Alteration of capsular type of encapsulated strains of *Staphylococcus aureus* during freeze-drying and storage

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Summary. Remarkable alteration was shown in capsular type antigen production in encapsulated strains of *Staphylococcus aureus* stored by lyophilization for 10 years. This alteration was further elucidated by antibody production in rabbits immunized with the altered strain and by absorbing the antibodies with representative capsular type strains.

Although biological and biochemical alterations during storage of bacterial strains have been known, no paper has reported the conversion of the capsular type of encapsulated bacterial strains. Ten years ago, we isolated a number of encapsulated strains of *Staphylococcus aureus.* The capsular types were determined and the strains stored by lyophilization. Recently, the capsular types were reexamined and significant alterations were observed.

Material and methods. 44 encapsulated strains of *S. aureus* were isolated from human clinical specimens in 1972. Encapsulation of the organisms was identified according to the description of Yoshida[1] and Witte[2]. Cell suspensions prepared with 10% skim milk were lyophilized, sealed under vacuum and stored at room temperature. In 1982, the following examination was performed to observe whether or not the capsular types had changed. 5 lyophilized strains were suspended in saline. After appropriate dilution with saline, they were directly inoculated in a serum-soft agar medium and cultured at 37 °C overnight according to the method of Finkelstein and Sulkin[3]. 5 colonies exhibiting diffuse-type growth were isolated and their capsular types were determined by the serum-soft agar technique reported by Yoshida[4], using the rabbit anticapsular sera used in 1972. These antisera had been stored at −70 °C and no change in serological activity was shown. Since similar capsular patterns were exhibited in all 5 colonies, the remaining 39 lyophilized strains were cultured in brain heart infusion broth at 37 °C overnight. Capsular typing of single colonies exhibiting diffuse-type growths in the medium was performed using the rabbit antisera noted above.

Results. In 1972, 26 (59.0%) out of 44 strains were monovalent capsular type A. However, in 1982 only 1 strain showed a similar capsular type and 1 strain in each case became capsular type C and non-typable, respectively; the others were bivalent or trivalent-type, exhibiting a variety of reactivity to the antisera. One monovalent capsular type B strain was trivalent-type and showed decreasing activity to antisera. Similar capsular types were observed in 3 out of 14 monovalent capsular type C strains and the remaining strains were shown to be trivalent types. Of previously 2 bivalent strains, 1 strain became bivalent and non-typable, and 1 trivalent-type strain converted to a bivalent-type as shown in the table.

Further experiments were performed to observe whether the polyvalent capsular type strain can produce specific antibodies against corresponding capsular type antigens in rabbits. A rabbit was immunized with strain NS-4, originally a monovalent capsular type A strain converted to trivalent capsular type, by the method described by one of the authors (K.Y)[4]. Tentatively designating 0.1 ml of anticapsular rabbit antisera containing the minimum amount of serological activity to convert diffuse to compact type growth in serum-soft agar as 1 unit, strain NS-4 antiserum was seen to possess 0.125, 0.5, 1.0 and 1.0 unit of activity against capsular type strains A, B, C, and homologous strain, respectively. Next, representative capsular type strains were cultured on brain heart infusion broth at 37 °C overnight, washed twice with saline and lyophilized. They were combined with rabbit antiserum containing 1 unit of activity against homologous strain NS-4 and the minimum amounts of organisms capable of completely absorbing out the serum activity which converted diffuse to compact type of the strains in serum-soft agar were measured. In this experiment, the minimum amounts of organisms to absorb the serum activity of anticapsular type A, B, C and homologous strains were 32, 128, 256 and 256 mg, respectively. These results indicated that capsular type antigen production demonstrated by the regular typing method was reliable and that the capsular type conversion truly occurred.

Discussion. Although loss of capsule by stock strains of encapsulated bacteria is a common phenomenon, investigations concerning alteration of the properties of encapsulated organisms during storage are few. We noted the decrease of mouse virulence in a strain of *Klebsiella pneumoniae* and the enhancement of enzyme production with in increased appearence of unencapsulated *S. aureus* during storage[5-7]. However, to our knowledge no paper concerning the antigenic substance of bacterial capsules has been published. At present, the biochemical properties of the capsular type antigen of *S. aureus* are not known; however, conversion of the capsular type, which is located at the outermost layer of the cell wall[1], was certainly shown to have occurred in these experiments using the same antisera used before storage. Several papers[8-10] describe the alteration of the agglutinating activities of some bacterial strains to antisera during storage. This alteration has not been observed during short storage times. Changes in the struc-

Alteration of capsular-type of 44 encapsulated strains of *Staphylococcus aureus* after storage for 10 years

January 1972 Capsular type	Number of strains	July 1982 Capsular type	Number of strains
A (+++)	26	A (++)[a]	1
		C (+++)	1
		A (++)/B (++)	2
		A (+++)/C (+)	1
		A (+)/C (+++)	1
		B (++)/C (+)	4
		A (+)/B (++)/C	1
		A (++)/B (++)/C (++)	6
		A (++)/B (++)/C (+++)	1
		A (++)/B (++)/C (+)	7
		Non-typable	1
B (+++)	1	A (++)/B (++)/C (++)	1
C (+++)	14	C (+++)	3
		A (++)/B (++)/C (+++)	5
		A (++)/B (++)/C (+)	5
		A (++)/B (++)/C (++)	1
A (+++)/C (+++)	2	A (++)/B (++)/C (+)	1
		Non-typable	1
A (+++)/B (+++)/ C (+++)	1	A (++)/B (++)	1

[a] (+++), (++) and (+) indicate perfect, weak and faint reaction against rabbit anticapsular sera, respectively.

tural sequence and degradation of nucleic acid have been noted[11-13], and our observation of the transformation of capsular type antigen of *S. aureus*[14] reflected classical studies described by Avery[15]. Genetic mechanisms are probably involved in cellular injuries due to freezing and drying in addition to the unknown factor of maintenance for a long time[16]. While biochemical investigations are required to elucidate this finding further, these observations already cast doubt on whether the capsular types of bacterial strains widely used are reliable, and emphasize the need to reexamine representative capsular type strains and reference antisera.

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On the interchromosomal connections in plants[1]

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Summary. Using Giemsa C-banding technique, the existence of interchromosomal connections in well spread root tip metaphase plates in a variety of plant species has been demonstrated. Various types of interchromosomal connections are observed, involving satellite, telomeres, interstitial regions and in a few cases centromeres, too. The possible role of these interchromosomal connections in establishing the homologous and non-homologous association in the somatic chromosomes and maintaining the spatial relationship of the genetic apparatus is indicated. In the majority of the cases the connections are made up of constitutive heterochromatin.

The existence of interchromosomal connections during different stages of meiotic and mitotic cell cycle have been demonstrated in various cell types in animals using electron and light microscopic studies. These connections establish a supra-structure which govern the exact distribution of chromosomes during the cell cycle[3,4]. In several plant species Wagenaar[5] from his studies on telophase and early prophase in root tip cells suggested that chromosomes are attached end-to-end during interphase and these attachments form chain like interphase association. Almost similar type of associations among the chromosomes through heterochromatic interchromosomal connections have been reported in *Ornithogalum virens*[6] and induced and/or revealed by 4-aminouracil in *Vicia faba*[7].

While working on the Giemsa C-banding patterns in various plant species in Leguminosae the authors noted very thin interchromosomal connections in well spread metaphase plates from root tips. The following types of chromatin connections could be delineated: a) Intersatellited connections, b) thin end-to-end connections among chromosomes, c) interstitial connections, d) centromere-to-centromere connections, e) centromere-to-telomere-to-telomere connections and a combination of above types.

The frequency of various types of connections varies in different plant types. The end-to-end connections are most frequently observed and centromere-to-centromere connections are rare. Approximately 80% of the connections observed are composed of heterochromatin. However, a smaller percentage is euchromatic too, as evidenced by C-banding technique. In order to ascertain the existence of the connections, control preparations were made to avoid the possible chemical pretreatment effect (i.e. the effect of colchicine, etc.) and the resulting fixation artefact, if any. As such, the excised root tips were either directly fixed in Carnoy's fixative or fixed following a cold water treatment (near freezing temperature) for 24 h to facilitate chromosome separation, and processed as usual for C-banding[8]. In all such cases, connections were noted.

In view of these observations and a survey of a wide variety of plants with small and large sized chromosomes, it appears that these interchromosomal connections may be universally present either in a detectable or undetectable state. However, the frequency and ease with which these connections can be manifested, depend upon the technique of squashing pertaining to mechanical pressure applied, since such thin connections easily break. If one examines carefully a large number of cells in a monolayer group of cells (not the too much scattered and isolated cells) in a squash preparation made by applying uniform gentle pressure either on a macerated root tip tissue or the hydrolyzed small pieces of root tip followed by Giemsa C-band staining, the connections should be observed at least in a few cells. The monolayer small group of cells is especially suited for such an observation as it involves the minimum possible distortion of chromosomal material in a cell vis-a-vis revealing the chromosomal features.

The very fact that interchromosomal connections are more clearly observed after C-banding as compared to orcein or Feulgen staining suggests that C-banding may cause a swelling or dispersion of basic chromatin fiber to allow their visibility at the level of light microscope. In fact, such a situation was recorded by Schwarzacher[9] who suggested