	Enzymatic activity (U/ml/min)	Increase of the activity (%)
E. coli E20 (parental)	0,980	3,60	-
E. coli E13 (parental)	0,920	3,00	-
E. coli T20.6 Ap^r (transformant)	0,970	6,28	74
E. coli T13.6 Ap^r (transformant)	0,920	4,89	63
E. coli T20.7Ap^r (transformant)	0,750	6,81	89
E. coli T13.7Ap^r (transformant)	0,910	6,43	114
E. coli T20.17Ap^r (transformant)	0,910	6,68	85
E. coli T13.17Ap^r (transformant)	0,940	6,51	117

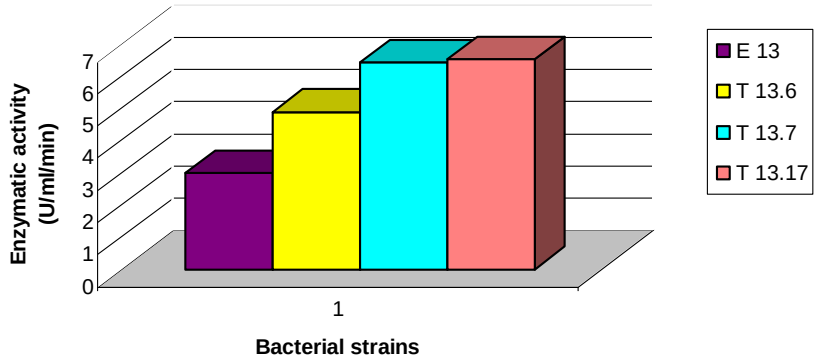

Figure 4. The improvement of L-asparaginase activity of E. coli E13 by genetic transformation with recombinant DNA

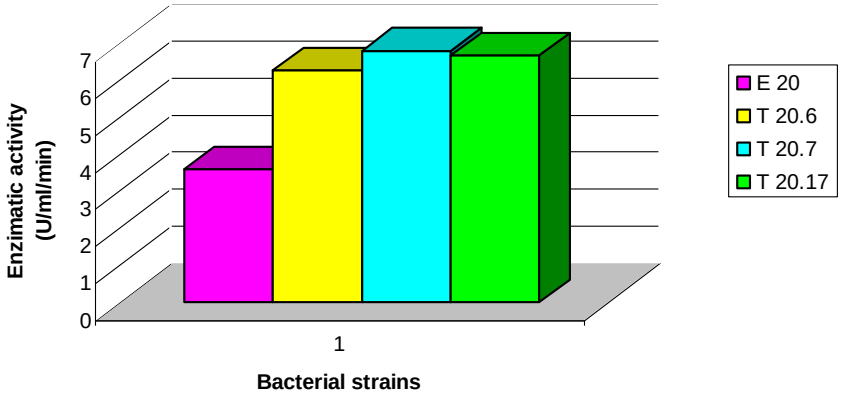

Figure 5. The improvement of L-asparaginase activity of E. coli ICCF 20 by genetic transformation with recombinant DNA

Conclusions

The purpose of our works was to establish an experimental model based on recombinant DNA, useful in improving L-asparaginase biosynthesis in *E. coli*. Some conclusions could be presented:

1. The concentration and the purity of chromosomal and plasmidial DNA was high enough for restricting enzyme digestion, ligation and transformation.
2. 24 recombinant *E. coli* HB101 strains were obtained and the recombinant plasmids they harbor were isolated and transferred in two *E. coli* recipient strains.
3. Six of the transformants have shown a level of asparaginase II higher with 63 -117% than the parental stains.

Acknowledgement

Partial support of this work by the Ministry of Education and Research is gratefully acknowledged.

References

1. D.T. BONTHRON, 1990, *Gene*, **91**,.101-105
2. C.P. CORNEA, A.BARBU, 1998, *Lucrări practice de inginerie genetică*, Tipografia USAMV Bucureşti
3. C.P. CORNEA, I. LUPESCU, I. VĂTAFU, V.G. SĂVOIU, G. CÂMPEANU, 2000, *Roum. Biotechnol. Letts.*, **5**, 471-478
4. T. DEL CASALE, P. SOLLITTI, RH. CHESNEY, 1983, *J. Bacteriol*., **157**, 513-515
5. R. GULATI, R.K. SAXENA, R. GUPTA, 1997, *Lett. Appl. Microbiol*., **24**, 23-26
6. E. HARMS, A. WEHNER, M.P. JENNINGS, K.J. PUGH, I.R. BEACHAM, K.H. ROHM, 1991, *Protein Expression and Purification*, **2**,.114-150
7. M.P. JENNINGS, I.R. BEACHAM, 1990, *J. Bacteriol*, **172**, 1491-1498
8. KARCHER, S.J., 1995, *Molecular Biology. A project approach,* Academic Press
9. J. SAMBROOK, E. F. FRITSCH, T. MANIATIS, 1989, *Molecular Cloning. A laboratory manual*, Cold Spring Harbor Laboratory Press