SUMMARY

A *Lactobacillus* that produces large mucoid colonies on sucrose-gelatin agar was found to occur commonly in meat curing brines. Members of this variety or species were also encountered as spoilage microorganisms in ham and other cured meats that were subjected to extended smoking periods at favorable growing temperatures.

These lactobacilli were related to, but distinct from, other homofermentative *Lactobacillus* species that have low temperature limits for growth. Other distinguishing characteristics were their rather high salt tolerance, their low limiting pH in glucose broth, and their general inability to ferment the pentoses, maltose, raffinose, the higher alcohols, or inulin.

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Hereditary Changes of Some Physiological Properties in *Streptomyces rimosus*

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Received for publication September 22, 1958

The study of the physiology of antibiotic-producing organisms has shown that their ability to produce antibiotic substances reveals itself only in definite and strictly specified conditions. Slight modifications in the medium composition or cultivation conditions greatly affect the ability of strains to synthesize the antibiotics, with even closely related strains responding differently to these modifications (Savage, 1949; Garner and Koffler, 1953; Pittenger and McCoy, 1953; Levitov *et al.*, 1958).

Many actinomycetes, for example streptomycin, chlortetracycline, and oxytetracycline producers, are known to synthesize considerable amounts of the antibiotics in media containing 0.25 to 0.5 per cent of corn steep liquor (dry weight). A small increase in the amount of the corn steep liquor in the medium leads to a marked inhibition of the antibiotic synthesis due to the increase in the concentration of soluble inorganic phosphorus, the surplus of which has a negative effect on the antibiotic production (Brinberg and Grabovskaya, 1958; Prokofieva-Belgovskaya and Orlova, 1958; Prokofieva-Belgovskaya and Popova, 1959; Orlova and Verkhovtzeva, 1959b).

Various mutagenic factors are known to produce marked changes in certain morphological and physiological properties of actinomycetes. However we do not yet know any actinomycetes strain, the antibiotic-producing ability of which would remain unaffected with a marked increase in amount of corn steep liquor in the medium.

In this paper the cases concerning the occurrence of such variants in *Streptomyces rimosus*, which produces oxytetracycline, are reported.

MATERIALS AND METHODS

As starting material, a highly active strain *S. rimosus* LS-T118 was used. This culture was obtained by selection of ultraviolet-induced variants of *S. rimosus* strain 8229. Two biochemical mutants of strain 8229 were used also, as well as their recombinants.

Ultraviolet light was used for the selection of the antibiotic-producing property, the source of radiation

being a bactericidal lamp BUV-30 emitting rays with a wave length of 2537 A.

The techniques of ultraviolet irradiation and selection of active variants, as well as the medium composition and fermentation conditions, are described in the paper dealing with selection of *S. rimosus* (Mindlin and Alikhanian, 1958).

Results and Discussion

After ultraviolet irradiation of the spores (dose 2500 ergs per mm^2), LS-T118 variant no. 293 (named later strain LS-T293) was selected from among 450 survivors as the most active one.

The study of strain LS-T293 and the comparison of its activity with that of the starting strain LS-T118 revealed a phenomenon which would at first sight seem rather strange, namely, the advantages of strain LS-T293 were observed only in media containing certain batches of corn steep liquor (when its concentration in the medium was 0.5 per cent). In media containing corn steep liquor taken from other batches, strain LS-T293 was even inferior in activity to strain LS-T118. Later, it was found that the optimal concentration of corn steep liquor was different for each strain and dependent on the batch of the corn steep liquor. The typical data on the production of oxytetracycline by strains LS-T118 and LS-T293 in media containing different amounts of corn steep liquor taken from two batches are presented in table 1.

TABLE 1

Oxytetracycline production by Streptomyces rimosus strains LS-T118 and LS-T293 in media containing different amounts of corn steep liquor

Corn Steep Liquor Batch No.	Oxytetracycline Production (μg/ml)					
	Strain LS-T118			Strain LS-T293		
	Corn steep liquor in the medium (%)					
	0.25	0.5	1.0	0.25	0.5	1.0
165	3425	3500	2475	1460	2950	4460
12	3310	2820	465	2640	3650	3360

It is evident from table 1 that, for strain LS-T118, the optimal concentrations of corn steep liquor are 0.25 to 0.5 per cent with batch no. 165, and 0.25 per cent with batch no. 12. The optimal concentrations of corn steep liquor for strain LS-T293 are 1.0 and 0.5 per cent, respectively; that is, at least twice as high as those for strain LS-T118. It is interesting to note that the selection of strain LS-T293 was performed on the medium containing 0.5 per cent corn steep liquor from batch no. 12, that is, in conditions that were not optimal for the antibiotic production by strain LS-T118.

The comparison of growth and development characteristics of the two strains, as well as their abilities to produce oxytetracycline in media containing 0.25 to 0.5 per cent corn steep liquor, showed that in such conditions, strain LS-T293 was characterized by poor growth, slow carbohydrate utilization, and low levels of antibiotic production (Orlova and Verkhovtzeva, 1959a).

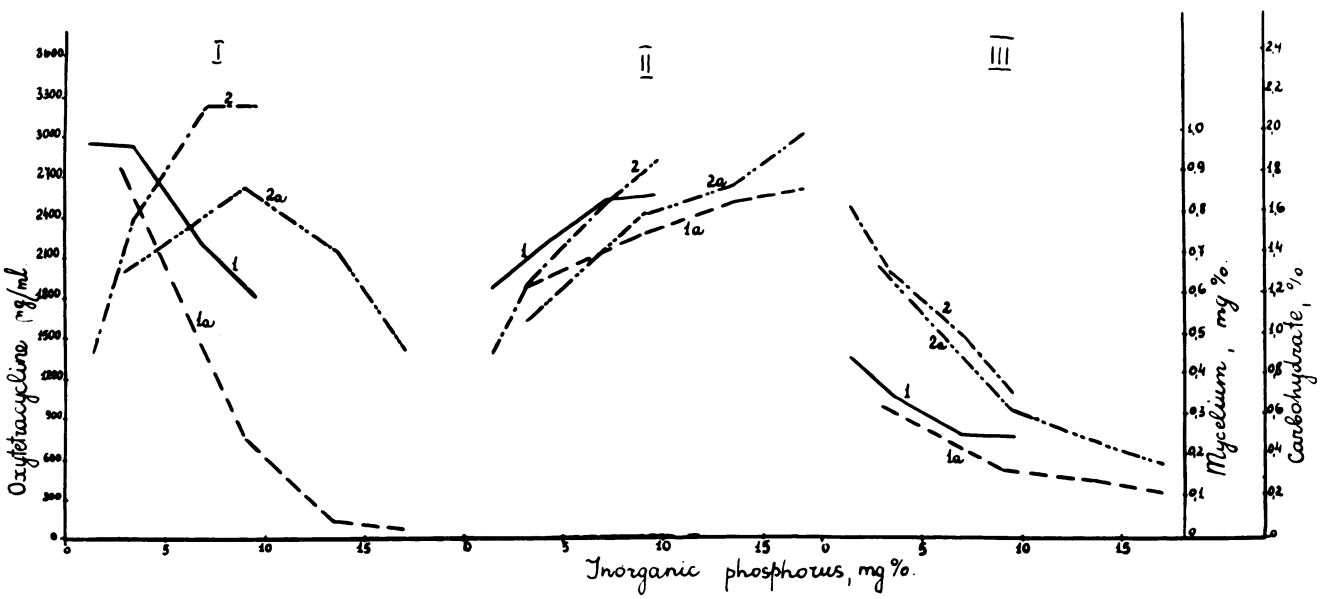

Figure 1. The dependence of growth, carbohydrate utilization, and oxytetracycline production by *Streptomyces rimosus*, strains LS-T118 and LS-T293, on the inorganic phosphorus content in corn steep liquor medium. *I* = maximum production of oxytetracycline; *II* = maximum weight of mycelia; and *III* = the carbohydrate level in the medium after 68 hr of fermentation characterizing the rate of their utilization. *1*, *1a* = strain LS-T118; *2*, *2a* = strain LS-T293; *1*, *2* = medium with 0.25 to 1.0 per cent of corn steep liquor containing 1.02 per cent of inorganic phosphorus. *1a*, *2a* = medium with 0.25 to 1.0 per cent of corn steep liquor containing 2.25 per cent of inorganic phosphorus.

In connection with the published data on the importance of inorganic phosphorus for the biosynthesis of oxytetracycline and other antibiotics, a comparative study of strains LS-T118 and LS-T293 was carried out with the use of two media containing various amounts of corn steep liquor taken from two batches differing in the content of inorganic phosphorus (figure 1).

It is evident from figure 1 that the increase in the amount of corn steep liquor and, consequently, inorganic phosphorus concentration in the medium has a negative effect on oxytetracycline synthesis by strain LS-T118 and a positive effect (to a certain limit) on oxytetracycline synthesis by strain LS-T293. Strain LS-T293 produces about the same amount of oxytetracycline in corn steep liquor medium as strain LS-T118, provided the content of soluble inorganic phosphorus in the medium is 7 to 9 mg per 100 ml, whereas the optimal concentration of inorganic phosphorus for strain LS-T118 is approximately 3 mg per 100 ml.

It should be noted that the growth of strain LS-T293 and its carbohydrate utilization depend to a larger extent on the amount of corn steep liquor in the medium as compared with strain LS-T118. The growth of strain LS-T293 in media containing small amounts of corn steep liquor is poorer, whereas in media containing large amounts of corn steep liquor, this strain produces more mycelia. Special emphasis should be laid to the fact that a marked inhibition of the antibiotic synthesis, accompanied by an increase of the inorganic phosphorus concentration, is observed in both strains when the amount of the mycelia is constant or in some cases even increased.

This fact seems to be related to an important change in the hereditary mechanism of strain LS-T293 which is directly connected with the mechanism of oxytetracycline synthesis.

A comparative study of the two strains on synthetic media supplemented with corn steep liquor ash containing different amounts of soluble inorganic phosphorus gave the same results.

To study the decisive role of inorganic phosphorus, experiments were carried out in which inorganic phosphorus in the form of potassium phosphate was added to the medium containing small amounts of corn steep liquor.

As was expected, the addition of inorganic phosphorus led to an inhibition of oxytetracycline synthesis by strain LS-T118 and to stimulation of oxytetracycline synthesis by strain LS-T293. Moreover, the addition of inorganic phosphorus, just as the increase in amount of corn steep liquor, accelerated the growth and carbohydrate utilization.

Furthermore, it is necessary to note that the addition of inorganic phosphorus stimulated oxytetracycline production by strain LS-T293 to a lesser extent than did the addition of corn steep liquor. This indicates that, for the production of large amounts of the antibiotic, strain LS-T293 requires not only phosphorus but other nutrients that are contained in corn steep liquor.

It is of interest that batch no. 12 of corn steep liquor used for the selection of strain LS-T293 contained considerable amounts of inorganic phosphorus (2.0 per cent) which could not but affect the properties of this strain.

Since strain LS-T293 requires higher concentrations of nutrients in the medium, it was possible to suppose that this strain requires higher rates of aeration (table 2).

The data presented in table 2 show that, if strain LS-T293 is cultivated under conditions unfavorable for its growth (0.5 per cent of corn steep liquor in the medium), the aeration rate has no effect on the antibiotic synthesis; whereas, strain LS-T118, if cultivated in the same medium, responds to the reduction in the aeration rate by slower growth and decreased oxytetracycline synthesis. The same phenomenon, although not as pronounced, is observed when strain LS-T293 is cultivated in media containing 0.75 per cent corn steep liquor, that is, in conditions favorable for the antibiotic synthesis.

Thus, strain LS-T293, named as a "phosphorus" mutant, has a number of essential physiological properties. It is characterized by poor growth, slow carbohydrate utilization, and low levels of antibiotic production in the medium optimal for the production of oxytetracycline by strain LS-T118, from which it is derived. The acceleration of growth, carbohydrate utilization, and biosynthesis may be achieved by an increase in the amount of corn steep liquor in the medium up to 0.75 to 1.0 per cent. In this case, the content of inorganic phosphorus in the medium plays the decisive role. For strain LS-T293 to utilize carbohydrate at the same rate as strain LS-T118, it is necessary to increase the concentration of soluble inorganic

TABLE 2

Influence of aeration conditions on oxytetracycline synthesis by Streptomyces rimosus strains LS-T118 and LS-T293

Strain LS-T	Corn Steep Liquor (dry wt)	Amt of Medium in 750-ml Flask	Oxytetracycline Yield	Mycelium Weight (max)
	%	*ml*	*μg/ml*	*mg/100 ml*
118	0.5	60	3270	746
		125	3330	739
		250	2440	667
293	0.5	60	1870	700
		125	2040	665
		250	2040	626
293	0.75	60	3400	834
		125	3240	781
		250	2960	787

phosphorus in the medium to 7 to 9 mg per 100 ml, as compared to 2 to 4 mg per 100 ml optimal for strain LS-T118.

It is interesting that, in spite of quite different requirements for the biosynthesis of the antibiotic by the two strains, they utilize just the same amount of carbohydrate and produce about the same amount of mycelia in conditions optimal for the maximal production of oxytetracycline.

The occurrence of variants similar in their physiological properties to the "phosphorus" mutant LS-T293 was observed by us not only as a result of treating the strain with mutagenic factors (ultraviolet light in our experiments) but in cases of hybridization. The hybrid (recombinant) forms in *S. rimosus* arising from the mixed culture of two biochemical mutants were described earlier (Alikhanian and Mindlin, 1957). In one of our combinations we used biochemical mutants derived from strain 8229 (the ancestor of strain LS-T118). One of these biochemical mutants (no. 1361) required threonine and the other (no. 310) required valine and isoleucine. From this combination, two types of prototrophic recombinants differing in their morphological features arose. The study of the antibiotic activity of these recombinants revealed, in addition, great physiological differences between them. While the antibiotic activity of the recombinants of one type (II), just as the activity of their parent strain 8229, decreased with the increase in amount of corn steep liquor in the medium, the antibiotic activity of the recombinants of the other type (I) in similar conditions increased; that is, their behavior resembled that of the "phosphorus" mutant LS-T293. The antibiotic activity of strain 8229 and of the two recombinant types obtained from combination 310 + 1361 in media containing different amounts of corn steep liquor (batch no. 12) is presented in table 3.

TABLE 3

Comparative activity of Streptomyces rimosus strain 8229 and the two types of recombinants between biochemical mutants of this strain in media containing different amounts of corn steep liquor

Strain	Oxytetracycline Yield (μg/ml)		Ratio of Activity in 0.5% Corn Steep Liquor Medium to Activity in 0.25% Corn Steep Liquor Medium (%)
	Corn steep liquor in the medium (%)		
	0.25	0.5	
Streptomyces rimosus 8229	1470	625	43
Recombinant 310 + 1361 of type II	1600	1145	72
Recombinant 310 + 1361 of type I	740	1555	210

As is evident from table 3, the activity of the recombinant of type I with the increase in amount of corn steep liquor from 0.25 to 0.5 per cent increased almost twofold, whereas the activities of strain 8229 and the recombinant of type II greatly decreased (by 57 and 28 per cent, respectively).

Thus, marked changes of hereditary physiological properties may be brought about not only with the treatment of strains by various mutagenic factors but also with the use of the recombination phenomenon.

SUMMARY

The occurrence of "phosphorus" mutation in *Streptomyces rimosus* strain LS-T293, under the action of ultraviolet radiation, is described. For the maximal production of oxytetracycline, this strain is shown to require increased amounts of inorganic phosphorus in the medium.

Similar strains, as far as their response to varying amounts of inorganic phosphorus in the medium is concerned, are obtained by means of hybridization (recombination).

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