Mutation Research, 64 (1979) 249—258

THE L-ARABINOSE-RESISTANCE TEST WITH *SALMONELLA TYPHIMURIUM* STRAIN SV3 SELECTS FORWARD MUTATIONS AT SEVERAL *ARA* GENES

CARMEN PUEYO * and JUAN LOPEZ-BAREA **

Laboratory of Environmental Mutagenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 (U.S.A.)

(Received 13 March 1979)
(Accepted 5 April 1979)

Summary

A new assay has been described for mutagenicity testing using an L-arabinose-sensitive strain of *Salmonella typhimurium*. The test strain SV3 and several L-arabinose-resistant mutants selected therefrom are characterized in the present study by 3 different criteria: inhibition of growth by L-arabinose, accumulation of keto-sugars, and activities of the enzymes involved in L-arabinose catabolism. Strain SV3 (*ara-531*) shows high levels of inducible L-arabinose isomerase (EC 5.3.1.4) and L-ribulokinase (EC 2.7.1.16) activities, but is deficient in L-ribulose-5-phosphate 4-epimerase (EC 5.1.3.4), the enzyme encoded in *Escherichia coli* by gene D in the *araBAD* operon. Addition of L-arabinose to SV3 growing in glycerol or casamino acids stops growth. D-Glucose only partially reverses this inhibition. Reversion of the *ara-531* mutation restores different levels of epimerase activity and resistance to L-arabinose. However, the great majority of the L-arabinose-resistant mutants do not utilize L-arabinose. The physiological and enzymatic properties of these L-arabinose non-utilizing mutants suggest that L-arabinose resistance is due to forward mutations in at least 3 other genes, *araA*, *araB* and *araC*, blocking steps prior to L-ribulose 5-phosphate accumulation.

In the screening of environmental mutagens a reversion test using a set of specialized strains detects very specific mutational events. On the contrary, a forward-mutation test utilizes a unique strain to detect a variety of mutational

* Present address: Cátedra de Genética, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Córdoba, Córdoba (Spain).
** Present address: Departamento de Bioquímica, Facultad de Veterinaria, Universidad de Córdoba, Córdoba (Spain).

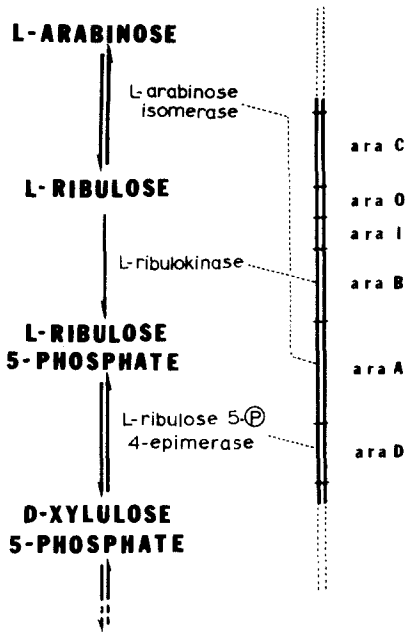

Fig. 1. The L-arabinose gene—enzyme system of *E. coli* [10].

changes [1,22]. The sensitivity of such a system is directly related to the portion of the genome target for the mutagens. Thus, it is crucial to determine the number of genes involved in the phenotypic change chosen as indicator of genetic activity.

In *Escherichia coli* B/r L-arabinose is converted to D-xylulose 5-phosphate by 3 enzymatic reactions (Fig. 1). Genes *araA*, *araB* and *araD* are the structural genes for L-arabinose isomerase, L-ribulokinase and L-ribulose-5-phosphate 4-epimerase respectively. These genes together with their controlling sites *araI* and *araO* constitute an operon, regulated by gene *araC* and localized between the genetic markers threonine and leucine [8]. *E. coli araD* mutants are strongly inhibited by L-arabinose, and this sensitivity can be relieved by an additional mutation in several genes (*araA*, *araB* and *araC*) of the *araBAD* operon [4,9].

The L-arabinose system of *Salmonella typhimurium* is considerably less well known. Genes *araB* and *araC* have been mapped in a position homologous to that of *E. coli* [23]. *S. typhimurium* strain SV3 is unable to use L-arabinose as the sole carbon source, and fails to grow in the presence of L-arabinose plus glycerol. The selection of L-arabinose-resistant mutants in strain SV3 has been proposed as a simple and convenient forward-mutation assay for routine screening of environmental mutagens [22]. The assay has been calibrated against a set of mutagens using a variety of protocols, and its sensitivity has been increased by the addition of excision repair and/or deep rough mutations [20,21].

L-Arabinose sensitivity in strain SV3 is the consequence of a mutation (*ara-531*) which is cotransducible with auxotrophy for leucine, suggesting a mutation in the *araBAD* operon which is closely linked to *leu*. Simultaneous mutation to L-arabinose resistance and leucine auxotrophy after *N*-methyl-*N'*-nitro-

N-nitrosoguanidine treatment, has suggested that the vast majority of mutations leading to L-arabinose resistance are also closely linked to *leu* [22].

The present study has two main purposes: To characterize the mutation *ara-531* carried by the test strain *S. typhimurium* SV3, and to determine the number of genes leading to L-arabinose resistance in this system. Physiological and enzymatic criteria related to L-arabinose catabolism have been used with strain SV3 and several L-arabinose-resistant mutants selected therefrom.

Materials and methods

Media. Luria broth [19], mineral casein hydrolyzate and Eosin methylene blue (EMB) L-arabinose media [13], minimal M9 salts [19] and Davis-Mingioli salts [12] have previously been described. Minimal M9 and Davis-Mingioli media contained either D-glucose or glycerol plus L-arabinose at 2 g/l each. Any required amino acid was added at 20 mg/l, and uracil at 10 mg/l.

Bacterial strains. Salmonella typhimurium SV3 is derived from *S. typhimurium* strain JL386 (*trp-294, thr-115, pyrB92*) in which the *ara-531* mutation was induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [22]. *Escherichia coli* Sb3107 (*araB* at 37°C) and *E. coli araD139* were obtained from Nancy L. Lee of the University of California, Santa Barbara. *Lactobacillus plantarum* strain 124-2 was from the American Type Culture Collection, Rockvillle, MD (A.T.C.C. 8041).

Isolation of L-arabinose-resistant mutants. 125-ml flasks containing 15 ml of Luria broth were inoculated with approximately 100 viable cells from a stationary-phase culture of *S. typhimurium* strain SV3 and shaken at 37°C for 15 h. The cells were spun down and resuspended in 15 ml of M9 salts. Aliquots of 0.1 ml from these suspensions were spread on M9 L-arabinose plates and incubated for 72 h at 37°C. Two colonies of different sizes were picked from each plate, purified, and streaked on Luria plates. Their tryptophan, threonine and uracil requirements for growth were confirmed on M9 D-glucose plates lacking the appropiated supplement. Their ability to use L-arabinose as a sole carbon source was checked on EMB L-arabinose plates. The L-arabinose-negative mutants were numbered 1 to 24 (Table 2). The two L-arabinose-utilizing revertants were designated SV22 and SV23 (Table 1). All the L-arabinose-resistant mutants were of spontaneous origin.

L-arabinose growth inhibition and keto-sugar accumulation. 10 ml of M9 salts containing 2 g/l of casamino acids (Difco) as the carbon source were supplemented with the auxotrophic requirements of the strains (threonine, tryptophan and uracil), inoculated with 0.1 ml of a stationary-phase culture and distributed in two test tubes (18 × 150 mm). L-Arabinose (filter-sterilized) was added to one of these tubes at 2 g/l and both were then incubated at 37°C for 4 h on a roller drum. The optical density (O.D.) at 425 nm was measured using a Spectronic 20 colorimeter (Bausch and Lomb) adapted to these growth tubes. Aliquots of 0.2 ml from each culture were then dispensed in tubes containing 0.9 ml of 0.1 M HCl and the keto-sugars were determined by the cysteine carbazole test [5]. The color was developed at room temperature for 20 min and measured at 540 nm in 0.5 inch Spectronic tubes. D-Ribulose (Sigma) was employed as a standard. The growth inhibition is expressed as: (O.D. of the cul-

ture without L-arabinose — O.D. of the culture with L-arabinose) divided by the O.D. of the culture without L-arabinose. The keto-sugar accumulation is expressed as micromole of D-ribulose per ml of culture divided by the O.D. of the same culture.

Preparation of cell-free extracts. Cells were grown in three 1-l Florence flasks, each containing 300 ml of casein hydrolyzate medium supplemented with the appropriate auxotrophic requirements and 0.05 mM of $MnCl_2$. The inoculum was 1% by volume of an overnight Luria broth culture. The flasks were shaken at 37° C until the O.D. at 425 nm reached approximately 0.6 (measured in 18 × 150 mm test tubes). At this time of logaritmic growth, the L-arabinose operon was induced by adding L-arabinose at a concentration of 4 g/l and continuing the incubation for an additional 3—4 h. The cells were then harvested by centrifugation at 10 000 × *g* for 10 min and washed with 10 mM potassium phosphate buffer, pH 7.4 (1 mM EDTA and 1 mM glutathione). The cell pellet was resuspended in the same phosphate buffer to yield a 14% suspension on a wet weight basis. The cell suspension was sonicated for 10 min in a Sonic 300 apparatus (Artek Systems Co., Farmingdale, NY), and the cellular debris was removed by centrifugation at 20 000 × *g* for 20 min.

The preparation of extracts for L-ribulose-5-phosphate 4-epimerase assays included two purification steps, $MnCl_2$ precipitation and heat treatment, as described previously [9]. We assayed the L-arabinose isomerase in these same partially purified extracts. L-Ribulokinase was assayed after $MnCl_2$ precipitation and 40—50% ammonium sulfate fractionation [17].

Enzymatic activity assays. L-Arabinose isomerase [7], L-ribulokinase [18], and L-ribulose-5-phosphate 4-epimerase [11] assays were performed as previously described for *E. coli* extracts. L-Ribulose used in L-ribulokinase assays was produced from L-arabinose (Sigma) using cells of an L-arabinose-isomerase hyperproducer, L-ribulokinase thermosensitive mutant of *E. coli* (strain Sb3107) [7]. L-Ribulose 5-phosphate used in epimerase assays was produced from L-ribulose using a partially purified L-ribulokinase preparation from an L-ribulose-5-phosphate 4-epimerase-less mutant (*araD139*) of *E. coli* [2]. The coupling enzyme phosphoketolase (EC 4.1.2.9) used in the epimerase assays was a partially purified extract ($MnCl_2$ precipitation, acetone treatment, and ammonium sulfate fractionation) from *Lactobacillus plantarum* [14]. One unit of activity was defined as the amount of enzyme which transformed one micromole of substrate per hour. Protein concentration was determined either by the Lowry method with bovine serum albumin as a standard, or by the method of Warburg in the extracts for ribulokinase assay [16].

Results

Inhibition by L-arabinose of the growth of S. typhimurium strain SV3 (ara-531)

The growth of the mutant strain SV3 was severely inhibited by L-arabinose in minimal medium containing casamino acids as the sole carbon source (Fig. 2, top). This inhibition was only partially released by D-glucose. The same L-arabinose inhibition and D-glucose release were observed if L-arabinose was added at other points along the growth curve instead of at zero time. Similar results

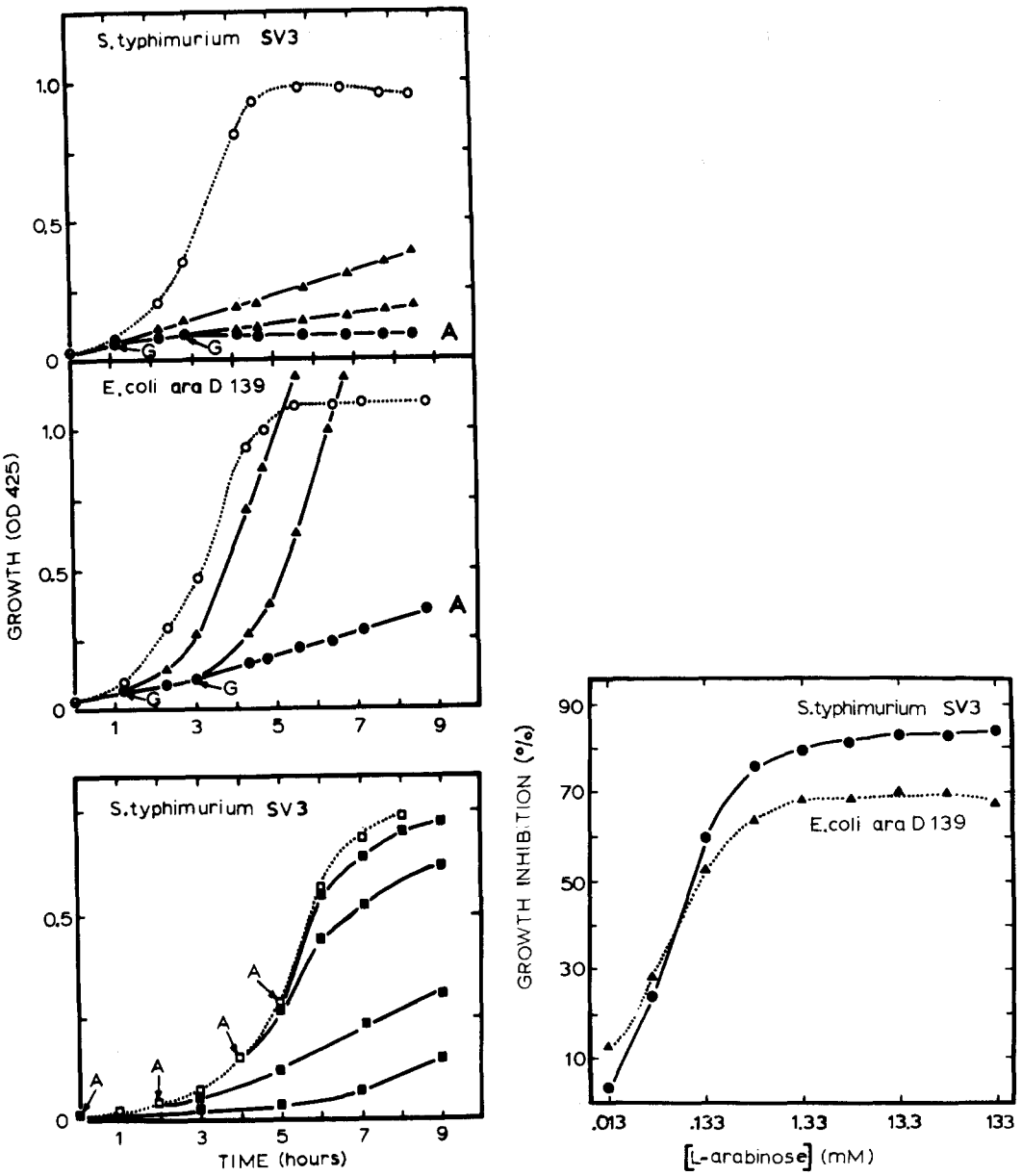

Fig. 2. Inhibitory effects of L-arabinose on cell growth. The inoculum was a washed sample from a stationary-phase culture in Luria broth. Top: Cells in M9 salts with casamino acids (○) as carbon source were inhibited by L-arabinose (A) (●) and the inhibition was released by adding D-glucose (G) (▲). Bottom: Cells in M9 salts growing in D-glucose (□) were inhibited by addition of L-arabinose (■) at the time indicated by the arrows. Casamino acids, L-arabinose and D-glucose were used at a final concentration of 2 g/l.

Fig. 3. Growth inhibition at different concentrations of L-arabinose. L-Arabinose growth inhibition was determined as described in Materials and Methods; strain SV3 (*ara-531*) (●) and *araD139* (▲).

were also obtained if casamino acids were replaced by glycerol as the carbon source. No loss of viability, defined as colony-forming ability on M9 D-glucose plates, was observed during L-arabinose growth inhibition. An enrichment for L-arabinose-resistant mutants was detected, however, since the frequency of such mutants increased from 2 to 69 per 10^8 viable cells after 4 h of L-arabinose exposure. The *E. coli* mutant *araD139*, in which growth inhibition by L-arabinose has previously been reported [9], was used as a control. The inhibitory effect of L-arabinose on the growth of strain SV3 was even noticeable with cells preadapted to D-glucose metabolism (Fig. 2, bottom).

The inhibitory effects of different L-arabinose concentrations are shown in Fig. 3. The growth inhibition of strain SV3 increased with the concentration of L-arabinose up to 1.33 mM, at which maximal inhibition was reached, around 81%. *E. coli araD139* behaved similarly but reached a lower maximal growth inhibition, around 69%.

Characterization of S. typhimurium strain SV3 (ara-531)

Neither L-arabinose isomerase nor L-ribulokinase activities were detected in extracts from strain SV3 uninduced by L-arabinose. After induction (Table 1), strain SV3 showed high isomerase and kinase activities as the parental strain JL386. However, SV3 L-ribulose-5-phosphate 4-epimerase activity was much lower. This enzymatic deficiency in the L-arabinose pathway was correlated with high sensitivity to growth inhibition by L-arabinose. The reversion of *ara-531* mutation to strains SV22 and SV23 resulted in different degrees of restoration of the epimerase activity with a concomitant decrease in the growth inhibition by L-arabinose. The revertants maintained high levels of L-arabinose isomerase and L-ribulokinase activities.

Characterization of L-arabinose resistant mutants selected in S. typhimurium strain SV3 (ara-531)

More than 98% of the L-arabinose-resistant mutants selected in strain SV3 were still unable to utilize L-arabinose as a carbon source, in contrast to the revertants SV22 and SV23. The L-arabinose-resistant mutants were classified into 4 groups by the criteria of growth inhibition by L-arabinose, keto-sugar accumulation, and L-arabinose isomerase and L-ribulokinase activities (Table 2). Type I mutants showed very low levels of both isomerase and kinase activities, accumulated small quantities of keto-sugars, and were only slightly inhibited by L-arabinose (about 10%). Type II mutants showed variable but high levels of isomerase activity and no detectable L-ribulokinase; they accumulated large quantities of keto-sugars, and their growth inhibition by L-arabinose was more severe than in Type I mutants, about 35%. Type III mutants showed very low L-arabinose isomerase activities but very high levels of L-ribulokinase, with little accumulation of keto-sugar and around 25% of growth inhibition by L-arabinose. Type IV mutants showed variable but low levels of both isomerase and kinase activities; they accumulated varying quantities of keto-sugars, and showed different extents of L-arabinose inhibition.

Growth of Type III mutants was inhibited on standard M9 L-arabinose plates in which 3% of the L-arabinose was converted upon autoclaving to keto-sugar, as determined by the cysteine carbazole test [5]. They were however isolated

TABLE 1

PHYSIOLOGICAL AND ENZYMATIC PROPERTIES OF *S. TYPHIMURIUM* STRAINS

Strain	Arabinose genotype	Growth inhibition (%)	Expt. number	Enzymatic activities (unit/mg protein)		
				Isomerase	Kinase	Epimerase
S. typhimurium						
JL386	wild-type	0	1	288	67	2.56
			2	186	nt	6.13
SV3	*ara-531*	88	1	153	64	<0.05
			2	218	nt	<0.16
			3	167	33	nt
SV22	*ara-531* revertant	35	2	464	nt	0.20
			3	140	74	nt
SV23	*ara-531* revertant	0	2	387	nt	2.20
			3	145	50	nt
E. coli	*araD139*	82	2	477	nt	<0.16
			3	542	45	nt

The L-ribulose-5-phosphate 4-epimerase in expts. 1 and 2 were measured using two different phosphoketolase preparations (nt, not tested).

TABLE 2

PHYSIOLOGICAL AND ENZYMATIC PROPERTIES OF L-ARABINOSE-RESISTANT MUTANTS SELECTED IN *S. TYPHIMURIUM* STRAIN SV3 (*ara-531*)

Mutant number	Growth inhibition (%)	Keto-sugar accumulation	Enzymatic activity (u/mg)		Type
			Isomerase	Kinase	
SV3	88	1.22	179	49.0	
2	12	0.07	1.0	<0.8	I
3	14	0.03	1.0	<0.8	
4	7	0.14	3.0	<0.8	
1	32	1.26	67	<0.8	II
5	32	1.54	40	<0.8	
6	34	1.77	137	<0.8	
7	40	1.72	83	<0.8	
12	34	1.87	169	<0.8	
15	36	1.77	343	<0.8	
16	36	1.70	269	<0.8	
17	25	0.04	1.3	167.0	III
18	23	0.02	0.7	48.0	
19	16	<0.01	1.4	53.0	
20	29	0.04	0.7	72.0	
21	29	0.04	0.7	72.0	
22	25	0.04	0.7	47.0	
23	22	0.06	2.4	62.0	
24	27	<0.01	1.2	152.0	
14	19	1.07	47	0.8	IV
11	17	1.05	29	1.6	
8	18	0.21	75	9.3	
13	3	0.17	31	11.0	
10	11	0.11	7	7.8	
9	6	0.10	4	5.2	

Type I, II and IV mutants were isolated on M9 L-arabinose plates as indicated in Materials and Methods. Type III, L-arabinose-resistant L-ribulose-sensitve mutants, were isolated by plating a culture of strain SV3 on Davis-Mingioli L-arabinose medium and further replica onto the same medium supplemented with

after modifying the selective medium. L-Arabinose was sterilized by filtration through a 0.22 μm millipore filter and added to Davis-Mingioli agar plates containing glycerol.

The effect of M9 minimal salts and the autoclaving of L-arabinose on the inhibition of growth was investigated with different types of L-arabinose-resistant mutants. The 29% inhibition of strain 20 in Table 2 increased to 40% if the L-arabinose was autoclaved but diminished to 19% if Davis-Mingioli salts were used. No change in growth inhibition was observed with SV3 or Type I or II mutants.

Discussion

The results presented in this paper show that the strain SV3 of *Salmonella typhimurium* carrying mutation *ara-531* is affected in the L-ribulose-5-phosphate 4-epimerase, the enzyme encoded in *Escherichia coli* by gene D of the *araBAD* operon [9]. Mutant strain SV3 displays nearly wild-type activities of the enzymes encoded by genes *araA* and *araB*, L-arabinose isomerase and L-ribulokinase, but is deficient in epimerase activity (Table 1). Additional deficiencies are unlikely since the inducilibity of the isomerase and kinase activities in strain SV3 suggests the presence of a functional L-arabinose transport system and typical *araBAD* operon regulation [3]. Furthermore, the growth of strain SV3 is strongly inhibited in the presence of L-arabinose and a carbon source which allows L-arabinose utilization (Fig. 2). D-Glucose, the best carbon source preventing L-arabinose catabolism [3,9], partially releases this inhibition. Two mutants of *E. coli*, *araD53* and *araD139*, have previously been reported to behave in a similar way [9]. In comparison with *E. coli araD139*, the growth of *S. typhimurium* strain SV3 shows stronger L-arabinose inhibition and weaker D-glucose release of this inhibition (Fig. 2).

Fig. 3 shows that 50% of maximal growth inhibition of strain SV3 is induced at a concentration of L-arabinose around 50 μM. A similar concentration has been reported to produce 50% induction of the *E. coli araBAD* operon [6]. Our data might indicate that maximal inhibition in strain SV3 requires the full expression of the L-arabinose catabolic pathway.

Besides the L-arabinose-utilizing revertants (Table 1), the L-arabinose-resistant mutants selected in *S. typhimurium* strain SV3 (Table 2) are deficient in either one or both of the first two enzymes, L-arabinose isomerase and L-ribulokinase, involved in L-arabinose metabolism. These results agree with the fact that L-arabinose inhibition of *E. coli araD* mutants is related to the accumulation of L-ribulose 5-phosphate and relative resistance is gained by any mutational event preventing this accumulation (Fig. 1) [4,9].

Based on the structural organization and regulation of the L-arabinose gene–enzyme system of *E. coli* [4,7,8,10], the different types of mutants in Table 2 are interpreted. Most Type I mutants with the lowest isomerase and kinase activities, could carry mutations in the regulatory gene *araC*, turning off altogether L-arabinose uptake and *araBAD* operon expression. *E. coli araC* mutants are similarly uninhibited by L-arabinose and fail to accumulate ketoses [7]. The possibility that some Type I mutants were initiator mutants (*araI*), or even *araB*

mutants exhibiting absolute polarity on gene *araA* [24] cannot, however, be ruled out.

Type II mutants lack kinase activity. As typical *E. coli araB* mutants they are slightly inhibited by L-arabinose and accumulate large quantities of keto-sugars [7]. Type III mutants lack L-arabinose isomerase, the enzyme encoded by gene *araA*. They differ from Type I mutants (*araC*) in their slight inhibition by L-arabinose and from Type II mutants (*araB*) in their lack of keto-sugar accumulation. Some Type II mutants (Table 2) show lower isomerase activities than strain SV3; these might be explained by polarity of *araB* mutations on the adjacent *araA* gene [24]. The increases in isomerase or kinase activities of some Type II and Type III mutants may be explained by: (a) better induction of these L-arabinose-resistant mutants compared with strain SV3, severely inhibited by the L-arabinose used as inducer (Fig. 2), and, (b) lack of catabolite repression in comparison with the Ara^+ strain JL386 [15].

Finally, Type IV (Table 2) is constituted by mutants less easy to classify. They could be *araB* mutants with strong polar effect on the isomerase gene, but they could also carry any *araC* or *araI* mutations leading to diminished expression of the *araBAD* operon [10,24]. Since 3 permeases are involved in the L-arabinose transport of *E. coli* [8], it seems unlikely that some of the Type I and IV could rather be transport mutants.

Mutants Type I, II and IV, but not Type III, are selectable on M9 minimal agar plates containing glycerol plus autoclaved L-arabinose. Since about 3% of the L-arabinose is converted into keto-sugars upon autoclaving, these plates might also contain L-ribulose at 60 mg/l (0.4 mM), which should induce maximal growth inhibition in *araA araD* double mutants (Fig. 3). *E. coli araA* mutants had previously been reported to be severely inhibited by L-arabinose in the presence of a low ratio of K^+ and/or Mg^{2+} to NH_4^+ [8]. Thus Type III, L-arabinose-resistant and L-ribulose-sensitive mutants are not inhibited in Davis-Mingioli mineral salts medium whose ion ratio is more favorable than that of M9 for isomeraseless mutants (if not contaminated with L-ribulose). Hence we propose to modify the selective medium previously used [20—22] to: Davis and Mingioli minimal agar plates containing glycerol to which L-arabinose filter-sterilized must be subsequently added.

Based on the diversity of mutant types, we conclude that the selection of L-arabinose resistance in strain SV3 of *Salmonella typhimurium* is an assay for detection of forward mutations in at least 3 different *araC*, *araB* and *araA* genes.

Acknowledgements

We thank N.L. Lee for the *E. coli* strains and M.E. Case, E. Cerdá-Olmedo, J.W. Drake, R.G. Harvey, C.H. Langley and C.Y. Lee for their critical editing of the manuscript.

C. Pueyo was supported by a postdoctoral fellowship from the Program of the U.S.A.—Spain Joint Committee for Educational and Cultural Affairs.

References

1 Ames, B.N., The detection of chemical mutagens with enteric bacteria, in: A. Hollaender (Ed.), Chemical Mutagens: Principles and Methods for their Detection, Vol. 1, Plenum, New York, 1971, pp. 267—282.

2 Anderson, R.L., L-Ribulose 5-phosphate, in: S.P. Colowick and N.O. Kaplan (Eds.), Methods in Enzymology, Vol. IX, Academic Press, New York, 1966, pp. 48—51.

3 Bahttacharya, A.K., and M. Chakravorty, Induction and repression of L-arabinose isomerase in *Salmonella typhimurium*, J. Bacteriol., 106 (1971) 107—112.

4 Boyer, H., E. Englesberg and R. Weinberg, Direct selection of L-arabinose negative mutants of *Escherichia coli* strain B/r, Genetics, 47 (1962) 417—425.

5 Dische, Z., and E. Borenfreund, A new spectrophotometric method for the detection and determination of keto-sugars and trioses, J. Biol. Chem., 192 (1951) 583—587.

6 Doyle, M.E., C. Brown, R.W. Hogg and R.B. Helling, Induction of the *ara* operon of *Escherichia coli* B/r, J. Bacteriol., 110 (1972) 56—65.

7 Englesberg, E., Enzymatic characterization of 17 L-arabinose negative mutants of *Escherichia coli*, J. Bacteriol., 81 (1961) 996—1006.

8 Englesberg, E., Regulation in the L-arabinose system, in: H.J. Vogel (Ed.), Metabolic Pathways, Vol. 5, Academic Press, New York, 1971, pp. 257—296.

9 Englesberg, E., R.L. Anderson, R. Weinberg, N. Lee, P. Hoffee, G. Huttenhauer and H. Boyer, L-Arabinose sensitive, L-ribulose-5-phosphate 4-epimerase-deficient mutants of *Escherichia coli*, J. Bacteriol., 84 (1962) 137—146.

10 Englesberg, E., and G. Wilcox, Regulation: positive control, Annu. Rev. Genet., 8 (1974) 219—242.

11 Gielow, W.O., and N. Lee, L-Ribulose-5-phosphate 4-epimerase from *Escherichia coli*, in: S.P. Colowick and N.O. Kaplan (Eds.), Methods in Enzymology, Vol. XLI, Academic Press, New York, 1975, pp. 419—423.

12 Green, M.H.L., and W.J. Muriel, Mutagen testing using Trp^+ reversion in *Escherichia coli*, Mutation Res., 38 (1976) 3—32.

13 Gross, J., and E. Englesberg, Determination of the order of mutational sites governing L-arabinose utilization in *Escherichia coli* B/r by transduction with phage Plbt, Virology, 9 (1959) 314—331.

14 Heath, E.C., J. Hurwith, B.L. Horecker and A. Ginsburg, Pentose fermentation by *Lactobacillus plantarum*, J. Biol. Chem., 231 (1958) 1009—1029.

15 Katz, L., and E. Englesberg, Hyperinducibility as a result of mutation in structural genes and self-catabolite repression in the *ara* operon, J. Bacteriol., 107 (1971) 34—52.

16 Layne, E., Spectrophotometric and turbidimetric methods for measuring proteins, in: S.P. Colowick and N.O. Kaplan (Eds.), Methods in Enzymology, Vol. III, Academic Press, New York, 1957, pp. 447—454.

17 Lee, N., and I. Bendet, Crystalline L-ribulokinase from *Escherichia coli*, J. Biol. Chem., 242 (1967) 2043—2050.

18 Lee, N., and E. Englesberg, Dual effects of structural genes in *Escherichia coli*, Proc. Natl. Acad. Sci. (U.S.A.), 48 (1962) 335—348.

19 Miller, J.H., Experiments in molecular genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1972, pp. 431—434.

20 Pueyo, C., Forward mutations to arabinose resistance in *Salmonella typhimurium* strains. A sensitive assay for mutagenicity testing, Mutation Res., 54 (1978) 311—321.

21 Pueyo, C., D. Frezza and B. Smith, Evaluation of three metabolic activation systems by a forward mutation assay in Salmonella, Mutation Res., 64 (1979) 183—194.

22 Ruiz-Vázquez, R., C. Pueyo and E. Cerdá-Olmedo, A mutagen assay detecting forward mutations in an arabinose-sensitive strain of *Salmonella typhimurium*, Mutation Res., 54 (1978) 121—129.

23 Sanderson, K.E., and P.E. Hartman, Linkage map of *Salmonella typhimurium*, edition V, Microbiol. Rev., 42 (1978) 471—519.

24 Sheppard, D.E., and D.A. Walker, Polarity in gene *araB* of the L-arabinose operon in *Escherichia coli* B/r, J. Bacteriol., 100 (1969) 715—723.