HOKKAIDO UNIVERSITY

Title	ISOLATION OF NON-PILIATED CLONE OF CORYNEBACTERIUM RENALE STRAIN 115
Author(s)	OKAMOTO, Ryoichi; TAKAI, Shinji; YANAGAWA, Ryo et al.
Citation	Japanese Journal of Veterinary Research, 29(3-4), 79-82
Issue Date	1981-12-05
DOI	https://doi.org/10.14943/jjvr.29.3-4.79
Doc URL	https://hdl.handle.net/2115/2239
Type	departmental bulletin paper
File Information	KJ00003436417.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Jpn. J. Vet. Res., **29**, 79–82 (1981)

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

(Received for publication, September 18, 1981)

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^-.

References

1) HONDA, E. & YANAGAWA, R. (1974): Agglutination of trypsinized sheep erythrocytes by the pili of *Corynebacterium renale Infect. Immun.*, **10**, 1426–1432
2) HONDA, E. & YANAGAWA, R. (1975): Attachment of *Corynebacterium renale* to tissue culture cells by the pili *Am. J. Vet. Res.*, **36**, 1663–1666
3) HONDA, E. & YANAGAWA, R. (1978): Pili-mediated attachment of *Corynebacterium renale* to mucous membrane of urinary bladder of mice *Ibid.*, **39**, 155–158
4) KUMAZAWA, N. & YANAGAWA, R. (1972): Chemical properties of the pili of *Corynebacterium renale Infect. Immun.*, **5**, 27–30
5) KUMAZAWA, N. &. YANAGAWA, R. (1973): Comparison of the chemical and immunological properties of the pili of three types of *Corynebacterium renale Jpn. J. Microbiol.*, **17**, 13–19
6) LANCEFIELD, R. C. (1928): The antigenic complex of *Streptococcus haemolyticus* I. Demonstration of a type-specific substance in extracts of *Streptococcus haemolyticus J. Exp. Med.*, **47**, 91–103
7) TAKAI, S., YANAGAWA, R. & KITAMURA, Y. (1980): pH-dependent adhesion of piliated *Corynebacterium renale* to bovine urinary bladder epithelial cells *Infect. Immun.*, **28**, 669–674
8) YANAGAWA, R., BASRI, H. & OTSUKI, K. (1967): Three types of *Corynebacterium renale* classified by precipitin reaction in gels *Jpn. J. Vet. Res.*, **15**, 111–120
9) YANAGAWA, R. & OTSUKI, K. (1970): Some properties of the pili of *Corynebacterium renale J. Bacteriol.*, **101**, 1063–1069
10) YANAGAWA, R., OTSUKI, K. & TOKUI, T. (1968): Electron microscopy of fine structure of *Corynebacterium renale* with special reference to pili *Jpn. J. Vet. Res.* **16**, 31–38

Explanation of plate

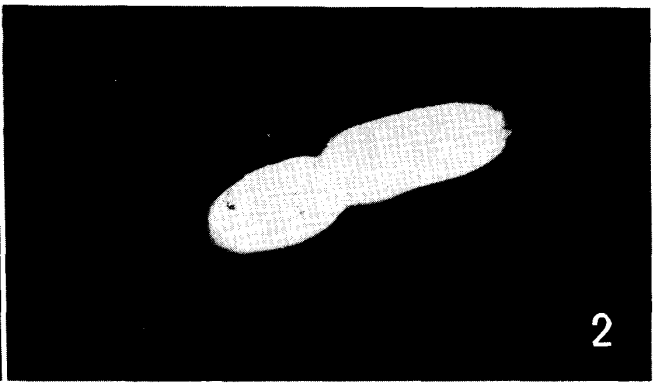
Fig. 1 Electron micrograph of P^+ (×20000)

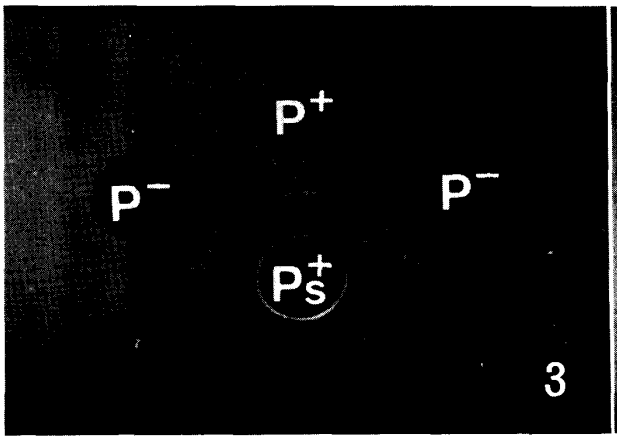
Fig. 2 Electron micrograph of P^- (×20000)

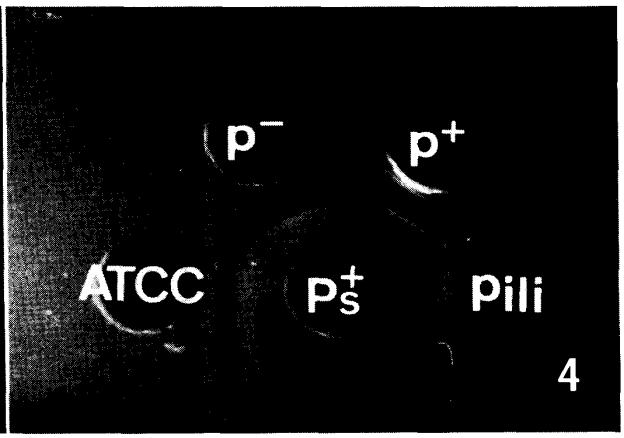
Fig. 3 Immunodiffusion of anti-P^+ serum (central well) with the antigens of P^+ and P^-

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

1


2


P+
P−
P−
Ps+
3


P−
P+
ATCC
Ps+
pili
4