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ISOLATION OF NON-PILIATED CLONE OF *CORYNEBACTERIUM RENALE* STRAIN 115

Ryoichi OKAMOTO, Shinji TAKAI, Ryo YANAGAWA and Hisaaki SATO

Department of Hygiene and Microbiology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

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A non-piliated (P^-) clone was isolated from 368 colonies of a densely piliated (P^+) clone of *Corynebacterium renale* strain 115 by means of a very weak agglutination with anti-pili serum. Lack of pili in P^- was confirmed by immunodiffusion and electron microscopy.

INTRODUCTION

Corynebacterium renale was found to be the first Gram-positive bacteria which possessed pili[9,10]. The pili of *C. renale* have been studied in the authors' laboratory morphologically[9,10], chemically[4,5], and immunologically[4,5,9]. Adhesion properties of the piliated corynebacteria have been reported[1,2,3]. Clones of *C. renale* possessing numerous and no pili have been thought to be essential for studies of the function of the pili in the bacteria. The present communication deals with isolation of a non-piliated (P^-) clone from a densely piliated (P^+) clone of *C. renale* strain 115.

MATERIALS AND METHODS

Strain *C. renale* strain 115 was used. This strain possessed pili[1], agglutinated trypsinized sheep erythrocytes[1], and adhered to tissue cultured cells[2] and the mucous membrane of the urinary bladder of mice[3].

Medium and cultivation The organisms were cultivated on nutrient agar (pH 7.2) at 37°C for 24 hours.

Antiserum to *C. renale* Antisera against *C. renale* were prepared as reported[8].

Anti-pili serum Anti-pili serum was prepared as previously reported[1]. Anti-strain 115 serum was absorbed with the organisms of *C. renale* ATCC 19412, which possessed only a few pili, and the somatic antigen identical with strain 115. The cells of ATCC 19412 collected from the culture on the agar medium were mixed and incubated with 1 ml of anti strain 115 serum at 37°C for 2 hours. The mixture was centrifuged, and the supernatant was filtered through a membrane filter (Millipore Corp., 450 nm pore size). The procedure was repeated until the absorption was completed, which was confirmed by immunodiffusion.

Slide agglutination test using anti-pili serum One drop of the anti-pili serum was mixed on a slide glass with a drop of saline suspended with bacteria. Normal rabbit serum was used instead of the anti-pili serum for control. Agglutination was recorded as positive soon after the mixing.

Purification of pili Pili of P^+ was partially purified according to the method described previously[5].

Electron microscopy Examination by electron microscopy was done as reported[9].

Immunodiffusion Antigens for immunodiffusion were extracted from organisms of *C. renale* with acid[6]; bacteria were suspended in 1 ml of saline containing N/20 HCl, bathed in boiling water for 15 min, cooled, and after being sedimented by centrifugation, the supernatant was used as the antigen after being neutralized with 1 N NaOH.

RESULTS

Seven arbitrarily selected colonies of *C. renale* strain 115 were found without exception to be strongly agglutinated by the anti-pili serum. And electron microscopy revealed that they possessed pili. One of the arbitrarily selected clones which clonized twice was again examined; all 15 colonies of this clone were agglutinated by the anti-pili serum and possessed pili. One of the colonies (s-13-1-5) was designated as P^+; its electron micrograph is shown in figure 1.

The colonies of P^+ were examined by slide agglutination using anti-pili serum to determine if any were not agglutinated by the antiserum. Of the 368 colonies examined, only one was very weakly agglutinated by the anti-pili serum, and it appeared only after a prolonged period of time after mixing. Electron microscopically, the organisms of this colony did not possess pili (fig. 2). This clone (s-13-1-5-P^--16) was designated as P^-.

There was no difference between the colonial features of P^+ and P^-; both colonies were smooth, slightly yellowish, glistening and moist.

In immunodiffusion using anti-P^+ serum, P^+ produced 3 lines while P^- formed 2 lines, which were common to those formed by P^+. P^- lacked the third line produced by the P^+ adjacent to the antigen well (fig. 3). This line corresponded to that produced by purified pili (fig. 4). ATCC 19412, which possessed only a few pili on a very limited number of organisms, also lacked the line produced by the pili.

DISCUSSION

Since their discovery in 1968[10], the pili of *C. renale* have been studied morphologically[9,10], chemically[4,5], and immunologically.[4,5,9] Adhesion of *C. renale* to trypsinized sheep erythrocytes, tissue cultured cells and the mucous membrane of the urinary bladder of mice mediated by the pili was disclosed by inhibition of the adhesion by anti-pili serum.[1,2,3] These experiments were done, however, using densely and slightly piliated

strains of *C. renale*. If we could have used piliated and non-piliated clones derived from the same strain, the results would probably have been clearer.

The P^- clone of *C. renale* strain 115, which was isolated by means of very weak agglutination with anti-pili serum and verified by immunodiffusion and electron microscopy in the present study, will be a useful tool for studies on the biological function of the pili of *C. renale*. As a matter of fact, our recent study of pH dependent adhesion of piliated *C. renale*[7] was completed with the aid of P^-.

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Explanation of plate

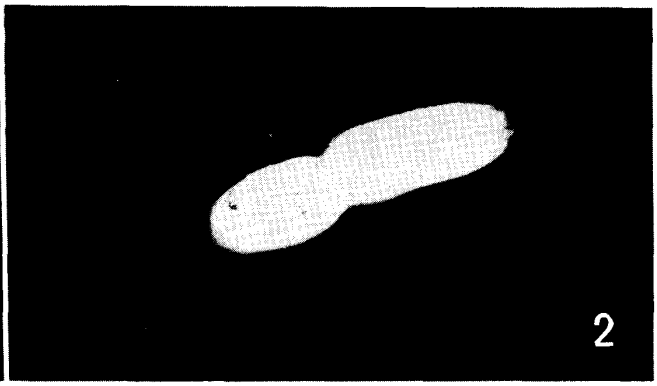
Fig. 1 Electron micrograph of P^+ (×20000)

Fig. 2 Electron micrograph of P^- (×20000)

Fig. 3 Immunodiffusion of anti-P^+ serum (central well) with the antigens of P^+ and P^-

Fig. 4 Immunodiffusion of anti-P^+ serum (central well) with the antigens of P^+, P^-, ATCC 19412 and purified pili of P^+


1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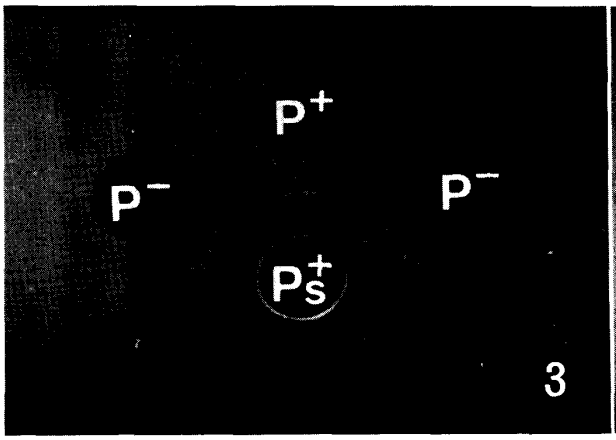
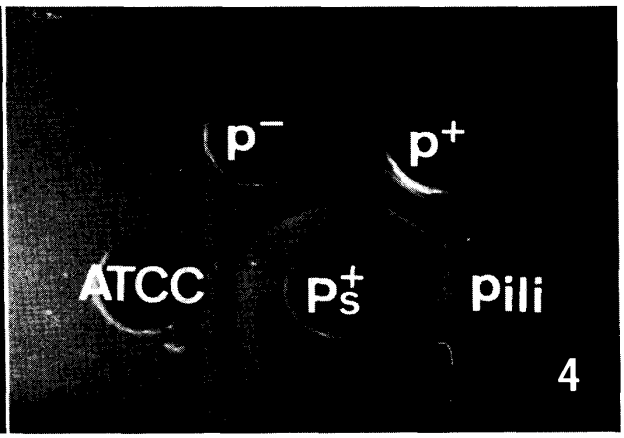

P+
P−
P−
Ps+
3


P−
P+
ATCC
Ps+
pili
4