Transformation of Streptococci in Chemically Defined Media.

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This report describes a chemically defined medium which fosters development of competence in cells of group H streptococcus (strain Challis) and which supports all steps involved in transformation. In addition, bacterial products which increase competence in a poorly transformable strain of streptococci are found in culture filtrates of strain Challis grown in this medium.

Previous reports on the transformation of group H streptococcus, and on the production of similar competence factor indicated that complex media were necessary (Pakula, 1965).

Materials and Methods

Cultures of group H streptococci used were previously described (Leonard et al. 1966).

Transforming DNA was prepared from cells of group H streptococcus, strain SBE:12 resistant to 2 mg streptomycin/ml and transformants to streptomycin resistance were scored as described by Leonard et al. (1966).

Competence-provoking factors were tested by the procedure of Pakula (1965A). Antisera to competent and non-competent cells were prepared as described by Pakula (1965B).

Two chemically defined media were used in these studies. Both media were prepared by autoclaving together (15 lbs., 15 min.) all the components shown in Table 1, except for the last 3 salts and glucose. These were each sterilized by filtration through Millipore filters (0.45μ) and added aseptically. $NaHCO_3$ was added to the medium just prior to its use. The growth medi-

um, designated MS6, was used for preparation of inocula and for maintenance of stock cultures. MS6 medium supports excellent growth of strain Challis (about

Table 1. Chemically defined media.

Component	Amount g/liter		Component	Amount g/liter	
	MS6	MS6-C		MS6	MS6-C
L-amino acids			Purines and pyrimidines		
Arginine	0.6	0.28	Adenine	0.01	0.01
Methionine	0.2	0.34	Guanine	0.01	0.01
Valine	0.2	0.82	Uracil	0.01	0.01
Leucine	0.2	0.70			
Isoleucine	0.2	0.54	Salts		
Lysine	0.2	0.74			
Serine	0.2	1.92	NaCl	0	5.0
Tyrosine	0.4	0.62			
Cysteine	0.2	0.025	K_2HPO_4	12.0	1.0
Histidine	0.2	0.14			
Cystine	0.05	0.60	KH_2PO_4	5.2	0
Phenylalanine	0.2	0.14			
Glycine	0.2	0.20	$(NH_4)_2SO_4$	2.0	0
Tryptophan	0.2	0.05			
Glutamic acid	0.5	2.84	$MgSO_4.7H_2O$	0.2	0.2
Alanine	0.2	0.48			
Proline	0.2	0.82	$MnSO_4.H_2O$	0.01	0.01
Threonine	0.2	0.50			
Aspartic acid	0	0.74	$FeSO_4.7H_2O$	0.01	0.01
Vitamins			$NaHCO_3$	5.0	5.0
Niacinamide	0.01	0.01	Glucose	10.0	1.0
Thiamine	0.01	0.01			
Riboflavin	0.01	0.01			
Ca-Pantothenate	0.01	0.01	Water (double distilled) to make 1 liter.		
Biotin	0.003	0.003			
p-aminobenzoic acid	0.002	0.002			
Pyridoxal.HCl	0.002	0.002			
Pyridoxamine	0.002	0.002			
Folic acid	0.002	0.002	pH 7.6		

All the components (except for the last 3 salts and glucose) were autoclaved together at 15 lb. pressure for 15 min. These were each sterilized by filtration and added aseptically.

2×10^9 cells/ml) and it has been transferred at least 50 consecutive times in MS6. The competence-development medium, designated MS6-C, supports very poor growth of strain Challis.

The following procedure was used for transformation of strain Challis: 4.5 ml of MS6-C medium were inoculated with 0.25 ml from an overnight culture grown in MS6 medium. The tubes were incubated for about 60 min. or until the original optical density about doubled as measured with a Coleman Junior spectrophotometer set at 570 mμ. Transformations were then carried out by mixing together 0.5 ml of competent cells, 0.4 ml of MS6-C medium and 0.1 ml DNA (20μg). The tubes were incubated for 90 min before adding 40 μg of pancreatic deoxyribonuclease (1 x crystallized, Worthington) and incubation continued for 15 min. The samples were then diluted and plated. Appropriate controls with a mixture of DNA and deoxyribonuclease were always included (Leonard et al. 1966).

Results and Discussion

The effect of time of incubation in MS6-C on the development of competence by cultures of strain Challis is shown in Table 2. Cells became highly competent after approximately 60 min incubation and transformation frequencies of over 10% were obtained routinely. Upon further incubation, competence was gradually lost; and after $4\frac{1}{2}$ hr incubation no significant transformation was obtained.

Table 2 also shows that strain Challis grew poorly in MS6-C medium, as measured by plate counts, even though the optical density increased 2 to 3 fold in the first 90 min of incubation. Under our test conditions, competent cells of strain Challis were obtained in chemically defined media only when glucose and phosphate concentrations were low and when poor growth occurred. All efforts to obtain competent cells in MS6 (the growth medium) failed, although many modifications and conditions were tested. On the other hand, once competent cells were obtained, their uptake of DNA occurred equally well in either MS6-C or MS6 medium. The relationship between inoculum size, growth, nutrients and development of competence requires further studies.

Addition of $NaHCO_3$ is not required for growth of strain Challis in either

Table 2. Transformation of group H streptococcus, strain Challis in a chemically defined medium.

Incubation time in MS6-C before addition of DNA*	Recipient cells**	Transformation to Streptomycin resistance	
min.	total counts/ml	transformants/ml	%
0	1.0×10^8	0	0
30	9.8×10^7	7.2×10^4	0.07
60	1.3×10^8	1.9×10^7	14.6
90	1.5×10^8	1.7×10^7	11.4
120	1.7×10^8	4.7×10^6	2.8
150	1.8×10^8	2.1×10^6	1.2
180	2.1×10^8	1.1×10^6	0.5
210	2.0×10^8	4.5×10^5	0.2
240	2.5×10^8	7.0×10^4	0.03
270	2.4×10^8	1.1×10^3	0.0005

* Strain Challis was grown in MS6-C medium for the indicated time, then 0.5 ml samples were exposed to 0.1 ml DNA for 90 min before scoring for transformants to streptomycin resistance.

**Total recipient cell counts were made after each indicated incubation time in MS6-C

MS6, MS6-C, or complex media. However, addition of $NaHCO_3$ to MS6-C is required for development of competence by strain Challis cells. A concentration of 0.5% $NaHCO_3$ was found optimal. Also, addition of $NaHCO_3$ to MS6, the growth medium, helped in maintaining the ability of stock cultures of strain Challis to transform well in the absence of serum. The role of $NaHCO_3$ in the development of competence remains to be elucidated.

Strain Wicky is a poorly transformable strain, but addition of culture filtrates from competent cells of strain Challis (culture filtrates from MS6-C medium with or without serum) increased its transformation over 3,000 fold (Table 3). The increased transformation of strain Wicky provoked by added competence factor could be blocked by the addition of antisera to competent

cells but not by antisera to non-competent cells (Table 3). Similar results were reported by Pakula (1965B) and, in pneumococcus, by Tomasz (1965).

Table 3. Effect of competence provoking culture filtrates on the transformation of group H streptococcus, strain Wicky.

Culture of strain Wicky*	Three hour old culture filtrates of strain Challis		Antisera to cells of strain Challis**	Transformation to streptomycin resistance
ml	media	ml	type of cells used for immunization	%
0.5	-	0	none	0.0006
0.5	-	0	non-competent	0.0006
0.5	-	0	competent	0.0006
0.5	MS6-C	0.1	none	2.1
0.5	"	0.1	non-competent	2.3
0.5	"	0.1	competent	0.0006
0.5	MS6-C + serum***	0.1	none	2.3
0.5	"	0.1	non-competent	2.3
0.5	"	0.1	competent	0.0007

Cultures of strain Wicky were grown for 2 hr in brain heart infusion broth with 2.5% horse serum added. Then 0.5 ml of these cells (1.5×10^8 cells/ml) were exposed to 0.1 ml culture filtrates (from 3 hr old cultures of competent strain cells of Challis grown in the indicated media) for 20 min before addition of 0.1 ml DNA (20 μg). Incubation was continued for 90 min before scoring for transformants to streptomycin resistance.
* Controls of strain Wicky cells with DNA also had added 0.3 ml of fresh MS6-C medium or fresh MS6-C medium with 2.5% horse serum.
** These samples had 0.1 ml antisera added when the competence provoking factor was added and the samples were incubated 30 min before adding DNA.
*** MS6-C with 2.5% horse serum added.

Cells of Challis from MS6-C medium with or without serum transformed equally well (frequencies of about 12%). Yet the activity of competence factor produced in MS6-C medium could not be demonstrated consistently due to an apparent instability of such factor. On the other hand, the activity of competence factor produced in MS6-C with serum was stable and stored well for over 4 weeks at 4 C. Since the demonstration of competence factor was

greatly improved by the presence of stabilizing factors in serum, we should emphasize the importance of the components of the medium used for competence development in such studies. It is therefore conceivable that, in any bacterial system, the failure to demonstrate competence factor(s) in culture filtrates of competent cells may depend on failure to achieve stabilizing conditions for such factor(s), rather than their actual absence.

Transformation in chemically defined media has been reported for several other microorganisms including pneumococcus (Tomasz, 1964) and haemophilus (Ranhand and Herriott, 1966). These reports, in addition to our present report on a chemically defined medium for the transformation of streptococcus, should make more feasible studies on the development of competence and on the mechanism of action of competence factor(s).

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