

Arsenite resistance in *Listeria monocytogenes*

R. L. Buchanan*, L. A. Klawitter, S. Bhaduri and H. G. Stahl

Microbial Food Safety Research Unit, US Department of Agriculture, Research Service, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 19118, USA

Received 21 January 1991

A total of 74 isolates of *Listeria* (53 *L. monocytogenes*, ten *L. innocua*, three *L. ivanovii*, six *L. welshimeri* and two *L. seligeri*) were evaluated for their ability to grow in the presence of 100 $\mu\text{g ml}^{-1}$ sodium arsenite. Six *L. monocytogenes* and one *L. innocua* strains were arsenite-resistant. There appeared to be a relationship between arsenite resistance and serotype, with five of the six arsenite-resistant strains of *L. monocytogenes* being serotype 4, and the incidence of arsenite resistance being substantially higher in type 4 *L. monocytogenes* than type 1 (63% vs 3%). Plasmid profiles of selected strains indicated that arsenite resistance is not a plasmid-linked trait in *Listeria*.

During a screening of potential selective agents for *Listeria monocytogenes*, it was observed that strain Scott A was able to grow in the presence of elevated levels ($\geq 200 \mu\text{g ml}^{-1}$) of sodium arsenite. This strong arsenite resistance prompted us to determine if this characteristic is common among *Listeria* isolates. A total of 74 *Listeria* isolates from clinical, food and environmental sources were examined including strains of *L. monocytogenes* (53), *Listeria innocua* (10), *Listeria ivanovii* (3), *Listeria welshimeri* (6), and *Listeria seligeri* (2) (Table 1). The isolates were screened for arsenite resistance using a solid medium assay system. After initial culturing in test tubes containing 5 ml of Tryptose Phosphate Broth (TPB) (Difco, Detroit, MI) for 18–24 h at 28°C, the isolates were streaked onto Brain Heart Infusion Agar supplemented with sodium arsenite (100 $\mu\text{g ml}^{-1}$) (BHIA + As). The plates were prepared by adding 20 ml of a filter-sterilized solution of NaAsO₂ to autoclaved BHIA after it had

equilibrated to 50°C. All BHIA + As plates were incubated for 24 h at 37°C and examined for growth. Incubation for an additional 24 h did not increase the number of strains positive for arsenite resistance. Preliminary studies indicated that comparable results were achieved when arsenite resistance was assessed using a liquid medium system. Arsenite resistance was detected in six of 53 (11%) *L. monocytogenes* and one of ten *L. innocua* (10%) isolates. No resistant strains of *L. ivanovii*, *L. welshimeri*, or *L. seligeri* were observed, though a substantially fewer number of isolates of these species were screened.

A majority of the *L. monocytogenes* strains were also screened for reactivity with commercially available (Difco) type 1 and type 4 *Listeria* antisera according to the manufacturer's directions. There appeared to be a degree of correlation between arsenite resistance and serotype in that five of the six resistant *L. monocytogenes* strains reacted with type 4 antiserum. However, the relationship was not absolute since there were other type 4 *L. monocytogenes*

*Corresponding author.

Table 1. Ability of *Listeria* isolates to grow in the presence of 100 µg ml⁻¹ sodium arsenite.

Species	Strain	Source	Reactivity with commercial antisera	Arsenite resistance
<i>L. monocytogenes</i>	Scott A	Clinical, FDA	4	+
	15313	ATCC	ND ^a	-
	Murray B	Clinical, FDA	4	+
	V7	Milk, FDA	1	-
	V37	Milk, FDA	4	+
	RMII	Milk, FDA	4	+
	GVN5-V-G	Meat, USDA	1	-
	MF2-L-P	Fish, USDA	1	-
	7644	ATCC	1	+
	RMI	Milk, FDA	4	+
	V3-L-T	Meat, USDA	1	-
	F4651	CDC	1	-
	F3-V-G	Fish, USDA	1	-
	HO-VG-S	Meat, USDA	1	-
	H2-V-G	Meat, USDA	ND	-
	H1-L-G	Meat, USDA	ND	-
	F4243	CDC	4	-
	F4258	CDC	4	-
	F2-V-G	Fish, USDA	1	-
	F2L2	Fish, USDA	1	-
	LA-23	Cheese, FDA	ND	-
	F4260	CDC	1	-
	F4245	CDC	1	-
	GLA2-L-S	Meat, USDA	1	-
	590-PORT	U. of Pennsylvania	ND	-
	LA-18	Cheese, FDA	1	-
	476-PORT	U. of Pennsylvania	ND	-
	BRIE-1	Cheese, FDA	ND	-
	GLB3-L-S	Meat, USDA	1	-
	GLB1-V-S	Meat, USDA	1	-
	GLB3-V-S	Meat, USDA	1	-
	H4NG	Meat, USDA	1	-
	GVN4-VG	Meat, USDA	1	-
	V3-V-T	Meat, USDA	1	-
	F2-L-G	Fish, USDA	1	-
	F2NG	Fish, USDA	1	-
	F3NG	Fish, USDA	1	-
	S9NS	Meat, USDA	1	-
	S9-V-S	Meat, USDA	1	-
	F4259	CDC	1	-
	GLA2-V-S	Meat, USDA	1	-
	GLB1-L-S	Meat, USDA	1	-
	GLB5-L-S	Meat, USDA	1	-
	LG3-L-S	Meat, USDA	ND	-
	GLA6-L-S	Meat, USDA	1	-
	GVN5-L-G	Meat, USDA	1	-
	GLA3-L-S	Meat, USDA	1	-
GLA2-L-S	Meat, USDA	1	-	
GLA1-L-S	Meat, USDA	1	-	
81-Li63	Washington State U.	1	-	
78-Li89	Washington State U.	1	-	

Table 1. Continued

Species	Strain	Source	Reactivity with commercial antisera	Arsenite resistance
<i>L. innocua</i>	SA3-V-T	Meat, USDA	4	-
	FRI-931	U. of Wisconsin	— ^b	+
	FRI-683	U. of Wisconsin	ND	-
	SH3-VJ	Meat, USDA	ND	-
	H2-L-G	Meat, USDA	ND	-
	LA-1	Cheese, FDA	ND	-
	FRI-386	U. of Wisconsin	ND	-
	FRI-770	U. of Wisconsin	ND	-
	TYPE 4	CDC	ND	-
	LG5-L-S	Meat, USDA	ND	-
<i>L. ivanovii</i>	KCL114	CDC	—	-
	F5999	CDC	ND	-
	KCCL1714	CDC	ND	-
<i>L. welshimeri</i>	FRI-101	U. of Wisconsin	ND	-
	CF2-L-P	Shellfish, USDA	ND	-
	CCR3-V-G	Shellfish, USDA	ND	-
	CF1-V-P	Shellfish, USDA	4	-
	CCR1-V-G	Shellfish, USDA	ND	-
	CCR8-V-G	Shellfish, USDA	1	-
<i>L. seligeri</i>	TYPE 1	CDC	ND	-
	F4080	CDC	ND	-

^a ND, Not determined.

^b — Did not react with either type 1 or 4 antisera.

strains that were arsenite-sensitive. When considered on a percentage of total *L. monocytogenes* strains serotyped, 63% of the type 4 strains were arsenite-resistant, whereas only 3% of the type 1 isolates had this phenotype. The single resistant strain of *L. innocua* did not react with either type 1 or 4 antisera. It may be worthwhile to determine if there is any correlation between arsenite resistance and the isolation of *L. monocytogenes* from clinical sources.

Arsenite resistance has been observed in isolates of a number of bacterial species associated with foods, including *Salmonella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The phenotype is often associated with a plasmid-linked gene(s) that

confers resistance to arsenite and antimony, and is commonly accompanied by a second gene that confers resistance to arsenate (Novick and Roth 1968, Hedges and Baumberg 1973, Smith 1978, Silver et al, 1981, Mobley et al. 1983). The plasmid-associated arsenite-resistance gene in *E. coli* and *S. aureus* appears to encode an anion translocating ATPase that confers resistance by exclusion of the arsenite ion (Silver and Keach 1982, Mobley and Rosen 1982, Rosen and Barbolla 1984, Chen et al. 1985, 1986, Tisa and Rosen 1990).

The possibility that arsenite resistance in *Listeria* is a plasmid-mediated characteristic was assessed by profiling a battery of resistant and sensitive strains. Ten strains were selected to encompass

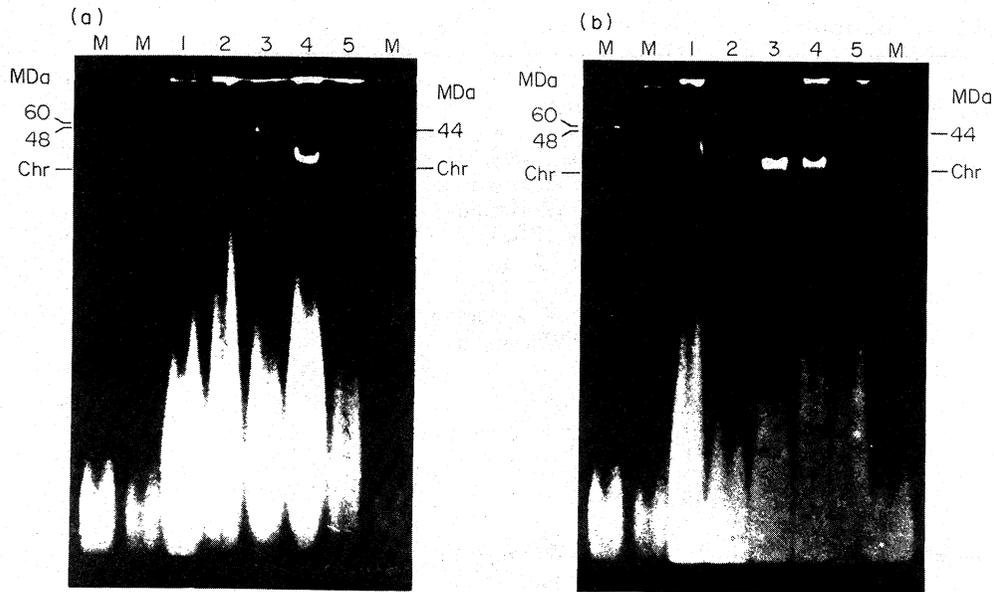


Fig. 1. Plasmid DNA profiles of arsenite-sensitive and arsenite-resistant strains of *Listeria*. Lanes: M, molecular mass standards (plasmids from *Escherichia coli* 45753-35, 60 MDa; *Yersinia enterocolitica* GER, 48 MDa, and *L. monocytogenes* 78-Li89, 44 MDa; Chr, chromosomal DNA. (a) Photograph of arsenite-resistant *Listeria*. Lanes: 1, *Listeria innocua* FRI-931; 2, *L. monocytogenes* Murray B; 3, *L. monocytogenes* 7644; 4, *L. monocytogenes* V37; and 5, *L. monocytogenes* Scott A. (b) Photograph of arsenite-sensitive *Listeria*. Lanes: 1, *L. innocua* SA3-V-T; 2, *L. monocytogenes* F4258; 3, *L. monocytogenes* V7; 4, *L. monocytogenes* BRIE-1; 5, *L. monocytogenes* F2L2. Profiles for *L. ivanovii* KCL114 and *L. welshimeri* CF1-V-P are not shown. The results of the plasmid profiles are summarized below:

<i>Listeria</i> species	Strain	Serotype	Arsenite resistance	Plasmid (MDa)
<i>L. monocytogenes</i>	Scott A	4	+	-
	F2L2	1	-	-
	V37	4	+	-
	BRIE-1	1	-	+(47)
	V7	1	-	-
	F4258	4	-	-
	7644	1	+	+(47)
	Murray B	4	+	-
	78-Li89	4	-	+(44)
<i>L. innocua</i>	FRI-931	^a	+	+(47)
	SA3-V-T	4	-	-
<i>L. ivanovii</i>	KCL114	^a	-	-
<i>L. welshimeri</i>	CF1-V-P	4	-	-

^a Did not react with either type 1 or type 4 antisera.

four of the *Listeria* species including strains of *L. monocytogenes* serotypes 1 and 4 (Fig. 1). *L. monocytogenes* 78-Li89, an isolate known to carry a 44 MDa plasmid (Flamm et al. 1984),

served as a positive control. The plasmid DNA was extracted from the *Listeria* isolates using a modification of the protocol of Flamm et al. (1984). After growing the isolates aerobically for 24 h

at 37°C, the 200 ml cultures were centrifuged at 16 270 g for 30 min. Cells were washed with 5.0 ml of 0.1 SSC, suspended in 9.0 ml of 0.01 M Na₂HPO₄/20% sucrose buffer (pH 7.0) with lysozyme (5 mg ml⁻¹), and incubated for 45 min at 37°C. A 1.0-ml portion of a 50 mM glucose, 100mM EDTA, 250 mM Tris hydrochloride (pH 8.0) solution, and 20 ml of a 0.2 N NaOH/1% SDS solution were added, and the mixture was held on ice for 5 min. A 15-ml portion of 3 M sodium acetate (pH 4.8) was added, and the mixture held on ice with occasional mixing for another 30 min. The preparation was centrifuged at 16 270 g for 30 min. The plasmid DNA was precipitated from the supernatant by adding a 0.58 volume of isopropanol and incubating for 30 min at -20°C. The precipitate was centrifuged at 16 270 g for 30 min at 4°C, and the pellet dried under nitrogen. The DNA was resuspended in 17 mM EDTA (pH 8.0) 14% glycerol/0.002% bromophenol blue (tracking dye) prior to agarose gel electrophoresis using 40 mM Tris, 20 mM sodium acetate, and 1 mM EDTA adjusted to pH 7.5 with glacial acetic acid. The DNA was electrophoresed in 0.7% (w/v) agarose for 7 h at 60 V, and then visualized by ethidium bromide staining and UV illumination. DNA preparations from *L. monocytogenes* 78-Li89, *Yersinia enterocolitica* GER, and *E. coli* 45753-35 (O157:H7), which contain 44, 48, and 60 MDa plasmids, respectively, were used as markers. The DNA from *Y. enterocolitica* and *E. coli* was extracted using the technique of Bhaduri (1990).

Plasmids having approximate molecular weights of 47 MDa were detected in

three of the *Listeria* isolates, *L. monocytogenes* 7644 and BRIE-1 and *L. innocua* FRI-931 (Fig. 1); one sensitive and two resistant strains. No plasmids were detected in strains Scott A, Murray B, or V37 three other isolates that were strongly arsenite-resistant. *L. monocytogenes* 78-Li89, the positive control for the plasmid profiling, was arsenite-sensitive. *L. monocytogenes* 81-Li63, a strain which has been reported to harbor a 32 MDa plasmid (Flamm et al. 1984), was also arsenite-sensitive (Table 1). While the possibility of a very low copy number plasmid in the resistant strains cannot be unequivocally ruled out, the current results suggest that arsenite resistance is not plasmid-linked in *Listeria*. This is somewhat surprising considering the small percentage of isolates with this phenotype. The possibility that arsenite resistance is associated with some other factor (e.g. bacteriophage) will have to await the results of future research.

Extreme arsenite resistance has proven to be a highly useful trait for conducting experimentation in non-sterile food systems. For example, we have already taken advantage of this characteristic to follow the fate of *L. monocytogenes* Scott A in raw ground beef (Buchanan and Klawitter 1990), a product that is often difficult due to the presence of staphylococci and enterococci, micro-organisms that often interfere with the quantitative detection of *L. monocytogenes*. The incorporation of 200 µg ml⁻¹ sodium arsenite into Modified Vogel Johnson Agar produced such a highly selective medium that only the inoculated Scott A was able to grow, thereby allowing the strain to be followed using direct plating techniques.

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