Electrophoretic Analysis of Proteins from *Mycoplasma capricolum* and Related Serotypes Using Extracts from Intact Cells and from Minicells Containing Cloned Mycoplasma DNA

By HANS ANDERSEN, GUNNA CHRISTIANSEN and CLAUS CHRISTIANSEN

1 Institute of Medical Microbiology, Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark
2 The Genetechology Group, Building 227, The Technical University of Denmark, DK-2800 Lyngby, Denmark

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The acidic proteins of six different mycoplasma serotypes causing bovine or caprine pleuropneumonia were compared by two-dimensional gel electrophoresis of extracts of 35S-labelled cells. The organisms investigated were *Mycoplasma mycoides* subsp. *mycoides* (PG1), *M. mycoides* subsp. *mycoides* (Y-goat), *M. mycoides* subsp. *capri* (PG3), *M. capricolum* (California kid), the unclassified bovine serogroup 7 of Leach (PG50) and the F38-like group (F38). The results suggested a close relationship between *M. capricolum* and F38 and a similarly close relationship between the different *M. mycoides* subspecies, whereas the two *M. mycoides* subspecies appeared to be quite distant from *M. capricolum* and F38. The representative strain of the bovine serogroup 7 of Leach was equally distant from F38, *M. capricolum* and the three strains of *M. mycoides*. Strikingly, all six mycoplasma strains apparently shared six proteins in the two-dimensional gels. In *Escherichia coli* minicells, DNA from strain PG50 cloned in the vector pBR325 gave rise to incorporation of radioactive label into proteins which were identified as mycoplasma proteins by two-dimensional electrophoresis and immunoprecipitation.

INTRODUCTION

The size of the genome in the genus *Mycoplasma* is about $5 \times 10^8$ daltons, which is approximately one-fifth of that of *Escherichia coli* (Ryan & Morowitz, 1969; Bak et al., 1969; Wallace & Morowitz, 1973); this is the smallest genome known in free-living prokaryotes. In the classification of mycoplasmas a number of serological, biochemical and genetic analyses have been used. Differences in genetic constitution have been determined directly by nucleic acid hybridization techniques (see for example Reich et al., 1966a, b; Askaa et al., 1978; Christiansen et al., 1979, 1981; Christiansen & Ernø, 1982), or indirectly by an investigation of cell proteins by polyacrylamide gel electrophoresis (Razin, 1968; Awaad et al., 1978), by two-dimensional gel electrophoresis (Rodwell et al., 1978; Rodwell & Rodwell, 1978), by immunoprecipitation tests (Archer, 1979) or by electrophoretic analysis of isoenzymes (Salih et al., 1983). Both one- and two-dimensional gel electrophoresis have been used to determine relationships between *Mycoplasma, Acholeplasma* and *Ureaplasma* strains (Rodwell & Rodwell, 1978; Howard et al., 1981; Mouches et al., 1981; Rodwell, 1982) and to determine the protein composition of *M. capricolum* 30S and 50S ribosomal subfractions (Kawauchi et al., 1982).

The acidic proteins of six mycoplasma strains representing *M. mycoides*, *M. capricolum* and two unclassified groups of mycoplasma with serological and other relations to *M. mycoides* and *M. capricolum* are analysed in this paper. The strains investigated have a base composition very close to 24 mol % G+C and by DNA–DNA hybridization experiments were found to have between 36% and 84% relatedness (Christiansen & Ernø, 1982).
The purposes of the electrophoretic analyses were twofold. Firstly, characterization of acidic proteins according to their mobility in two-dimensional gel electrophoresis gives information that can be used to determine the relationships between these mycoplasma strains, thus contributing to the classification of strains PG50 and F38. This application was used by Rodwell (1982) to study proteins from various strains of *M. mycoides*. The results obtained in the present study together with the findings of Rodwell (1982) suggest that electrophoretic analysis reflects the genetic relationships seen in DNA–DNA hybridization experiments. The second purpose of this study was to initiate a catalogue of the proteins in these mycoplasma strains, in order to obtain a reference for identification of mycoplasma proteins synthesized by *E. coli* containing cloned mycoplasma DNA. The results show that mycoplasma proteins can be synthesized in *E. coli* minicells and that such proteins can be identified in two-dimensional gel electrophoresis.

**METHODS**

**Strains and cultivation.** *Mycoplasma mycoides* subsp. *mycoides* (PG1), *M. mycoides* subsp. *capri* (PG3). *M. capricolum* (California kid), bovine serogroup 7 of Leach (PG50) and F38-like group (F38) were obtained from the FAO/WHO Collaborating Center for Animal Mycoplasmas, Institute of Medical Microbiology, University of Aarhus, Denmark. The cells were grown at 37 °C in 5 ml of medium, pH 7-8, containing: heart infusion broth, 2.2-2.3% (w/v); horse serum, 15-15.5% (v/v); yeast extract, 1.9-2% (w/v); thallium acetate 0.008%; benzyloxyacetic, 400 i.u. ml^-1; glucose, 0.62%; calf thymus DNA, 0.002%; and phenol red. 0.23%. The medium was sterilized by filtration. The cells were labelled with [35S]methionine [10 μCi ml^-1 (370 kBq ml^-1)] for 24 h, harvested by centrifugation and washed twice in PBS buffer (12 mM-sodium phosphate, 0.145 M-sodium chloride, pH 7.0).

**Media.** L-broth contained (l^-1): Bacto-tryptone (Difco), 10 g; Bacto-yeast extract (Difco), 5 g and NaCl, 5 g (Kennedy, 1971). M9 medium contained (l^-1): Na2HPO4·H2O, 6 g; KH2PO4, 3 g; NaCl, 0.5 g; NH4Cl, 1 g; glucose, 2 g, with the addition of 10 ml 1 M-MgSO4 and 10 ml 0.01 M-CaCl2. ‘Basic soup’ was composed according to Zubay et al. (1970). The individual components were stored in stock solutions at −70 °C and mixed immediately before use.

**SDS-gel electrophoresis of cell proteins.** The [35S]methionine-labelled cells were freeze-thawed four times and resuspended in 100 μl SDS-sample buffer [0-0625 M-Tris/HCl, pH 6-8; SDS, 2.3% (w/v); β-mercaptoethanol, 5% (v/v); glycerol, 10% (v/v); bromophenol blue, 0.05%] and heated to 100 °C for 2 min. Insoluble material was removed by centrifugation in a microcentrifuge before electrophoresis in a 10% (w/v) polyacrylamide gel. Samples containing about 900 Bq [35S] were applied to the gels.

**Two-dimensional gel electrophoresis of cell proteins.** The [35S]methionine-labelled cells were freeze-thawed four times and resuspended in 100 μl lysis buffer 9.5 M-urea; Nonidet 40 (Shell), 2% (w/v); amphotericin, pH 5-8, 1-6% (v/v) and amphotericin, pH 3-5-10, 0-4% (v/v); β-mercaptoethanol, 5% (v/v) and bromophenol blue, 0.05%.

**Samples containing 1200 Bq 35S 35S** were applied to a cylindrical pH 5-7-5 isoelectric focusing gel (165 × 2 mm) for separating acidic proteins (O'Farrell, 1975). The gels were run for 17.5 h at 400 V and 1 h at 1000 V. The technique used for the second dimension was first described by Laemmli (1970). The focusing gel was applied to a second dimension SDS-slab gel (final dimensions 130 × 50 × 0.8 mm) and electrophoresed for about 17 h at 60 V. Molecular weight standards were 14C-methylated proteins (Bethesda Research Laboratories): ovalbumin, 43000; α-chymotrypsinogen, 25700; β-lactoglobulin, 18400 and lysozyme, 14300. SDS-slab gels were fixed in 45% (v/v) methanol, 15% (w/v) TCA for 1 h after electrophoresis. Fluorography was done according to Bonner & Laskey (1974). The gel was immersed twice in DMSO for 20 min and for 2 h in DMSO with PPO (10%, w/v). The gels were washed in H2O and dried for 3 h. Fuji X-ray film, 18 × 24 cm, was used for autoradiography. The film was pre-fogged to an OD600 of about 0-1, placed directly in contact with the dried gel and kept at −70 °C for 7 and 14 d.

**Measurement of pH gradient in isoelectric focusing gels.** The isoelectric focusing gel was cut into 5 mm slices and placed in 1 ml 0-1 M-KCl, and the pH was measured after the gel slices were eluted (Archer et al., 1978).

**Molecular cloning of mycoplasma DNA.** All experiments were done under P2 containment in accordance with recommendations from the Danish Research Council, Committee for Registration of Recombinant DNA Experiments. Restriction enzymes were purchased from Boehringer-Mannheim or New England Biolabs and used according to the manufacturers' directions.

The cloning vector was pBR325 (Bolivar, 1978). It was cleaved with HindIII or EcoRI and treated with calf intestinal phosphatase. The phosphatase was heat-inactivated and the buffer was removed by ethanol precipitation of the DNA. The passenger DNA from mycoplasma strain PG50 was cleaved with HindIII or EcoRI, heated to 65 °C and ethanol-precipitated before use in ligation reactions.

Ligation of 1 μg vector DNA and 1 μg passenger DNA was done in a volume of 25 μl. The buffer was 0-08 M-Tris/HCl, pH 7-6, 0-01 M-MgCl2, 0-01 M-dithiothreitol, 0-5 mM-ATP and 2 Weiss units of T4 DNA ligase. The
reaction was incubated at 12 °C for 16 h. Escherichia coli strain HB101 was used as recipient in the transformation reaction after two treatments with 50 mM-CaCl₂. Transformed cells were plated on L-broth plates with 50 µg ampicillin ml⁻¹.

After incubation at 37 °C for 16 h the plates were replicated to L-broth plates with 25 µg tetracycline ml⁻¹ (HindIII clones) or 25 µg chloramphenicol ml⁻¹ (EcoRI clones). Tetracycline- or chloramphenicol-sensitive colonies were used for mini preparations of plasmids. Recombinant plasmids with inserts of more than 1 kb were purified and used for further studies. Plasmids are named pMYC and are consecutively numbered according to the time of isolation.

Transformation to E. coli minicells. The bacterial strain used was the minicell-producing E. coli K12 substrain DS410 (Dougan & Sherratt, 1977). Purified plasmid DNA (0.1–0.5 µg) was used in the transformation reaction; 200 µl competent cells and 300 µl 0.1 M-CaCl₂ were mixed and incubated on ice for 30 min (Mandel & Higa, 1979; So et al., 1975; Bolivar & Backmann, 1979). The cells were heated to 42 °C for 2 min after which 4-5 ml L-broth medium was added. Transformed cultures were incubated at 37 °C for 90 min and then transferred to 250 ml L-broth with ampicillin (25 µg ml⁻¹).

Isolation and labelling of minicells. Minicells were isolated as described by Kool et al. (1974) and Hallewell & Sherratt (1976). They were suspended in 1 ml labelling medium (30 µl ‘basic soup’ + 1 ml M9 medium) and pre-incubated for 30 min at 37 °C with shaking. Protein was labelled by adding 5 or 10 µCi [³⁵S]methionine (New England Nuclear; > 800 Ci mmol⁻¹) and incubation was continued for another 30 min. L-broth (5 ml) was added for chasing and incubation continued for a further 45 min. Minicells were then pelleted and washed twice in 5 ml of a medium containing NaCl, 0.85%; KH₂PO₄, 0.03%; Na₂HPO₄, 0.06%; gelatin, 0.01%. Pellets were prepared for gel electrophoresis as described above or frozen at −20 °C until use.

Immunoprecipitation. Precipitation reactions were done on a sample of the previously described minicell extract diluted to 25 µl with PBS (0.012 M-sodium phosphate, 0.145 M-sodium chloride, pH 7.0). Commonly 20 µl minicell extract was used for the reactions. To each sample was added 20 µl hyperimmune rabbit serum and the reactions were incubated at room temperature for 30 min. Finally 5 µl of a 50% (w/v) solution of polyethylene glycol 6000 was added and incubation was continued for an additional 30 min to precipitate the immunoglobulins with the attached antigens. Control reactions were run in parallel in each case substituting the rabbit antiserum with PBS. The vials were centrifuged for 10 min in a microcentrifuge. The radioactivity of the supernatant was determined by liquid scintillation counting.

RESULTS

Analysis of one- and two-dimensional gels of mycoplasma extracts

The electrophoretic patterns of whole cell extracts of [³⁵S]-labelled proteins from the six mycoplasma strains in a SDS-polyacrylamide gel are shown in Fig. 1. The molecular weights of the proteins in the gel range from 12000 to 160000. The majority of proteins are in the molecular weight range 30000 to 80000. It is possible to distinguish each strain on the basis of the band pattern in Fig. 1, but many similarities are recognizable especially between the three strains of Mycoplasma capricolum (PG3, PG1 and Y-goat). Autoradiograms of the two-dimensional gels are shown in Fig. 2(a–c) and Fig. 3(a–c). Panel (d) in each of these figures demonstrates results of paired comparisons of autoradiograms. Between 103 and 163 proteins were detected in each of the radiograms after 7 d exposure. Further exposure revealed only a few proteins not already detected. The inclusion of molecular weight standards in the second dimension and the determination of the pH gradient in the focusing dimension were the basis of further comparisons of the autoradiograms. Each autoradiogram was divided into 24 squares according to specific pH values and molecular weights. Proteins within a square were identified in terms of position and intensity. Overlaid identical areas of the autoradiograms allowed determination of identical polypeptides and these identical features were counted for each strain.

The two-dimensional protein patterns of each strain were compared with those of the other five strains. We found between 16 and 46 common polypeptide spots when two gels were compared (Table 1). The PG1 versus PG3 comparison is shown in Fig. 2(d), and the F38 versus California kid comparison in Fig. 3(d). Common proteins are indicated by circles. The cross-hatching of circles in Fig. 2(d) indicates proteins common to all strains of Mycoplasma mycoides. The shaded area in Fig. 2(d) and Fig. 3(d) indicates the position of six proteins shared by all six strains in this investigation. Five of these proteins have very similar molecular weights of 45000 to 50000 and pl values of 5, 6, 6-1, 6-8 and 7. The sixth protein has a molecular weight of 70000 and a pl value of 5.7. The protein patterns of Mycoplasma capricolum and F38 were highly congruent, with
46 identical proteins, while the relationship of *M. capricolum* to PG50 and the mycoides group (PG1, PG3 and Y-goat) seems to be more distant, with only 16 to 19 common proteins.

Comparison of the members of the mycoides group PG1/PG3, PG1/Y-goat and PG3/Y-goat showed a close relation, with 45, 41 and 40 common proteins, respectively (Fig. 2). The protein pattern of PG50 was equally distant from F38, *M. capricolum* and the three subspecies of *M. mycoides*.

The protein relation between two strains can be recorded as a percentage congruence using the formula \[
\frac{2N_{AB}}{(N_A + N_B)} \times 100,
\]
where \(N_A\) and \(N_B\) are the total number of spots on the fluorograms for strain A and strain B, respectively, and \(N_{AB}\) is the number of common spots (Rodwell & Rodwell, 1978). This relation is listed in Table 1, column b. The results of comparable experiments (Rodwell, 1982) are included in column c and the DNA–DNA hybridization data on the same strains (Christiansen & Ernø, 1982) are also included in the table. There is good agreement between the nucleic acid hybridization data and the comparative protein data; indeed an increase in DNA homology level is unequivocally accompanied by an increase in the percentage congruence.

**Analysis of cloned mycoplasma DNA in *E. coli* minicells**

The presence of plasmid DNA in minicells gave rise to synthesis of proteins detectable by incorporation of \([^{35}S]\)methionine. Figure 4 shows the autoradiogram of a polyacrylamide gel with extracts from minicells carrying pBR322, pBR325 and the recombinant plasmid pMYC107. The positions of the gene products for the tetracycline, ampicillin and chloramphenicol resistances in the gel are indicated. The extract from minicells with the
Protein homologies in Mycoplasma capricolum

Fig. 2. Two-dimensional electrophoretic pattern of cell proteins from the three *M. mycoides* strains: (a) *M. mycoides* subsp. *mycoides* (PG1); (b) *M. mycoides* subsp. *mycoides* (Y-goat); (c) *M. mycoides* subsp. *cupri* (PG3). The proteins were labelled with $[^{35}\text{S}]$methionine and added to the isoelectric focusing gel with a pH gradient of 5–7.5 and focused for 8000 V h. The second dimension electrophoresis was done for 17 h at 60 V on a 10% (w/v) polyacrylamide gel. Fluorograms were exposed for 7 d. Isoelectric focusing is in the horizontal direction with the basic proteins to the left. The second dimension SDS-gel electrophoresis is in the vertical dimension. pH values and positions of molecular weight standards are indicated by pointers. The diagram (d) indicates the positions of proteins common to strains PG1 and PG3. The shaded spots identify six proteins which were found in all six mycoplasma strains analysed. The hatched spots represent 12 proteins found to be common to all three *M. mycoides* strains.

A minicell plasmid containing inserted mycoplasma DNA showed five new proteins not seen in the lanes for minicells with vector plasmids alone. The new protein bands are indicated in Fig. 4 by arrows at 56000, 54000, 41000, 37000 and 33000 daltons.

Plasmid pMYC107 contains approximately 5-5 kb of inserted mycoplasma DNA. This is equivalent to a coding capacity of about 200000 daltons of protein. The molecular weight of the observed proteins slightly exceeds the coding capacity of the DNA insert. A total of 15 different plasmids with DNA from strain PG50 inserted in the *EcoRI* or the *HindIII* sites of pBR325 were tested.

In about half the cases the presence of recombinant plasmid DNA resulted in production of one or more new polypeptides in the minicells. The minicell extracts were tested for immunoreactivity with hyperimmune rabbit serum against PG50. (The serum was a gift from Dr H. Erne, Aarhus.) More than 50% of the radioactivity was precipitated after addition of antibodies in the experiments where the minicells contained a recombinant plasmid. With the vector plasmid alone less than 20% of the radioactivity was found in the precipitate. The immunoprecipitated material was dissolved and analysed on polyacrylamide gels. The radioactive proteins in these gels were mainly the mycoplasma proteins seen in the equivalent gel of minicell extracts, while proteins synthesized from the vector were absent or present in very small quantities.
Table 1. Protein and DNA homologies of six mycoplasma strains

Protein homologies are given as percentage congruence and are calculated from the formula \[ \frac{2N_{AB}}{(N_A + N_B)} \times 100 \], where \( N_A \) and \( N_B \) are the total number of spots on the fluorograms for strains A and B, respectively, and \( N_{AB} \) is the number of common spots (Rodwell & Rodwell, 1978). (a), Number of common protein spots; (b), percentage congruence; (c), percentage congruence determined by Rodwell (1982). DNA homologies are from Christiansen & Ernø (1982).

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Protein homologies in *Mycoplasma capricolum*

Fig. 3. Autoradiograms of two-dimensional electrophoretic gels of cell proteins from three mycoplasma strains: (a) F38-like group (F38); (b) bovine serogroup 7 of Leach (PG50); (c) *M. capricolum* (California kid). Conditions were as described in Fig. 2. The positions of molecular weight standards and pH values are indicated by pointers. The diagram (d) shows the identification of proteins common to strain F38 and strain California kid. The shaded areas show the positions of the six proteins which were found to be identical in all six mycoplasma strains. (See also Fig. 2.)

The proteins from pMYC107 were analysed in a two-dimensional gel and the five proteins already mentioned were seen (Fig. 5a). Comparing this gel to a gel of in vivo-labelled proteins from PG50 (Fig. 5b) allows identification (arrows) of the five proteins from pMYC107 in the total protein map for PG50.

**DISCUSSION**

The results of SDS-gel electrophoresis were very reproducible and at least 40 to 50 distinct protein bands were seen in the autoradiograms (see Fig. 1). Differences between strains were easily detected and with its high reproducibility the method is a valuable tool as a first examination of the relationship between new mycoplasma species. The six mycoplasma strains analysed showed similar electrophoretic patterns but distinct differences were apparent. The *M. mycoides* strains, however, appeared to be similar, while *M. capricolum*, PG50 and F38 appeared equally distinct.

Between 100 and 160 proteins were detected in the two-dimensional gels. The number of detected proteins was rather constant in different experiments on the same strain, but the present study did not examine variations in the conditions of growth of the cells. The labelling intensity of the different proteins will vary because of the $[^35]S$ methionine label as proteins without methionine will not be seen. The number of proteins is clearly less than expected on the basis of the coding capacity of the genome. The genome mass of mycoplasma is sufficient to code for about 640 proteins with an average molecular weight of 40000. About one-third of these
Fig. 4. Polyacrylamide gel electrophoresis of protein extracts from *E. coli* minicells. The minicell proteins were labelled with $^{35}$S-methionine. The minicells contained the following plasmids: A, pMYC107; B, pBR322; C, pBR325. The positions of molecular weight standards in the gel are indicated on the left and the positions of the gene products for tetracycline, ampicillin and chloramphenicol resistances are indicated on the right. The five mycoplasma proteins encoded by pMYC107 in the minicells are indicated by arrows in lane A.

Fig. 5. An autoradiogram of a two-dimensional electrophoresis of $^{35}$S-labelled proteins from minicells containing the plasmid pMYC107 (a). For comparison the autoradiogram of a two-dimensional gel of PG50 proteins labelled *in vivo* is shown (b). The five mycoplasma proteins from pMYC107, and their proposed counterparts, are indicated by the arrows.
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Proteins were observed in the two-dimensional gels. Unlabelled proteins and basic proteins probably accounted for the residual amount. Kawauchi et al. (1982) using \(^{14}\)C label found a total of 355 proteins in *M. capricolum*, with 202 in the acidic range. Rodwell & Rodwell (1978) compared the protein patterns of PG1 and PG3. These strains exhibited 132 and 142 protein spots, respectively, and extended exposure allowed detection of about 300 proteins with \(^{14}\)C label. We found in these strains a maximum of 136 and 163 proteins and prolonged exposure did not demonstrate the presence of more proteins. The acidic cell proteins were very easy to detect as they reached their isoelectric points rapidly and retained the band sharpness even after long periods of electrofocusing. The few moderately basic proteins which entered the focusing gel always produced streaks. In this study only the acidic proteins were included and with the \(^{35}\)S label this was sufficient for our purposes of comparing a number of mycoplasma strains and to detect the production of mycoplasma proteins in *E. coli* minicells.

The results demonstrate close relations between the different strains of *M. mycoides* and between *M. capricolum* and strain F38, while strain PG50 seems equally related to *M. mycoides* and *M. capricolum*. Rodwell (1982) compared approximately the same set of strains by analysis of \(^{14}\)C-labelled proteins. The two studies found similarities between the same strains, but our data indicate a smaller difference between related strains. This could be a result of the smaller number of proteins detected by the \(^{35}\)S label. A comparison of DNA homologies with the results of the comparative analyses of protein patterns shows that a high DNA homology between two strains is also reflected in the protein patterns of the same two strains. In our study we found a close relationship between *M. capricolum* and F38 and a close relationship within the mycoides group (strains PG1, PG3 and Y-goat). The results obtained in this study with respect to the F38 group and *M. capricolum* may suggest that F38 should be classified as a subspecies of *M. capricolum*.

In contrast, Salih et al. (1982) suggested, on the basis of their isoenzyme analysis, that the F38 group should be established as a new species, but they found in their dissimilarity matrix that F38 and *M. capricolum* were closer to each other than to any other strain in their study.

The molecular weight of the five proteins common to all strains (Fig. 2d and Fig. 3d) corresponds to the regions of common bands in one-dimensional SDS-gel electrophoresis (Fig. 1) at 50000 daltons. The sixth protein corresponds to the common protein band seen in the SDS-gel electrophoresis at about 70000 daltons. The function of these six proteins is unknown but they are all very prominent in the autoradiograms and therefore present in the cells in many copies. Comparison of our results to equivalent results from *E. coli* (Pedersen et al., 1978) might suggest the ribosomal protein S1 (MW 60000) and elongation factor Tu (MW 39000–47000) as candidates for equivalent proteins.

The results of analysis of recombinant plasmids containing DNA fragments from mycoplasma strain PG50 clearly show that mycoplasma proteins can be synthesized in *E. coli*. This conclusion is based on the presence of new proteins in SDS–polyacrylamide gels when mycoplasma DNA is present in minicells and on the immunoreactivity of these proteins. Further studies are necessary, however, to show whether the synthesis of these proteins is initiated from promoters on the mycoplasma DNA itself or whether it is the result of a read-through from a promoter on the vector plasmid. The detection of mycoplasma proteins from minicells in two-dimensional gels demonstrates the possibility of using such studies to assign proteins to regions of the mycoplasma genome. This method could be developed as an important tool in the study of the mycoplasma genome when classical genetic analysis may not be feasible.

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REFERENCES


