SYNTROPHISM IN Pasteurella pestis AND THE POSSIBLE BIOLOGICAL ROLE OF THIS PHENOMENON

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Data concerning intrapopulation and interpopulation syntrophism of Pasteurella pestis are described. The ability of auxotrophic bacterial populations to multiply on synthetic medium without amino acids through syntrophism with prototrophic mutants suggests that this is a factor contributing to survival of bacteria under unfavorable conditions.

Syntrophism is exhibited by certain microorganisms belonging to the same species and also by bacteria of different species, such as Escherichia coli and Proteus mirabilis [8] or between Pasteurella pestis and Sarcina [1]. Many workers have used the syntrophic test when studying amino-acid metabolism in microorganisms [7-9].

There is no information in the literature concerning intrapopulation and interpopulation syntrophism of Pasteurella pestis, a microorganism naturally dependent on certain amino acids [2]. The object of the present investigation was to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on strains of P. pestis whose characteristics are given in Table 1. The essential amino acids for these microorganisms were determined at 28° by seeding 10^3-10^8 bacterial cells on the surface of a synthetic medium [2]. Amino acids were added to the glucose-mineral basis up to a concentration of 10 μg/ml, with the exception of cysteine hydrochloride, the concentration of which was 25 μg/ml.

Intensity of growth was assessed after incubation of the cultures for 5 days, using as the standard (6+) growth of the bacteria on complete medium (Hottinger's agar, pH 7.1-7.2). The complement of amino acids which produced growth of the culture assessed at 2+ was regarded as minimal.

Syntrophism was produced by several methods: 1) by seeding a mixture (1:1) of bacterial suspension (10^9 cells/ml) of two strains on glucose-mineral medium; 2) by the replica method [9]; 3) by the stroke method [5]; 4) by the nephelometric method [6, 7]; and 5) by the method of syntrophism of colonies [5].

EXPERIMENTAL RESULTS

Intrapopulation syntrophism was detected by studying the amino-acid requirements of the plague bacilli. After seeding 10^7-10^8 bacterial cells on synthetic medium without amino acids or with one amino acid (phenylalanine, cysteine, valine) and incubation for 5-6 days, single colonies of prototrophic and mono-auxotrophic variants were grown with a frequency of between 10^{-3} and 10^{-8} [4]. A zone of weak growth of cells of wild type, dependent on two or three amino acids, were observed around these colonies (Fig. 1). As

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TABLE 1. Amino Acid Requirements of P. pestis and Detection of Syntrophism with Mutant EV-M

Strain	Amino-acid req. for intensive growth	Deg. of syn-trophism	Strain	Amino-acid req. for intensive growth	Deg. of syn-trophism
EV	[phe] met thr	2+	1228-M	[cys]	3+
EV-M	[lys]	–	1252	[phe met] cys thr	3+
1/1092	[pro] cys	2+	1252-M	[cys]	3+
101	[phe met] cys thr	3+	1253	[phe met] cys thr	3+
101-M	[cys]	4+	1253-M	[cys]	3+
102	[phe met] cys thr	3+	1258-M	Not determinable	–
102-M	[cys]	4+	1213	[orn cys]	4+
153	[met cys thr]	–	1213-M	[orn]	4+
153-M	[cys]	–	1468	[arg] met thr	4+
154	[phe met] cys thr	2+	1469	[arg met] cys thr phe	4+
154-M	[cys]	4+	1470	[arg met leu] cys thr phe	3+
175	[phe met] cys thr	2+	1471	[arg met] cys thr	4+
179	[phe met] cys thr	3+	1472	[phe met] cys thr	3+
179-M	[cys]	4+	1473	[arg met ieu] cys phe thr	3+
1228	[phe met] cys thr	3+	1484	[phe met] cys thr	3+

Note. [ ]: minimal requirement of amino acids; – : absence of syntrophism.

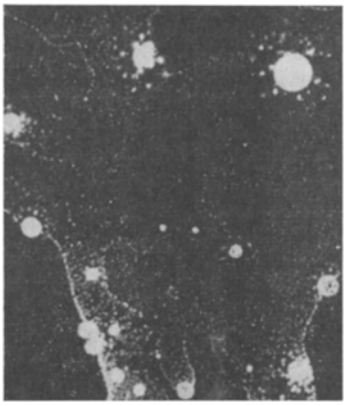

Fig. 1. Stimulation of growth of P. pestis EV-M cells by colonies of strain No. 1213 on synthetic medium with ornithine and cysteine.

the colonies of mutants developed, the zone of stimulation of growth of the polyauxotrophic cells widened. This evidently indicates excessive synthesis of definite amino acids by the prototrophic and monoauxotrophic variants. These amino acids, diffusing into the agar, enabled multiplication of the polyauxotrophic cells, constituting the majority of the bacterial population, to take place.

Syntrophism between strains of P. pestis with different nutrient requirements was found during their combined cultivation (Table 1). In seedings of bacterial mixtures of P. pestis EV-M (lys$^-$) and No. 1213 (orn$^-$ cys$^-$), continuous growth of the bacteria was observed on the 4th-5th day. No recombinants could be isolated from the mixed culture. Despite the fact that the bacterial cells multiplied on medium without amino acids, they remained auxotrophs with their previous characteristics. These observations suggested that syntrophism occurs between the bacteria of these strains. This was confirmed in later experiments by all tests for syntrophism. These showed that a lysine-dependent mutant proliferated in the presence of bacteria of strain No. 1213 (orn$^-$ cys$^-$). Further evidence of syntrophism and of its direction was given by the ability of the lysine-dependent mutant to proliferate on filtrates of a culture of P. pestis strain No. 1213 grown in liquid synthetic medium with ornithine and cysteine. The orn$^-$ cys$^-$ bacteria did not proliferate in a filtrate of strain lys$^-$ grown in medium with lysine.

As well as the pair of strains described above syntrophism of nineteen wild variants and of eight cystein-dependent mutants was studied relative to P. pestis EV-M (lys$^-$). As a result, syntrophism expressed to the same degree as in strain No. 1213, i.e., to the degree of 4+, was observed in 7 of the 27 tested cultures, to the degree of 3+ in 13, and 2+ in 4 cultures. No syntrophism was found with two strains and one cysteine-dependent mutant. The degree of syntrophism thus varied substantially, probably because of differences in the metabolism of the studied strains and differences in the localization of the genetic block of amino-acid synthesis. Since the lysine-dependent mutant was shown to grow on synthetic medium only in the presence of lysine, it must be assumed that some of the studied strains produce this amino acid or a closely related precursor of it, which would explain the trophic effect relative to cells of the lysine-dependent variant. This suggestion is confirmed by observations made by other workers [3], who determined lysine production by different auxotrophic mutants of E. coli and Micrococcus glutamicus, including mutants dependent on homoserine, phenylanaline, proline, and methionine.

The nonhomogeneity of populations of reference strains of P. pestis relative to their amino-acid requirements, described previously [4], evidently serves some definite biological purpose, in the light of these facts concerning intrapopulation syntrophism. Under conditions unfavorable for reproduction of the bacteria, the population can survive not only through the appearance of rare mutants with reduced nutritional requirements, but also through syntrophism between these mutants and wild-type cells.

Trophic synergism between bacteria thus contributes toward the stability of certain properties of the population, enabling survival to take place of microorganisms with the wild genotype, constituting the majority of this population.

LITERATURE CITED

1. A. E. Bokalo, S. V. Vedishchev, A. V. Sabinin, et al., Vestn. Mikrobiol., 10, No. 3, 241 (1931).
2. I. V. Domaradskii and V. A. Ivanov, Abstracts of Proceedings of an Inter-institute Scientific Conference on the Microbiology, Immunology, Laboratory Diagnosis, and Treatment of Particularly Dangerous Infections and the Production of Bacteriological Preparations [in Russian], Saratov (1955), p. 14.
3. L. A. Mineeva and S. I. Alikhanyan, Genetika, No. 8, 96 (1967).
4. Yu. G. Suchkov, I. V. Domaradskii, and E. A. Kanatova, in: Genetics, Biochemistry, and Immunochemistry of Particularly Dangerous Infections [in Russian], No. 1, Rostov-on-Don (1967), p. 153.
5. R. C. Clowes, J. Gen Microbiol., 18, 140 (1958).
6. B. Davis and E. Mingiolli, J. Bact., 60, 17 (1950).
7. W. Grabow and J. Smit, J. Gen. Microbiol., 46, 47 (1967).
8. J. Lampen et al., Arch. Biochem., 13, 55 (1947).
9. D. Smith, J. Gen. Microbiol., 24, 335 (1961).