REVERSAL OF THE D-SERINE INHIBITION OF GROWTH AND DIVISION IN A *FLAVOBACTERIUM*[1]

Norman N. Durham and Revelle Milligan

Department of Bacteriology
Oklahoma State University
Stillwater, Oklahoma

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Interference with the synthesis of the microbial cell wall results in a variety of unusual morphological forms. Tuttle and Gest (1960) reported several aberrant types when *Rhodospirillum rubrum* was grown in a D-amino acid containing medium. Lark and Lark (1959) reported D-amino acids induced protoplast-like forms in *Alcaligenes fecalis*. Grula (1960) noted that certain D-amino acids inhibited cell division in an *Erwinia* and the inhibition by D-serine could be reversed by several compounds including a toxic concentration of *p*-aminobenzoic acid.

We have studied the latter phenomenon using experimental conditions conducive to good growth in the presence of high concentrations of *p*-aminobenzoic acid (PAB). The organism used in this investigation was a *Flavobacterium* capable of oxidizing PAB as the sole source of carbon and energy for aerobic growth. All experiments were performed using an inorganic salts medium containing 0.2% of the indicated substrate (Durham, 1957). D-serine was sterilized by filtration and added aseptically. The pH of the medium prior to inoculation was 7.2. PAB-grown cells were used as the inoculum and all cultures were incubated at 37° C with constant shaking. Growth studies with the various D-serine containing media showed that dry cell mass and optical density (O.D.) were closely correlated during the first 10 to 12 hrs, but in certain media, osmotically sensitive spheres appeared and

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some lysis was evident after 12 hrs. All results are reported for 8 hr intervals.

Studies indicated that within 8 hrs following inoculation, cells growing in a succinate medium containing 0.916 mg/ml D-serine showed the formation of "chain-like" filaments ranging in length from 5 to 15 microns and a significant decrease in O.D. was evident when compared to the non-serine containing succinate control. Microscopic examination revealed the cells were unable to complete the division process as early as 2 to 4 hrs after inoculation. Chaining was also observed with lower concentrations of the amino acid but the effect on total mass, as measured by O.D., was not evident. Addition of larger quantities of D-serine showed much longer chains and drastically decreased the O.D. When "chain-like" filaments were transferred to a D-serine-free medium, all cells regained their normal morphology within 90 minutes indicating normal growth and division had occurred in the absence of the D-amino acid.

Similar findings were obtained with nutrient broth or when 0.2% L-aspartic acid or asparagine replaced succinate in the salts medium. L-serine in a molar ratio of 50/1 was unable to reverse the inhibition of growth and division by the D-isomer. The Flavobacterium cells were unable to oxidize D-serine as a source of carbon and energy for growth.

Additional studies indicated that the D-serine effect(s) could be reversed. Inoculation of the salts medium containing 0.2% PAB as the carbon and energy source showed that neither growth nor division was influenced by the presence of D-serine (0.916 mg/ml). Also, incorporation of PAB in the succinate medium containing D-serine reversed both the inhibition of growth and division since the culture showed no chain-like filaments and the density closely paralleled the reading in the control tube. Addition of 1.33 mg/ml D-serine to a medium containing 1.3 mg/ml PAB resulted in some chaining but the culture did not show a significant change in density (Table 1). When D-serine was added in a final concentration of 1.66 mg/ml a significant de-

TABLE 1

RELATIONSHIP BETWEEN P-AMINOBENZOIC ACID (PAB) AND D-SERINE DURING GROWTH AND DIVISION OF A FLAVOBACTERIUM.

PAB mg/ml	D-Serine mg/ml	O.D. (8 hrs)	Morphology* (8 hrs)
3.3	-	.30	-
	1.00	.27	-
	1.33	.26	-
	1.66	.26	ƒ
2.6	-	.29	-
	1.00	.28	-
	1.33	.29	ƒ
	1.66	.19	ƒƒ
2.0	-	.33	-
	1.00	.30	-
	1.33	.26	ƒ
	1.66	.17	ƒƒƒ
1.3	-	.32	-
	1.00	.32	ƒ
	1.33	.28	ƒƒ
	1.66	.14	ƒƒƒƒ

* - cells normal (1μ); ƒ occasional short chain (3-4μ); ƒ several short chains (3-4μ); ƒƒ numerous short chains-occasional long chain (12-15 μ); ƒƒƒ many long chains (12-15 μ); ƒƒƒƒ most long and several very long chains (30-50 μ).

crease in O.D. and more pronounced chaining were evident. These effects could be reversed by increasing the PAB concentration suggesting a competitive relationship between PAB and D-serine. This competitive effect was also observed in the PAB supplemented succinate medium. The ability of low serine concentrations to increase chaining without decreasing the density suggests that D-serine may influence growth and division by different mechanisms.

Maas and Davis (1950) reported that D-serine interferred with the conversion of β-alanine to pantothenic acid, but high levels of D-serine affected processes other than pantothenic acid synthesis. Since our findings suggested that D-serine might influence growth and division by different mechanisms, a number of compounds were added to the succinate medium containing D-serine in an attempt to reverse the inhibition of growth and/or inability of the cells to complete the division process. The results obtained with pantothenic and folic acid are presented in Table 2. The addition of folic acid (.008 and .416 mg/ml) did not reverse inhibition of growth or division

TABLE 2

INFLUENCE OF FOLIC AND PANTOTHENIC ACID ON INHIBITION OF GROWTH AND DIVISION OF A FLAVOBACTERIUM BY D-SERINE.

Succinate mg/ml	D-Serine mg/ml	Folic acid mg/ml	Pantothenic acid mg/ml	O.D. (8 hrs)	Morphology* (8 hrs)
1.66				.52	-
1.66	.91			.21	///
1.66		.416		.49	-
1.66	.91	.416		.25	///
1.66		.008		.54	-
1.66	.91	.008		.27	///
1.66			.16	.56	-
1.66	.91		.16	.48	///
1.66			.016	.54	-
1.66	.91		.016	.43	///

* - cell normal (1μ); /// many long chains (12-15 μ).

but it was not definitely ascertained that folic acid permeated the cell barrier. The addition of pantothenic acid reversed the inhibition of growth as indicated by O.D. readings but was unable to overcome inhibition of division.

These findings establish that D-serine affects both growth and cell division. Pantothenic acid reverses the inhibition of growth but is unable to reverse the inhibition of division. PAB can reverse both the inhibition of growth and division but folic acid can do neither. This would indicate that PAB functions other than as an integral part of folic acid, but this conclusion must await verification that folic acid penetrated the cell barrier. Purko et al. reported that PAB was required for the conversion of ∝-ketoisovalerate to ketopantoate, and, in view of the results obtained with pantothenic acid, this may be one way that PAB functions to overcome the inhibition of growth. However, since only PAB reversed the inhibition of division by D-serine, then PAB must also satisfy a nutritional requirement essential for completion of cell division.

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