NOTES

THE ANTIBACTERIAL POTENTIAL OF A PHOSPHO*ENOL*PYRUVATE: SUGAR PHOSPHOTRANSFERASE SYSTEM BLOCKING AGENT

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The phospho*enol*pyruvate: sugar phosphotransferase system (PTS) is involved in the transport and concentration of various carbohydrates across the plasma membrane of certain bacteria[1]. Since it is absent from eukaryotic cells, blockage of the PTS has been suggested as a potentially selective chemotherapeutic objective[2]. The present study was undertaken to assess this possibility. This was accomplished by determining what effects PTS blockage would have on bacterial growth and virulence. Since selective inhibitors of the PTS are unavailable, the effects of its blockage was studied in PTS blocked mutants of *Salmonella typhimurium* and compared with the parent strain.

The *S. typhimurium* strains used were the *pts*HI deletion mutants SB2309 and SB2950, and the parent strain trpB223[3]. These cultures were maintained on nutrient agar slants. For *in vitro* growth studies, inocula were grown overnight at 37°C, with aeration, in Medium A, modified according to CORDARO and ROSEMAN[3] and containing 0.2% sodium lactate and 10 μg/ml tryptophan. A 1% inoculum was used to initiate growth in Medium A containing 0.2% glucose instead of lactate, tryptophan and varying concentrations of heat inactivated fetal calf serum (KC Biological, Inc., Lenexa, Kansas). These cultures were either aerated by bubbling, or alternatively, incubated under static conditions, in 16×100 mm test tubes, at 37°C. Growth was measured turbidometrically; generation times calculated from the linear portion of plots of log A_{600nm} *vs.* time are presented. For virulence tests, cultures were grown overnight, under static conditions, at 37°C, in trypticase soy broth (BBL, Div. Becton, Dickinson & Co., Cockeysville, Maryland) and dilutions were made in the same medium. Hog gastric mucin (ICN Pharmaceuticals, Cleveland, Ohio) was added, as indicated, to the appropriate serial dilution in trypticase soy broth to give a final concentration of 5%. Swiss albino CD-1 mice weighing 18~20 g were infected intraperitoneally with 0.5 ml of the appropriate inoculum. The LD_{50} (mean lethal dose) of each bacterial strain was determined 7 days after infection.

An agent capable of specifically blocking the PTS would be expected to cause a physiological conversion of the growth characteristics and virulence of strain trpB223 to that of the mutants, SB2309 and SB2950. Results presented in

Table 1. Growth of *Salmonella typhimurium* strains

	Serum additions (% by vol)	Generation time (min)		
		Parent trpB223	Mutant strains	
			SB2309	SB2950
Aerated cultures	0	66	No growth	
	5	53	180	180
	10	48	140	140
	50	52	57	57
	100	56	60	57
Static cultures	50	58	324	324
	100*	52	204	204

* Glucose was omitted.

Table 2. Virulence titration of *Salmonella typhimurium* strains in mice

Expt. No.	Hog gastric mucin	LD_{50} (−Log dilution for 50% mean lethal dose)		
		Parent trpB223	Mutant strains	
			SB2309	SB2950
1*	No	2.8	1.1	1.3
	Yes	10.1	8.7	9.1
2*	No	4.7	3.5	2.7
	Yes	9	9	8.2
3*	No	3.7	2.4	1.7

* Adjusted cultures to A_{600nm}=1.1 before dilutions were made.

Table 1 show that the mutants were able to grow in medium supplemented with serum, and that at high serum concentrations the generation times for both the parent strain and mutants were about the same, when grown in aerated cultures. Only when oxygen was made limiting, *i.e.* static conditions, did the mutants grow more slowly than the parent (Table 1). The addition of serum to the growth medium was deemed logical in order to simulate *in vivo* conditions. Furthermore, the differences in growth of the mutants and their parent strain under oxygen limiting conditions may be due to a less efficient utilization of serum nutrients compared to glucose.

Results of our growth experiments are supported by a study on the virulence of the *S. typhimurium* strains for mice. Values of LD_{50} shown in Table 2 indicate that the mutants are virulent for mice, although the data suggest that this virulence may be somewhat lower than that of the parent strain.

Our results indicate that a PTS-blocking agent might block the growth of a pathogen *in vivo,* if the infection was confined to an anaerobic site. Its chemotherapeutic potential therefore, would be correspondingly limited. Since an antibiotic having a PTS-blocking mechanism of action is not known, our contentions cannot be tested directly with such a drug.

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References

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