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Factors Regulating Competence in Transformation of Streptococci

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ABSTRACT

PAKULA, ROMAN (University of Toronto, Toronto, Ontario, Canada). Factors regulating competence in transformation of streptococci. J. Bacteriol. **90**:1320–1324. 1965.—The highly transformable group H *Streptococcus* strain Challis produced an exocellular competence-provoking enzyme capable of converting to the state of competency incompetent cells of the homologous strain, and of the very poorly transformable strain Wicky. The competence-provoking activities of culture filtrates of strain Challis prepared at various periods of growth were tested on cells of strain Wicky. In the first 3 hr of growth, a strict correlation was found between the degree of competence and the competence-provoking activity. The period of maximal competency was followed by a rapid decline, although the competence-provoking activity of the filtrates remained at a maximum. The decay of competence was caused by a change in the structure of the cells which rendered them nonreceptive to the action of the competence-provoking enzyme.

Transformability of a bacterial strain is a genetically determined property and occurs under limited cultural conditions. Any deviation from these conditions may drastically reduce the fraction of transformed bacteria or even make transformation impossible. Under proper conditions, the bacteria absorb deoxyribonucleic acid (DNA) during a limited period of the growth cycle and undergo genetic transformation (Schaeffer, 1964). The physiological state of the culture in this particular period is designated as competence.

Experiments presented here indicate that the state of competence in a culture of a transformable *Streptococcus* strain is dependent on two factors: on the concentration of an exocellular protein in the culture, evidently an enzyme (Pakula and Walczak, 1963) capable of converting to the state of competency incompetent cells, and on the presence of receptive sites on the cell surface for this protein. The kinetics of competence can be explained by the above factors.

MATERIALS AND METHODS

Organisms. The group H streptococci, strains Challis and Wicky, employed in these experiments as recipients were received from the Central Public Health Laboratory in Colindale, London, England. Strain Challis, previously reported as a highly transformable *Streptococcus* (Pakula et al., 1958), produced the competence-provoking factor which was designated "competase" (Pakula, Can. J. Microbiol., *in press*). Strain Wicky did not produce measurable amounts of competase and was not transformable in various media suitable for transformation of other streptococci. However, 0.01 to 0.05% of transformants were occasionally found in cultures of strain Wicky which were grown in media containing beef-heart extract and serum and which were exposed to DNA for several hours. Both strains were sensitive to 25 μg/ml of streptomycin.

Media. Medium ET3 (Pakula and Walczak, 1963) was used for cultivation of strain Challis. Some batches were sterilized by filtration through Millipore filters, and others were sterilized at 121 C for 60 to 70 min. Strain Challis produced competase and acquired competence in the filtered medium in the absence of serum or serum albumin. In the heat-sterilized medium, neither competase nor transformation could be produced in the absence of serum factor. Occasionally, medium HE was employed. It contained the same ingredients as medium ET3, except that water was replaced by beef-heart extract. In some experiments, the above media were supplemented with 2.5% swine serum previously heated for 30 min at 62 C to destroy deoxyribonuclease activity.

Strain Wicky was grown in Brain Heart Infusion (Difco) broth (BHI) which contained 2.5% swine serum.

Transforming DNA. A mutant of strain Challis resistant to 2 mg/ml of streptomycin was used as donor of DNA which was prepared as previously described (Pakula, Walczak, and Shugar, 1962*b*).

Preparation of competase. Unless stated other-

wise, competase was prepared as follows. A 10-ml amount of medium ET3 was inoculated with 0.5 ml of a 16-hr culture of strain Challis. After 2 hr of growth at 37 C, the whole culture was used to inoculate 300 ml of filter-sterilized medium ET3. The peak of competence appeared usually after 1.5 or 2 hr, but growth was not interrupted until the end of the next hour. The culture was then centrifuged, and the supernatant liquid was filtered through a Millipore filter and checked for sterility. The filtrate converted incompetent cells of strain Challis and of strain Wicky to competency.

Transformation procedures. Streptomycin-sensitive bacteria were transformed to streptomycin resistance.

Strain Challis was transformed either in a natural way or by conversion to competency with previously prepared competase. For strain Wicky, only the second procedure could be used. In natural transformation, filter-sterilized medium ET3 with or without serum was inoculated with 7×10^5 to 10×10^5 colony-forming units per milliliter. The average unit contained about 2.5 cells. The culture was grown at 37 C. At given intervals, 1.8-ml samples were withdrawn and treated with 0.2 ml of a solution of 50 μg/ml of DNA. After 15 min, nonabsorbed DNA was destroyed by addition of deoxyribonuclease. The culture was grown for the next 110 min to allow phenotypic expression of the acquired streptomycin-resistance character, and 0.3-ml quantities of properly diluted samples were spread on the surface of blood-agar plates containing 250 μg/ml of streptomycin. The number of transformants was assayed after 40 hr at 37 C.

For conversion to competence, strain Challis was grown in heat-sterilized medium ET3, and strain Wicky was grown in BHI with serum. After 2 hr of growth, 0.25 ml of supernatant liquid of known competase activity was mixed with 2 ml of culture. Competase was allowed to act for 25 min at 37 C. The bacteria were then centrifuged, suspended in fresh medium, exposed to DNA, and treated further as in natural transformation.

RESULTS

Kinetics of competence. Figure 1 shows the kinetics of competence of strain Challis in medium ET3 and in medium HE supplemented with 2.5% swine serum. The cultures were started with 7.2×10^5 and 10.8×10^5 viable units per milliliter, respectively. A curve with one sharp peak was always observed in medium ET3. On the other hand, two peaks and a slightly concave plateau between them characterized the competence curve in medium HE. The period of maximal competency in the first medium did not exceed 15 min. In the second medium, high competency was observed over a period of about 1.5 hr. In both media these periods were followed by a rapid decline which occurred in the logarithmic phase of growth and well before the onset of the stationary phase.

Correlation between production of competase and development of competence. A culture of strain Challis in medium ET3 which contained serum was started with an inoculum similar in amount to that used in the experiment shown in Fig. 1. After various periods, samples of the culture were withdrawn, exposed to DNA, and assayed for the number of transformants. At the same times, sterile culture filtrates were prepared. These were used to convert to competency the incompetent cells of strain Wicky grown for 2 hr. Control tubes were supplemented with fresh medium instead of culture filtrates. The cells of strain Wicky did not acquire any competence after treatment with the medium. Thus, all the observed conversion of Wicky cells was due to competase activity of culture filtrates of strain Challis.

As shown in Fig. 2, the left arm of the curve illustrating development of competence in the Challis culture runs parallel to the line indicating numbers of Wicky cells converted to transformability through mediation of the culture filtrates prepared during the first 3 hr of growth. Both curves reached their maximum at approximately

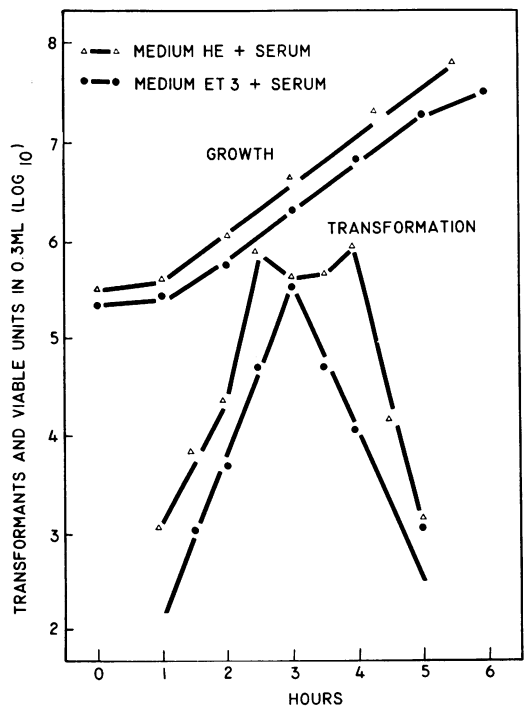

FIG. 1. *Transformation and growth of strain Challis in medium HE and ET3 supplemented with serum.*

the same time. However, whereas the concentration of competase in the culture did not decrease, even in the late stationary phase of growth, competence decayed very rapidly.

Absence of a transformation inhibitor in cultures of strain Challis. The decline of competence suggested that strain Challis might produce a substance which inhibits either conversion to competency or DNA uptake. To test these possibilities, sterile filtrates were prepared from 5-hr cultures that had passed the period of competence. Samples (2 ml) from 2-hr cultures of strains Challis and Wicky were centrifuged and suspended either in the 5-hr culture filtrates with the suspected inhibitor or in fresh medium. Competase and then DNA were subsequently added as in the routine procedure for conversion. After 15-min treatment with DNA, the bacteria were again collected by centrifugation, suspended in fresh medium, allowed to grow for phenotypic expression, and spread on streptomycin-blood-agar plates. As shown in Table 1, transformation of cells treated with the filtrates was as high as transformation of the controls. The results were the same with filtrates concentrated 8 to 10 times with Carbowax 20,000 (Union Carbide Corp., New York, N.Y.). The experiments shown in Table 1 were carried out with filtrates prepared from cultures in heat-sterilized medium ET3. However, similar results were obtained when filter-sterilized medium was used.

TABLE 1. *Transformation in the presence or absence of a 5-hr culture filtrate of strain Challis*

Transformed strain	Percentage of transformed viable units			
	Filtrate present		Filtrate absent	
	Expt 1	Expt 2	Expt 1	Expt 2
Challis.....	20.6	16.5	23.4	15.8
Wicky.....	24.8	30.3	27.3	32.6

TABLE 2. *Decline of susceptibility to competase of cells of strain Challis during growth*

Time of growth	Percentage of transformed viable units		
	Expt 1	Expt 2	Expt 3
hr			
1.5	12.1	9.3	11.8
2.5	11.8	14.4	22.0
3.5	1.1	0.7	0.8
4.5	0.28	0.43	0.21
5.5	0.03	0.30	0.07
17.0	0.05	0.02	0.10

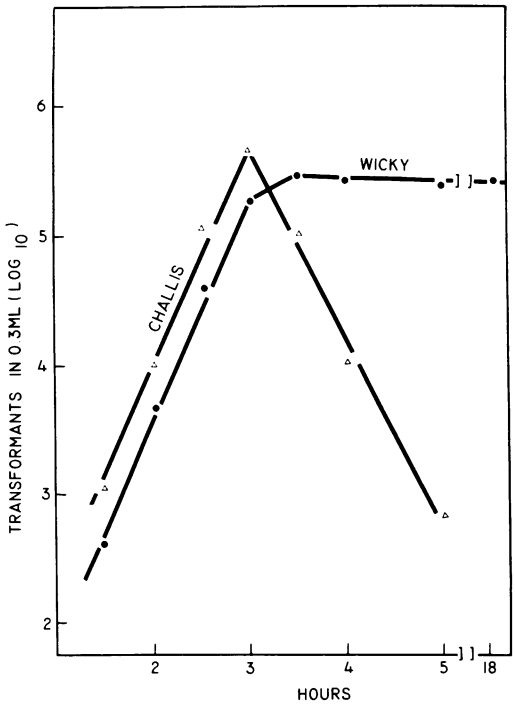

FIG. 2. *Correlation between competase activity and the efficiency of transformation.*

Decline of cell receptivity to competase during growth. Since the decay of competence was not caused by an inhibitor, the possibility that it resulted from a change of cell susceptibility to competase was investigated. Strain Challis was grown in heat-sterilized medium ET3, in which competase is not produced. At the intervals indicated in Table 2, proper volumes were withdrawn from the culture and centrifuged. The bacteria were then suspended in 0.85% NaCl to obtain a suspension with an optical density of 0.3 at 650 mμ (Bausch & Lomb Spectronic-20 colorimeter). The suspensions were then diluted 25-fold in medium ET3. From these dilutions, 2-ml samples were dispensed into tubes, and the bacteria were exposed to competase and then to DNA. Thus, competase acted on a similar number of cells withdrawn from the culture at various periods of growth. At the time of DNA addition, the number of viable units varied between 10^6 and 1.3×10^6 per milliliter. This was due to variation of the chain length occurring during the growth cycle. As shown in Table 2, the high susceptibility to competase of cells in the early phase of growth was followed by a rapid decline.

DISCUSSION

Recent data show that populations of transformable pneumococci, of *Bacillus subtilis*, and of streptococci acquire competence through the mediation of enzymes. Tomasz and Hotchkiss (1964) showed that the ability of pneumococci to absorb DNA is provoked by a protein, probably an enzyme. In the *B. subtilis* transformation system, competence seem to be related to the action of an autolytic enzyme (Young and Spizizen, 1963) recently identified as an acetyl-muramyl-L-alanine amidase (Young, Tipper, and Strominger, 1964). The chemical reactions provoked by the streptococcal competase are at present unknown. However, with C^{14}-labeled DNA it was shown that competase results in a change on the cell surface to allow DNA uptake (Pakula and Hauschild, Can. J. Microbiol., *in press*). Immunological methods lead to the same conclusion. Rabbits injected with competent cells of strain Challis and of strain Wicky produce specific globulins which inhibit transformation by blocking DNA uptake. Globulins from rabbits immunized with noncompetent cells of either strain are not inhibitory (Pakula, 1965). Differences in antigenic structure between competent and noncompetent pneumococci were previously reported by Nava, Galis, and Beiser (1963).

It was shown (Fig. 1) that competence of strain Challis in medium HE remained maximal for 1.5 hr. Thomas (1960) found that albumin prolongs the duration of this physiological state in cultures of pneumococci. It is, therefore, possible that the same effect is exerted by the proteins present in beef-heart extracts. In previous work with a group H *Streptococcus*, strain SBE, a 4-hr period of high competency was observed (Pakula et al., 1962*a*). This suggests that not all cells were competent at a given time, and that the long duration of competence might be due to a continuous turnover of the population (Hotchkiss, 1954).

The rapid decline of competency which occurs long before the onset of the stationary phase of growth cannot be explained by exhaustion of the medium. Tomasz and Hotchkiss (1964) found that sterile supernatant fluids of pneumococcal cultures, prepared after the period of high competency had passed, contain an inhibitor of transformation. They also showed that the decay of competence is related to its action. However, no inhibitor could be found in streptococcal cultures, although filtrates were prepared at various periods of the growth cycle. On the other hand, Table 2 shows that the decay of competence in streptococcal cultures is caused by a change of the cell structure. Young, Spizizen, and Crawford (1963) presented direct evidence which shows that quantitative chemical changes occur in the cell wall of transformable strains of *B. subtilis* during growth. The action of competase is of a narrow specificity. For instance, competase derived from cultures of the above-mentioned strain SBE has little activity on Challis cells and vice versa. It seems, therefore, that some minor changes on the cell surface render the cells nonreceptive to competase, resulting in loss of transformability.

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