

Tolerance of Homoserine by *Pseudomonas pisi* and Implications of Homoserine in Plant Resistance

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ABSTRACT

Homoserine utilization is a useful property for identification of *Pseudomonas pisi*, as all of 13 strains of this pathogen grew on it compared to only 1 of 117 strains from 14 species of other arginine-dihydrolase negative pseudomonads. Only *P. marginalis* among the arginine positive group tended to utilize homoserine. Lack

of utilization was apparently due to homoserine toxicity, which could be overcome by L-glutamic acid and to a lesser extent glycerol. Homoserine may be one of the factors in pea which prevents infection by many pseudomonad pathogens.

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Homoserine, reported as toxic to some bacteria (4), occurs in small amounts in many plants (1). In pea, however, levels increase rapidly after germination of seeds until it becomes the predominant free amino acid about 72 hr after germination. Amounts reach as high as 12% of the dry weight of 5-day-old shoot shafts (6), accounting for some 70% of the free amino acid pool (5). The concentration decreases somewhat thereafter, but homoserine remains a large part of the amino acid pool throughout the life of the plant. The effect of homoserine on bacterial plant pathogens of pea has not been investigated. In this study, the utilization of and tolerance to this compound by *Pseudomonas pisi* (a host-specific pathogen of pea) and related species are reported.

I tested homoserine utilization by incorporating 0.1% (w/v) filter-sterilized homoserine into a simple mineral-base medium (3) as the sole carbon source, placing a 0.03 ml drop of bacterial suspension on the medium, and observing the capacity of various pseudomonads to grow. The pseudomonad species and number of strains screened included *Pseudomonas pisi*, 13 strains; *P. cichorii*, 4; *P. viridiflava*, 1; *P. syringae*, 45; *P. coronafaciens*, 4; *P. eriobotryae*, 4; *P. tomato*, 7; *P. mori*, 5; *P. morsprunorum*, 4; *P. angulata*, 3; *P. tabaci*, 1; *P. lachrymans*, 7; *P. phaseolicola*, 24; *P. glycinea*, 5; *P. sesami*, 3; *P. marginalis*, 9; *P. fluorescens*, 16; *P. aeruginosa*, 5; and *P. putida*, 2.

Homoserine utilization appears to be a useful property for the identification of *P. pisi*, as all 13 strains utilized it as a source of carbon. Of the remaining 117 strains of arginine-dihydrolase negative pathogens, only one strain of *P. tomato* (*P. tom.* 1) utilized homoserine. This general inability to utilize homoserine was shared by the arginine-dihydrolase positive saprophytes, as only two (both *P. fluorescens* strains) of 23 strains grew on the medium. However, homoserine was utilized by four of nine strains of the arginine-dihydrolase positive pathogen, *P. marginalis*.

Whether lack of growth on homoserine was probably due to toxicity, or some other factor such as permeability, was determined by observation of

growth on different concentrations of homoserine either singly or in combination with L-glutamate, a substrate that can overcome homoserine toxicity (4), or glycerol, a substrate utilized by all the pseudomonads being tested. Homoserine was tested at 0.01%, 0.1%, and 0.2% concentrations. The combinations tested were 0.1% glycerol plus 0.001%, 0.01%, and 0.1% concentrations of homoserine, and 0.1% glutamate plus the three concentrations of homoserine. Pseudomonads tested included *P. pisi*, 6 strains; *P. cichorii*, 1; *P. viridiflava*, 1; *P. syringae*, 8; *P. coronafaciens*, 1; *P. tomato*, 4; *P. mori*, 2; *P. morsprunorum*, 2; *P. tabaci*, 1; *P. lachrymans*, 3; *P. glycinea*, 3; *P. sesami*, 1; *P. marginalis*, 2; *P. fluorescens*, 6; *P. aeruginosa*, 2; and *P. putida*, 1.

The lack of growth of many pseudomonads on homoserine appeared to be due to its toxicity. The rate of growth for most nonutilizing strains on homoserine decreased with increasing concentrations of homoserine (Table 1), indicating that lack of growth was due to toxicity. The arginine-dihydrolase negative group was in particular affected, as many strains did not grow in the presence of 0.1% homoserine supplemented with either glutamate or glycerol. In general, glutamate appeared to overcome the toxic effect of homoserine to a greater extent than glycerol. More strains grew at higher concentrations of homoserine when glutamate was added, and growth was faster when the same concentrations of homoserine were used.

The importance of homoserine tolerance or utilization by *P. pisi* at this point is largely conjectural. However, it appears significant from a host-parasite interaction standpoint that as far as is known, only pea contains a very large amount of homoserine (1), and that of the major group of fluorescent pseudomonad pathogens, it is essentially only *P. pisi*, the pathogen of pea, which tolerates large amounts of homoserine. Consequently, homoserine may possibly be one of the major factors in pea which normally prevents infection by most pseudomonad pathogens except *P. pisi*. This is not to say that other pathogens such as *P. syringae* cannot

TABLE 1. Growth of pseudomonads on homoserine with or without addition of glycerol or L-glutamic acid^a

Homoserine concentrations	Number of strains showing growth								
	<i>Pseudomonas pisi</i> (6 strains)			Arginine-dihydrolase negative strains (31 strains)			Arginine-dihydrolase positive strains (11 strains)		
	1 day ^b	4 days	8 days	1 day	4 days	8 days	1 day	4 days	8 days
0.2%		5	1		1 ^c			1	2
0.1%		5	1		1			1	2
0.01% plus 0.1% glycerol		6			1		1	2	
0.1%		5	1		3	7	2	8	1
0.01%	2	4		3	20		11		
0.001% plus 0.1% glutamate	2	4		2	23	2	11		
0.1%		6		1	14	9	6	5	
0.01%		6		3	22	6	11		
0.001%	3	3		20	10	1	11		

^a As carbon sources in a mineral base (2).

^b Length of incubation period at 28 C when growth was observed.

^c Strain P. tom. 1.

infect pea. Various amino acids differ in their ability to overcome homoserine toxicity (4). Also, the relative amounts of different amino acids change throughout the life of a plant. Thus, 48 hr after germination in pea, for example, glutamic acid is the predominant amino acid with serine next. The levels of these amino acids decrease thereafter, and by 15 days are less than asparagine or homoserine, which then predominate (9). Environment also can have an effect on levels of amino acids such as homoserine (2). Also, it is quite likely that other amino acids occurring in plants are selectively toxic, as many pseudomonad pathogens grow slowly or not at all on a number of naturally occurring amino acids on which saprophytes grow quite readily (8). Thus, as Lewis (7) has proposed in his "balance hypothesis", what might be termed the nutrient pool balance might be one of the more important considerations in determining whether infection occurs.

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