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FAILURE TO ISOLATE PLASMID DNA FROM ANTIBIOTIC RESISTANT STRAINS OF *MYCOPLASMA GALLISEPTICUM*

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SUMMARY: Resistance of *Mycoplasma gallisepticum* to spiramycin and tylosin was determined by plasmid isolation. Two techniques for isolation of plasmids were applied and their presence was examined by agrose gel electrophoresis. Plasmids were not detected from these strains which suggest that resistance to antibiotics was not attributed to plasmids in the strains examined.

Keywords: Plasmids, *Mycoplasma gallisepticum*

INTRODUCTION

Many different antibiotics have been used in an attempt to control airsacculitis and reduced egg production resulting from *M. gallisepticum* infection. In Japan, spiramycin (Sp) and tylosin (Ts) have been used for the control of avian respiratory mycoplasmosis in breeding flocks (Matsui *et al.*, 1967). However, with the widespread use of these antibiotics, *M. gallisepticum* acquired resistance to Sp and Ts (Kuniyasu *et al.*, 1973). Recently, it was found that resistance of *M. gallisepticum* to Sp and Ts was lost due to plasmids during storage (Kuniyasu, unpublished). There is now clear evidence that some of the resistance factors of bacteria are mediated by plasmids (Amyes, 1989).

Plasmids are extrachromosomal DNA molecules known to occur frequently in a wide variety of both gram-negative and gram-positive bacteria. Although plasmids have been detected in *Mycoplasma arthritidis, M. hominis* and *Acholeplasma laidlawii* (Harasawa and Barile, 1983), their functions are unknown. This present study attempts to isolate plasmids DNA from antibiotic resistant strains of *M. gallisepticum.*

MATERIALS AND METHODS

Four strains of *M. gallisepticum* for this study were provided by Dr. C. Kuniyasu. Strain S4A (resistant to Sp and Ts) and strain S6 (sensitive to Sp and Ts), were clinical isolates. Strains Sp23 and Ts18 were resistant to Sp and Ts. Their resistance was acquired artificially by passing 23 generations and 18 generations of *M. gallisepticum* strain KP-13 in Sp and Ts, respectively.

The Mycoplasma strains were grown from three to five days at 37°C in modified K-broth (Yamamoto *et al.*, 1992).

Escherichia coli strain V517 containing eight plasmids and *E. coli* NIHJ containing no plasmids were grown at 37°C in 10 ml of LB broth (Sambrook *et al.*, 1989).

Plasmid DNAs were isolated by methods described by Sekizaki (pers. comm.) and, Harasawa and Barile (1983). The Sekizaki's method (based on method by Kado and Liu (1981) which was mainly for enterobacteria was modified for Mycoplasma. The organisms were harvested from 40 ml broth cultures by centrifugation at 10,000 rpm (Hitachi RPR 20-4 rotor), at 4°C for 30 minutes; the cell sediments were suspended in 1.5 ml of EDTA-tris-glucose solution (solution 1), centrifuged at 15,000 rpm for 5 minutes at room temperature, and resuspended in 100 ml of the solution 1. Further procedures are as described by Kado and Liu (1981). The plasmids of *E. coli* V517 cells were used as molecular weight reference markers.

The samples were electrophoresed through a horizontal 0.7% agarose gel at 100V for 3 hours, using Tris-borate buffer (Uchida *et al.*, 1986). The gel was stained for 15 min in ethidium bromide, washed for 15 min in distilled water and viewed under ultra-violet illuminator.

RESULTS AND DISCUSSION

The results of the agarose electrophoresis showed that plasmids were not detected in the four strains of *M. gallisepticum* by both plasmid isolation techniques although both methods detected plasmids of *E. coli* V517.

Bacterial resistance to an antibiotic is sometimes acquired as a result of infection of the bacterial cell by a plasmid belonging to the class of resistance factor. In this study, no plasmids were detected. As both the techniques detected plasmids in *E. coli* V517, technical deficiencies or faults are unlikely to be responsible for the apparent absence of plasmids. It appears the *M gallisepticum* isolates rarely carry detectable plasmids.

The failure to find any plasmid-carrying isolates of *M. gallisepticum*, however, is somewhat surprising, as plasmids have been detected in 233 Mycoplasma strains (Harasawa and Barile, 1983). Since these *M. gallisepticum* strains appear to be free of plasmids, it raises two possibilities concerning Sp and Ts resistance in *M. gallisepticum*. First, such resistance may be due to mutation of a chromosomal gene, which modifies the structure of the cellular target (Stanier *et al.*, 1979); an example is mutationally acquired streptomycin resistance. Alternatively, the resistance may be mediated by a plasmid that integrates into chromosome, which had been reported in *H. influenza* (Mendelman *et al.*, 1984). Our current observations, however, indicate that antibiotic resistance in *M. gallisepticum*, is probably not mediated by plasmids.

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REFERENCES

Amyes, S.G.B. (1989). The success of plasmid-encoded resistance genes in clinical bacteria. *J. Med. Microbiol.* **28**: 73-83.

Harasawa, R. and Barile, M.F. (1983). Surveys of plasmids in various Mycoplasmas. *The Yale J. Biol. Med.* **56**: 783-788.

Kado, C.I. and Liu, S.T. (1981). Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol.* **145**: 1365-1375.

Kuniyasu, C., Yoshida, Y. and Takano, M. (1973). Spiramycin and tylosin - resistant strains of *Mycoplasma gallisepticum* isolated from breeding chickens and pipped eggs. *Nat. Inst. Anim. Hlth. Quart.* **14**: 48-53.

Matsui, K. *et al,* (1967). The *in vitro* sensitivity of *Mycoplasma gallisepticum* to antibiotics and nitrofurans. *Nat. Inst. Anim. Hlth. Quart.* **7**: 124-125.

Mendelman, P.M. Doroshou, C.A., Gandy, S.L. Syriopoulou, V., Weigen, C.P. and Smith, A.L. (1984). Plasmid mediated resistance in multiple resistant *Haemophilus influenza* type b causing meningitis: Molecular characterisation of one strain and review of the literature. *J. Infect. Dis.* **150**: 30-39.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Appendix A: Bacterial media, antibiotics and bacterial strains. In: Molecular Cloning - A Laboratory Manual. 2nd edn. p A 1. Cold Spring Harbor Laboratory Press, USA.

Stanier, R.Y., Adelberg, E.A. and Ingraham, J.L. (1979). Exploitation of microorganisms by Man. In: General Microbiology, p 831-853. The Macmillan Press Ltd. Great Britain.

Uchida, I., Hashimoto, K. and Terakado, N. (1986). Virulence and immunogenicity in experimental animals of *Bacillus anthracia* strains harbouring or lacking 110 MDa and 60 MDa plasmids. *J. of Gen. Microbiol.* **132**: 557-559.

Yamamoto, K., Zaini, M.Z., Tan. L.J. and Kuniyasu, K. (1992). Bacteriological and serological survey of avian mycoplasmosis in Peninsular Malaysia - *JARQ* **25**: 278-282.

RINGKASAN

KEGAGALAN MEMENCIL DNA PLASMID DARIPADA STRAIN MYCOPLASMA GALLISEPTICUM *TAHAN ANTIBIOTIK*

Ketahanan Mycoplasma gallisepticum *terhadap spiramisin dan tilosin ditentukan melalui pemencilan plasmid. Dua teknik untuk pemencilan plasmid telah diguna dan kewujudannya dikaji menerusi elektroforesis agar-agar agaros. Plasmid tidak dikesan daripada strain-strain ini, menyarankan yang ketahanan terhadap antibiotik itu bukan bersabitkan plasmid dalam strain-strain yang dikaji.*