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SIGNIFICANCE OF THE ELECTROPHORETIC ANALYSIS IN THE RAPID AND ACCURATE IDENTIFICATION OF AVIAN MYCOPLASMAS

(With 6 Figures)

By

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(Received at 30/1/1993)

أهميه التحليل الكهربائي في سرعه ودقه تصنيف عترات ميكوبلازما الدواجن

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بدراسه تسعه عترات مرجعيه وتسعه عترات اخرى معزوله محليا من ميكوبلازما الدواجن باجراء اختبار التحليل الكهربائي لهالوحظ اشتراك عترات ميكوبلازما جاليسبتكم جميعافي حزمتي بروتين بينما باقي الحزم تعتبر خاصة ومميزة لكل عتره على حده. وكانت عترات الميكوبلازما الدواجن : ميكوبلازما جاليسبتكم وجاليزم والبلورم والآنريز والجليبفونس في حزمة بروتين واحد بينما باقي الحزم تعتبر خاصة ومميزة لكل عترة على حده. وبمقاومة التحليل الكهربائي للعترات المعزولة محليًا للتحليل الكهربائي للعترات المرجعية تم تصنيف العترات المعزولة وثلاثة عترات ميكوبلازما جاليسبتكم (١س - ٦) وثلاثة عترات ميكوبلازما جاليزم وثلاثة ميكوبلازما بلورم بمقارنتهم بالتحليل الكهربائي للعترات المرجعية.

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SUMMARY

The electrophoretic patterns of avian mycoplasmas (9 reference strains and 9 locally isolated strains) were studied. Two common protein bands were detected among Mycoplasma gallisepticum reference strains, the remaining bands were considered as specific and characteristic for each strain. *Mycoplasma gallinarum*, M. pullorum, M. iners and *M.gallopavonis* (reference strains) were found to have a common protein band, while the remaining bands were considered specific for each strain. In comparison between the reference and locally isolated strains revealed the *M.gallisepticum* (3 strains) were similar to the homologus reference strain. *M.pullorum* (3 strains) were found to be identical with reference strain type C *M.pullorum.*

INTRODUCTION

Till now, Mycoplasmosis is still of the most important respiratory infections of chicken which when complicated with other pathogens results in the so known as "Chronic Respiratory Disease". This infection has a great economical importance due to poor growth gain, poor feed conversion, low grade carcass quality in broiler as well as lowering of egg production in laying birds *JORDAN (1979).* Because of such significant importance of the disease. Further investigations concerning the updating of the spread and diagnosis of the infection are required.

The present study was conducted to determine the significance of sodium dodecyl sylophate polyacrylamide gel electrophoresis (SDSPAGE) in the rapid and accurate identification of mycoplasma isolates.

MATERIAL and METHODS

1- Mycoplasma Strains:

A total of 18 mycoplasma strains were used in this study, these strains were as follows:

A- Five reference strains of *Mycoplasma gallisepticum* (ATCC 19610, A5969, S-6, F,R) together with one *Mycoplasma gallinarum* strain, one *M.gallopavonis* strain.

B- Nine locally isolated mycoplasma strains which were serologically identified as follows:
- Three *Mycoplasma gallisepticum* strains.
- Three *Mycoplasma gallinarum* strains.
- Three *Mycoplasma Pullorum* strains.

2- Solution A *(MAIZEL, 1969):*

Composed of 0.1 of prepared mycoplasma antigen and 0.9 ml. Triton X- 100.

3- Solution B *(Maizel, 1969):*

Sodium phosphate buffer (0.01)	1 ml
2-Mercaptoethanol	2 ml
Sodium dodecyl sulphate (SDS)	2 gm
Distilled water	up to 100 ml

4- Preparation of cell proteins:

Antigens for electrophoretic analysis were prepared as described by *RAZIN and TULLY (1975).* Purified media adapted mycoplasma cultures after being checked for bacterial ontaminations and growth score, were used as starting inoculation. The volume of the growth media was increased through passages at 72 hours intervals until 500 ml amounts of the broth medium were inoculated for the final propagation and harvesting of mycoplasma cells.

5- Polycrylamide gel electrophoresis:

Sodium dodecyl sulphate (SDS) technique was described by *STEPHENSON and STORZ (1975).* Gels and buffers were the same as described by *MAIZEL (1969).* The gels were poured and left for polymerization. Take 0.1 ml from the prepared mycoplasma antigen then add 0.9 ml from solution A to gain a total volume of 1 ml., then add 1 ml of solution B. This mixture is placed in boiling water bath for 5 minutes. After cooling take 0.25 ml of this mixture were taken and 20 ul. of sucrose 50% 10 ul. 2-mercaptoethanol and 10 ml bromophenol blue were added to it, then the suspension was shaked.

The gels were pre-run before application of samples for 1-2 hours (pre-electrophoresis) at 6 V /cm gel with positive electrode above and negative electrode below.

6- The relative mobility (Rm Values) of the prominent protein bands were determined as described by *AWAAD* et al. *(1978).* 100-200 ug of mycoplasma protein was applied over each gel. Electrophoresis was carried out at 6V/cm gel for 20 hours with the positive electrode below and the negative electrode above.

After the process was completed, the gels were extracted from the tubes and placed in 20% sulfosalicylic acid for 24 hours. The gels were placed in the staining solution for 12 hours then transferred to the destaining solution.

RESULTS

A total of 18 mucoplasma strains were used, prepared as previously described for sodium dodecyl sulphate polyacrylamide gel electropphoresis (SDS-PAGE).

A characteristic electrophoretic profile could be identified for each strain as shown in figure (1) The relative mobility (Rm values) of prominent protein bands were determined as described by *AWAAD* et al. *(1978).*

Two common protein bands between the Mycoplasma gallisepticum group (reference strains) could be observed. Other remaining bands considered as specific and characteristic for each strain (Fig. 2).

With respect to *Mycoplasma gallinarum, M.pullorum, M.iners* and *M.gallopavonis* (reference strains), a common protein band was detected. The remaining bands were considered specific for each strain (Fig. 3 and 4).

Concerning the group of local isolates (Fig. 5 and 6), the protein bands of the 3 *M.gallisepticum* strains were identical with S-6 reference strain. The 3 *M.pullorum* strains were similar to type C reference strain. The 3 *M.gallinarum* strains were identical with *M.gallinarum* reference strain.

DISCUSSION

The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique was used to study the protein make up of avian mycoplasmas. The SDS-PAGE profile were carried out to demonstrate the difference of protein patterns among five strains of *M.gallisepticum* (reference strains). All the strains were found to have two common protein bands (Fig. 1 and 2). These findings were helpful for rapid comparing a number of strains and as an aid for monitoring the interaction of vaccinal strains (F-strain) and wild type of *M.gallisepticum.* These results are more or less similar to those reported by *KHAN* et al. *(1987),* who concluded that F-strain protein banding pattern differed from the S-6 reference strain.

On the other hand, other workers *(MULLEGGER & GERLACH, 1974 and ROADES* et al., *1974)* were unable to demonstrate variability among strains of M.gallisepticum.

Mycoplasma gallinarum, M.pullorum, M.iners and *M.gallopavonis* (reference strains) were found to have a common protein bands (Fig. 3 and 4).

Finally we used the electrophoretic profiles to confirm the diagnosis of the locally isolated strains which were serologically identified as *M.gallisepticum, M.pullorum* and *M.gallinarum*. They were found to be identified with that of S-6 strain, *M.pullorum* (type C) and *M.gallinarum*.

In conclusion of our results ensure that avian mycoplasmas could be diagnosed and differentiated by SDS-PAGE profiles on the basis of their protein pattern. Such observations were reported by *RAZIN and ROTTEM (1967)* and AWAAD et al. *(1978)*.

REFERENCES

Awaad, M.H.H.; Krauss, H. and Schmatz (1978): Electrophoretic characterization of bovine mycoplasma and acholeplasma reference strains. Zbl. Bakt. Hyg., I. Abt. Orig. A 240: 359-365.

Jordan, F.T.W. (1979): Avian Mycoplasms, the mycoplasms Vol. II, 140.

Khan, M.I.; Lam, K.M. and Yamamato, R. (1987): Mycoplasma gallisepticum strain variations detected by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Avian Dis., 31(2): 315-320.

Maizel, J.F. (1969): Acrylamide gel electrophoresis of protein and nucleic acids. In: Habe, K. and N.P. Salzman (ed.), Fundamental techniques in virology, Academic Press Inc., N.Y.

Mullegger, P.H. and Gerlach, H. (1974): The differentiation of avian mycoplasmas by means of disc - electrophoresis. Zentralbl. Bakteriol. Orig. A 226: 119-136.

Razin, S. and Rottem, S. (1967): Identification of Mycoplasma and other micro-organisms by polyacrylamide gel electrophoresis of cell protein: J. Bacterio. 94: 1807-1810.

Razin, S. and Tully, J.G. (1975): Identification of Mycoplasma by electrophoretic analysis of cell proteins. World Health Org., Programme on Comparative Mycoplasmology, 7: 1-15.

Roades, K.R.; Phillips, M. and Yoder, H.W.Jr. (1974): Comparison of strain of Mycoplasma gallisepticum by polyacrylamide gel electrophoresis. Avian Dis. 18: 91-96.

Stephenson, E.H. and Storz, J. (1975): Protein profiles of five chlamydial strains of five chlamydial strains of animal origin. Am. J. Vet. Res., 36(7): 881-887.

ELECTROPHORETIC ANALYSIS & AVIAN MYCOPLASMAS

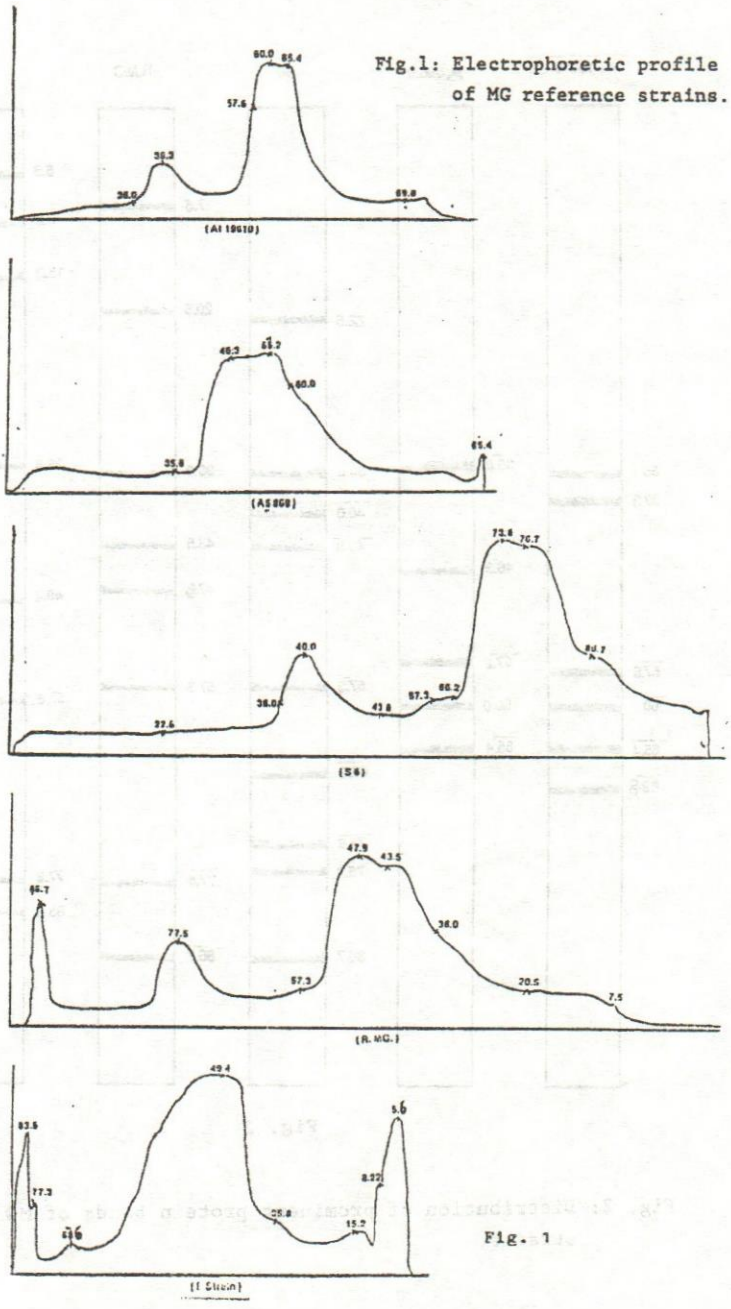

Fig.1: Electrophoretic profile of MG reference strains.

Fig. 1

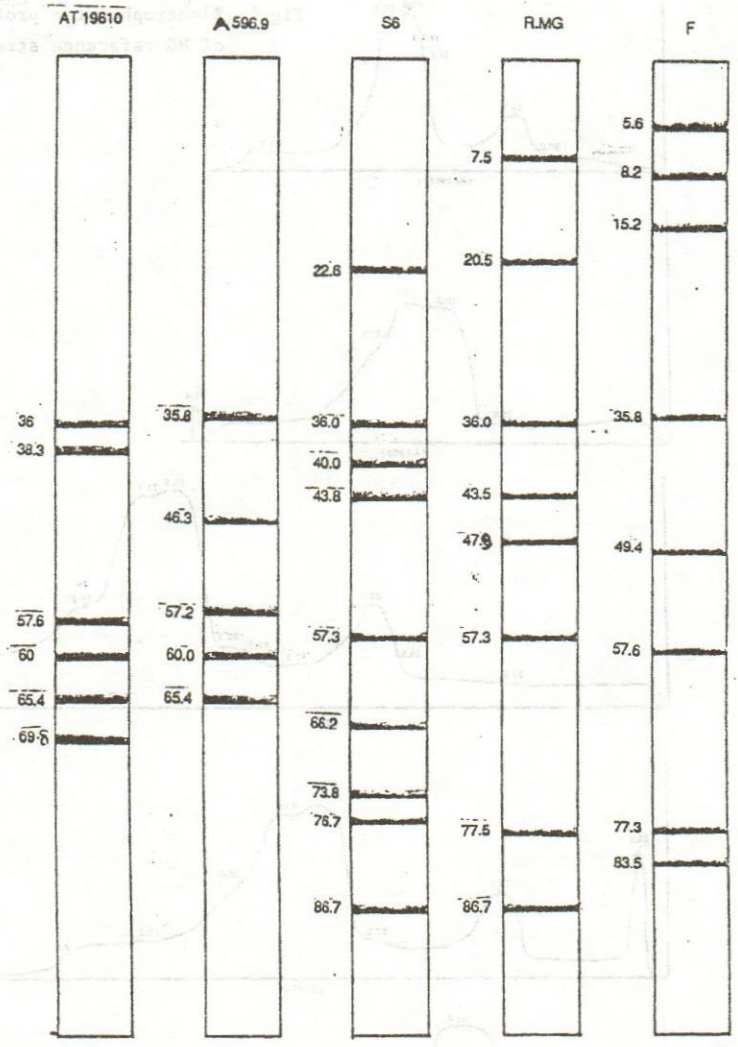

Fig. 2

Fig. 2: Distribution of prominent protein bands of MG reference strains.

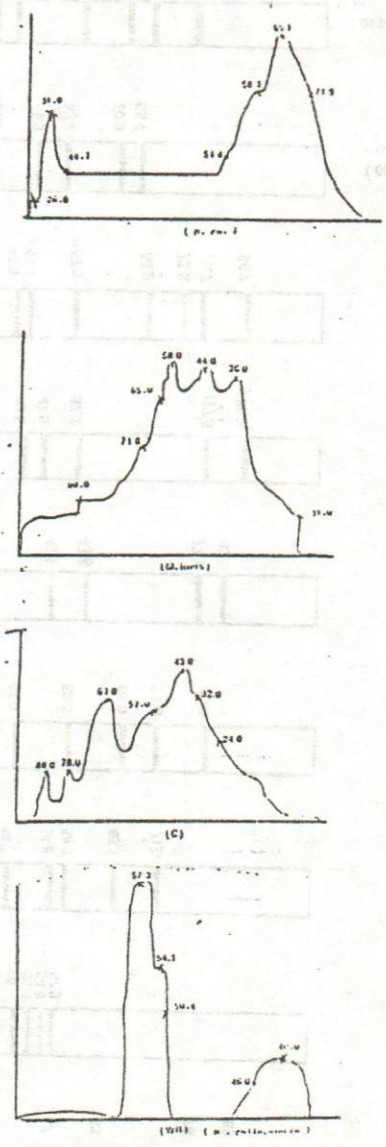

Fig. (3): Electrophoretic profile of avian mycoplasmas.

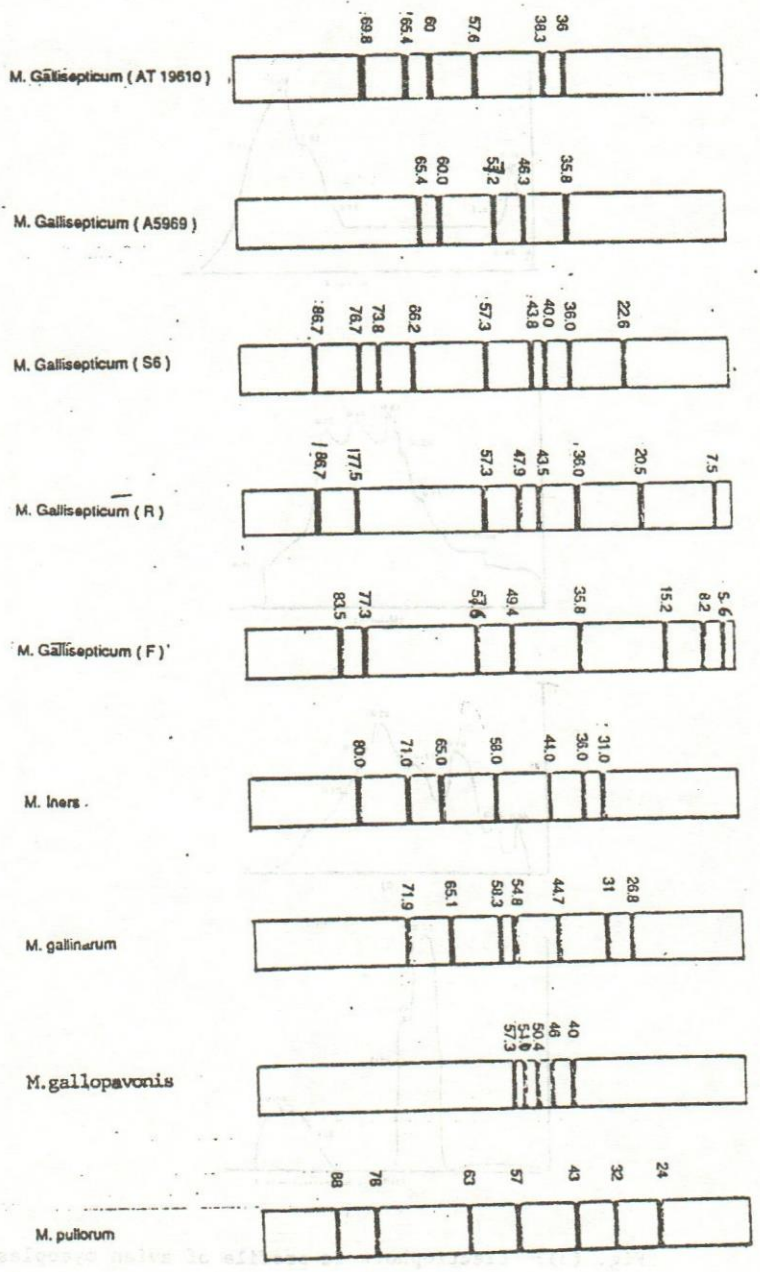
M. Gallisepticum (AT 19610)
69.8
65.4
60
57.6
38.3
36
M. Gallisepticum (A5969)
65.4
60.0
57.2
46.3
35.8
M. Gallisepticum (S6)
86.7
76.7
73.8
66.2
57.3
43.8
40.0
36.0
22.6
M. Gallisepticum (R)
86.7
77.5
57.3
47.9
43.5
36.0
20.5
7.5
M. Gallisepticum (F)
83.5
77.3
57.6
49.4
35.8
15.2
8.2
5.6
M. Iners
80.0
71.0
65.0
58.0
44.0
38.0
31.0
M. gallinarum
71.9
65.1
58.3
54.8
44.7
31
26.8
M. gallopavonis
57.3
51.0
50.4
46
40
M. pullorum
88
78
63
57
43
32
24

Fig. 4

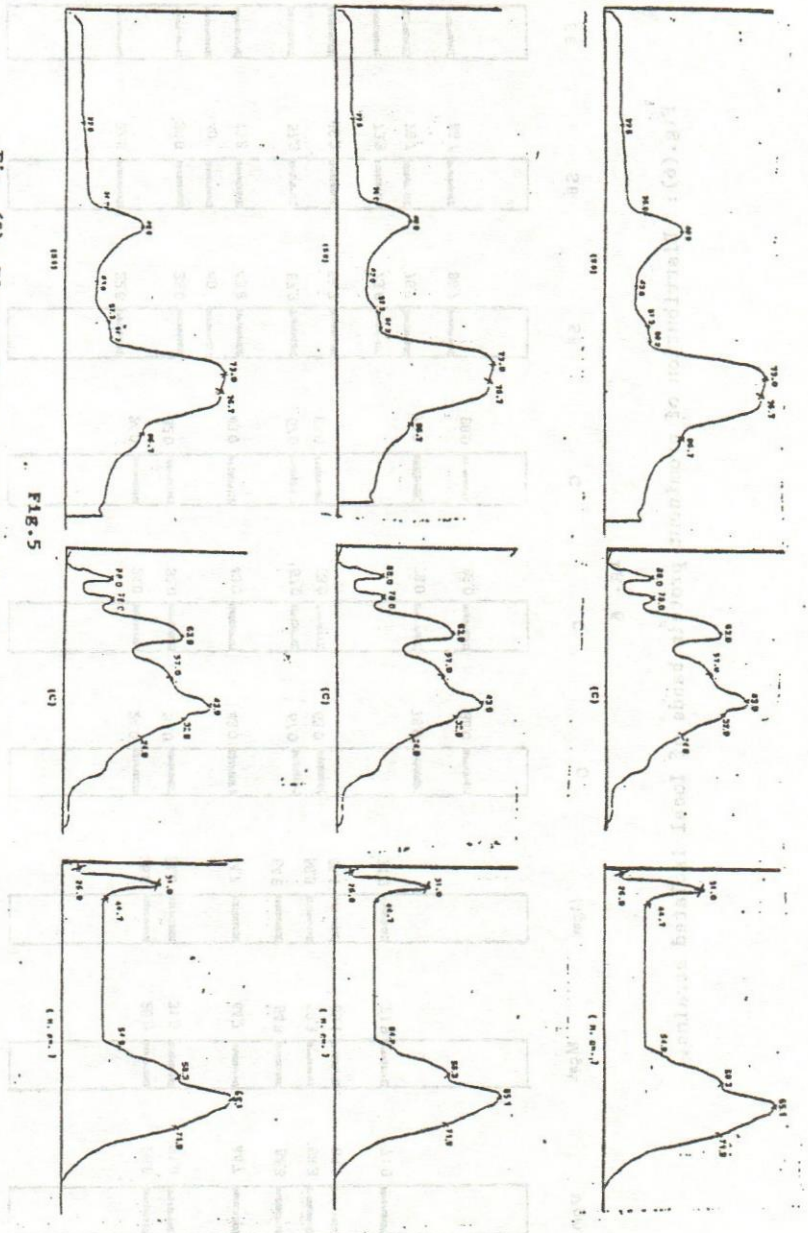

Fig. (5): Electrophoretic profile of S.6,C, and M. gallinarum local isolated strains.

Fig.5

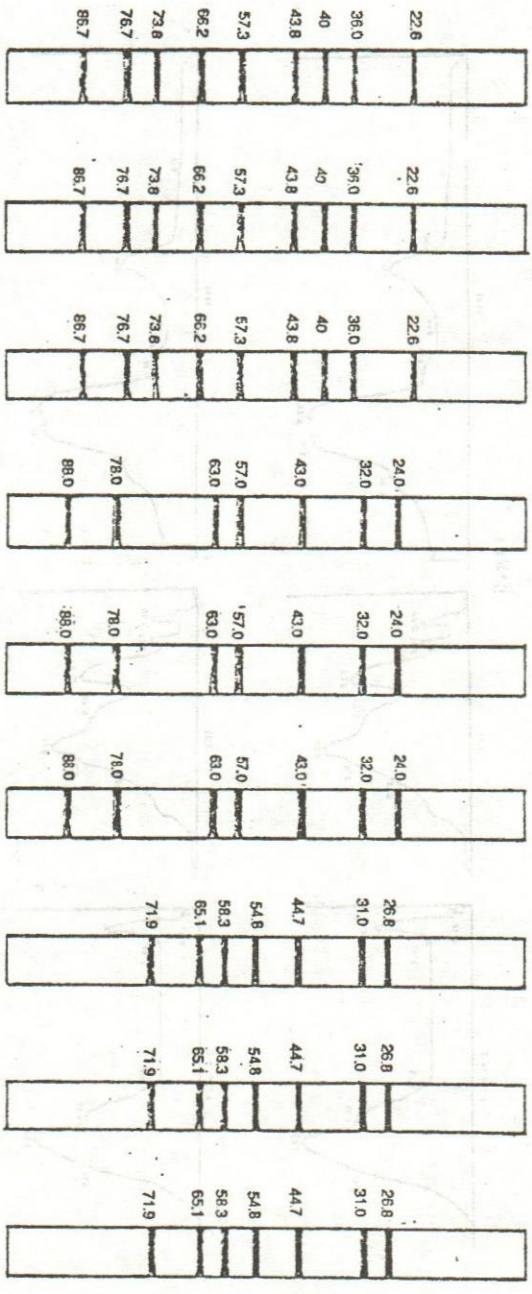

Fig. 6

Fig.(6): Distribution of prominent protein bands of local isolated strains.