Pulmonary Nocardiosis

Similarity to Tuberculosis

(A Bacteriological and Proteomics Study)

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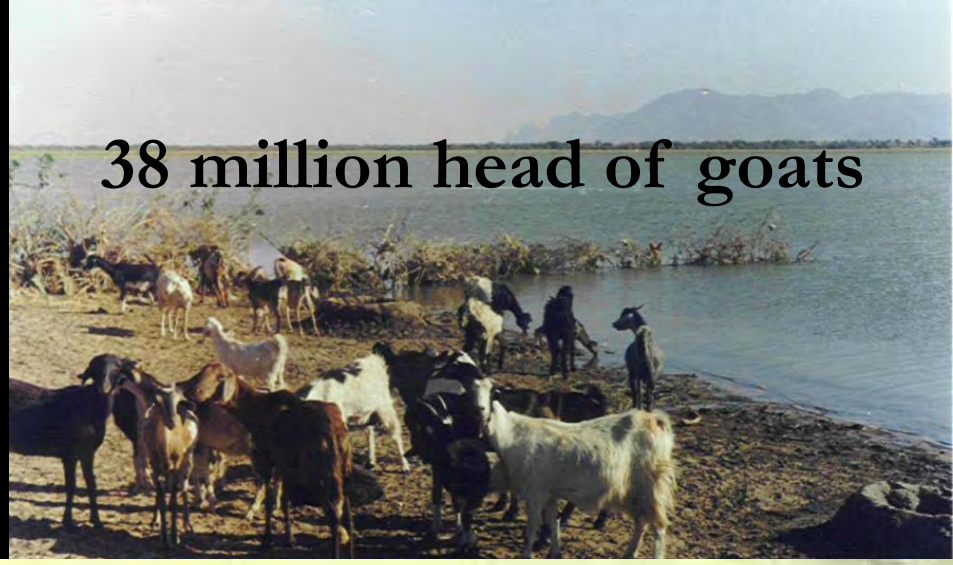
INTRODUCTION



37 million head of cattle

Data from the Animal Wealth Sector Performance Report, Federal Ministry of Animal Wealth in 2000

- Nocardiae, are actinomycetes which are epidemiologically saprophytic

- They include species forming parasitic association with animals and plants

- They occur in a wide range of man made and natural habitat including activated sewage sludge, soil, water and tissues of plants and animals including human.

- **Nocardiosis, is an important cause of morbidity and mortality in patients with HIV and cancer (immunocompramised).**

- **Nocardiosis is considered to be a late presenting community acquired infection, but there is growing evidence that the disease is transmissible.**

Pulmonary Nocardiosis

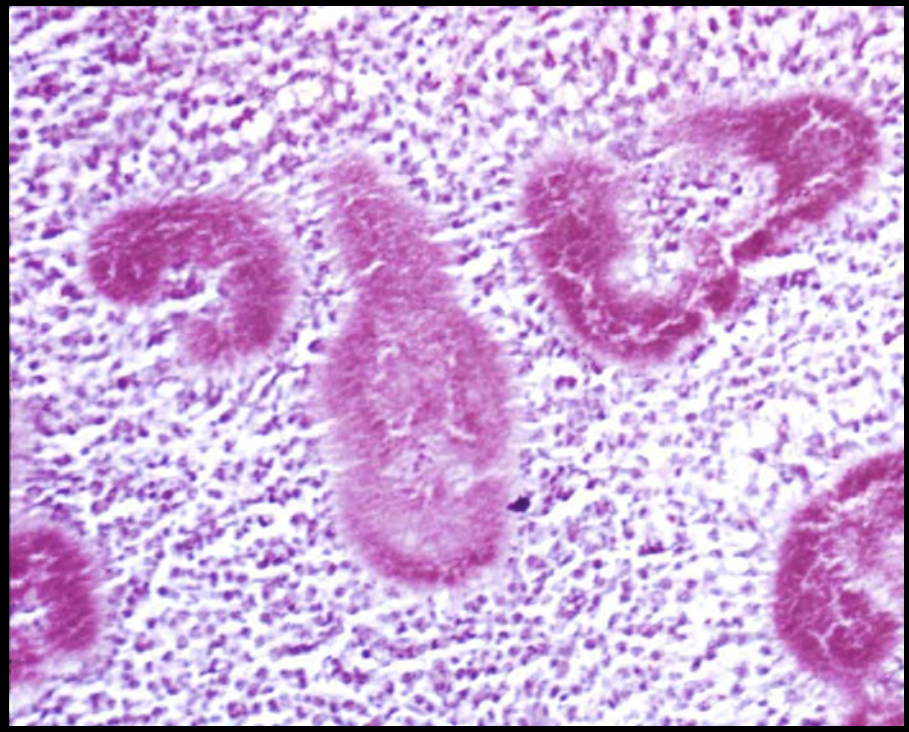
- **Primary pulmonary nocardiosis may be sub clinical or pneumonic; it may be chronic or acute with secondary involvement of other organs, mainly the brain.**

Pulmonary Nocardiosis

- **In non-tropical countries, most infections are caused by *N. asteroides, N. farcinica and N. nova*, relatively few *by N. brasiliensis, N.otitidiscaviarum* and *N. transvalenses.***

Nocardia and Diseases

Mastitis caused by *N. asteroides*



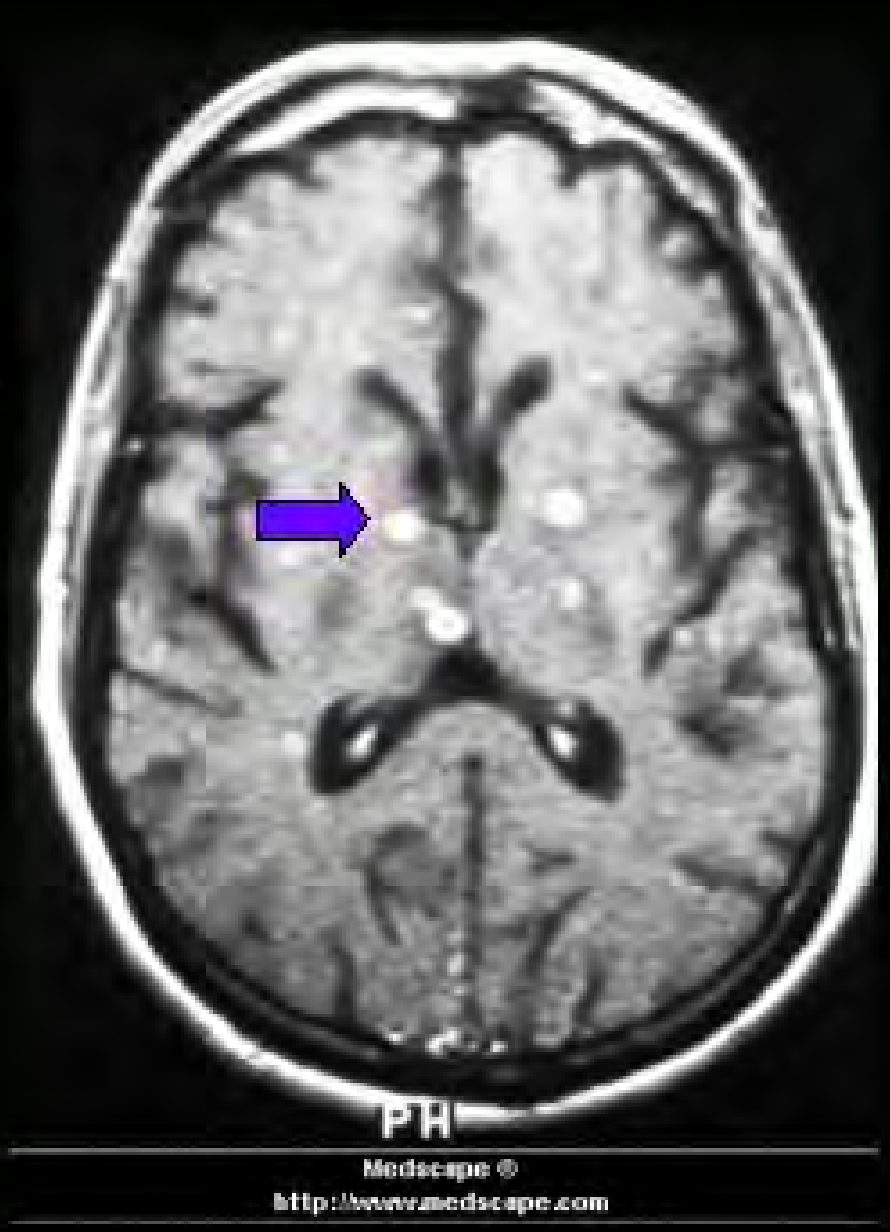
Mycetoma produced by *Nocardia brasiliensis*


Dr. K. Salfelder


Dr. K. Salfelder

CT Scan Image

Lesions caused by *Nocardia asteroides* infection in an HIV-positive individual



Nocardia africana

- ***Nocardia africana,*** **was isolated from sputum obtained from patients suffering from pulmonary infection admitted at Chest Unit of Khartoum Teaching Hospital in Sudan.**

- **The isolates showed morphological features of actinomycetes and they have assigned to the genus, *Nocardia*. According to their morphological criteria and chemical properties (Hamid *et al*., 2001).**

Proteomics

- **Recently, proteomics have been introduced as a new field of research for ultra study of protein structure and function**

- **Proteomics is the study of all proteins expressed by a genome. It involves the identification of proteins in the body and the determination of their role in physiological and pathophysiological functions.**

Rationale

- **New strategies are required for diagnosis, prevention and therapy of tuberculosis as well as chest infections due to MOTT or nocardiae as it is misdiagnosed by physicians;**
- **An imminent example in Sudan was *Nocardia africana* which was thought to be multi-drug resistant *Mycobacterium tuberculosis*.**

Objectives

General Objectives:

To analyze all proteins expressed by the genome of *Nocardia africana and Nocardia farcinica and to* compare them to one of the most important pulmonary pathogens: *Mycobacterium tuberculosis.*

Specific Objectives

. To compare the protein compositions of *Nocardia africana* strains with *Nocardia farcinica* and *Nocardia asteroides*.

. To compare the protein compositions of *Nocardia* spp. with *Mycobacterium tuberculosis*.

(A) Bacteriological Study

Methods

- Patients were examined for the presence of acid- fast bacilli.
- They were suspected to have tuberculosis infection according to the symptoms.

Methods

- Thin bacterial smears were prepared and stained by Ziehl Neelsen (ZN) stain.
- Samples were then cultured on (L.J) medium
- Growth was monitored daily during the first week to observe the presence of rapid growers and then weekly up to the 8th week
- Phenotypic characterization was performed by using different biochemical tests.

(B) Proteomics Study

Test Strains

Protein Purification

Protein analysis

Preparation of Lysate

- ***Nocordia species were*** **cultured on (GYEA) for 2 days and a lysate was prepared.**
- **Protein was purified and up-concentrated using the CleanUp Kit from BioRad**

Protein Purification

- Two-dimensional gel electrophoresis was performed according to general protocols of BioRad with only slight modifications.

- 17 cm pH3-10 IPG strips were actively rehydrated and gels were stained with Coomassie Brilliant Blue and subsequently analyzed with PDQuest 6.2 (BioRad).

Protein analysis

- Prominent spots were excised and tryptically digested in the gel .
- For matrix-assisted laser desorption/ionization (MALDI)-MS, samples were purified using ZipTips C18
- Peptide maps were generated with MALDImicroMX instrument .
- α-cyano-4-hydoxycinnamic acid was used as matrix and 1 µl was applied.

Protein analysis

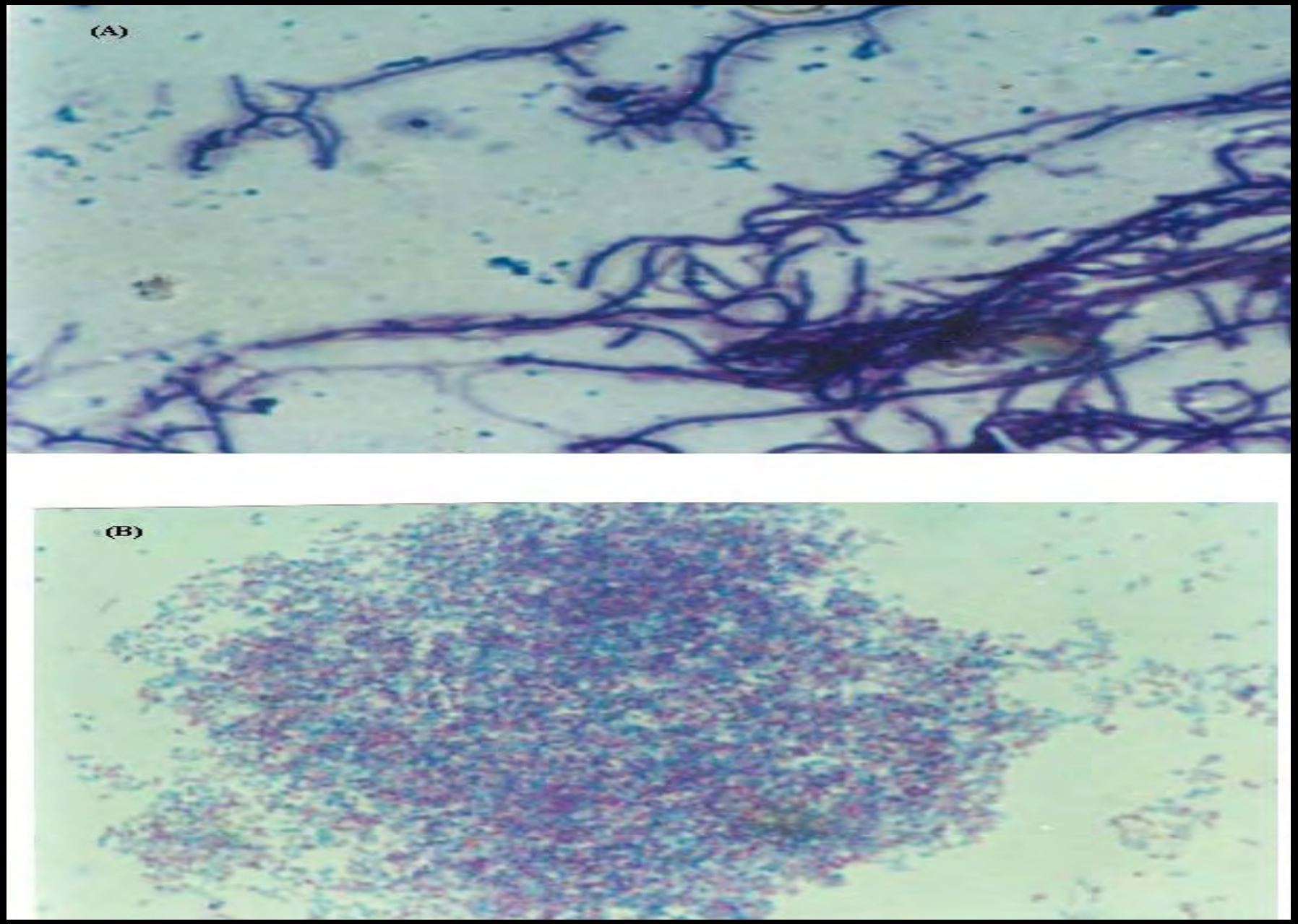
- Database queries were carried out using Mascot in-house with species limitation on bacteria.
- All data can be obtained from the NoDaMS site (Nocardia database on Mass spectrometry analyses) on the following web address: http://ifg-izkf.uni-muenster.de/proteomik/.

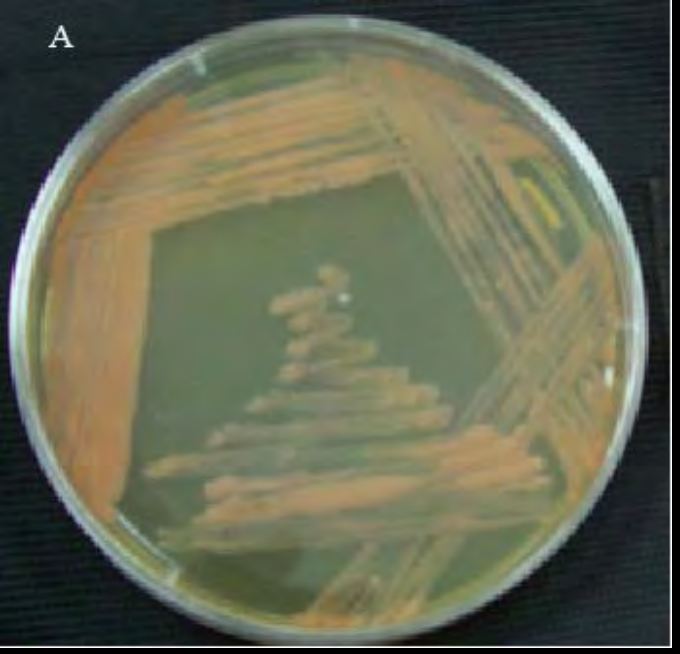
RESULTS AND DISCUSSION

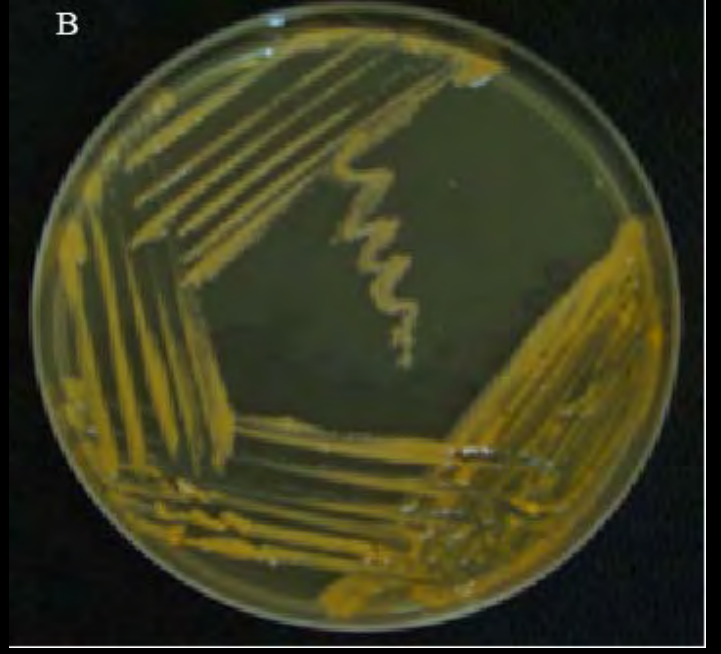
BACTERIOLOGICAL RESULTS

- **The colonial morphology of 319 (97%) appeared as rough, friable, warty, granular and grey in color with irregular margins and showed the appearance of AFB when stained again (indirect smear).**
- **The 319 isolates were initially identified as members of the *M. tuberculosis* complex.**

- Ten (3%) of the LJ slopes reveled the growth of small orange filamentous colonies, (within 2-3 days) which were tentatively considered to be nocardiae. Selected biochemical tests were performed to confirm this findings
- The 10 nocardia isolates, were then designated SD1001, SD1002, SD1003, SD1004, SD1005, SD1006, SD1007, SD1008, SD1009 and SD10010


(A)
(B)


A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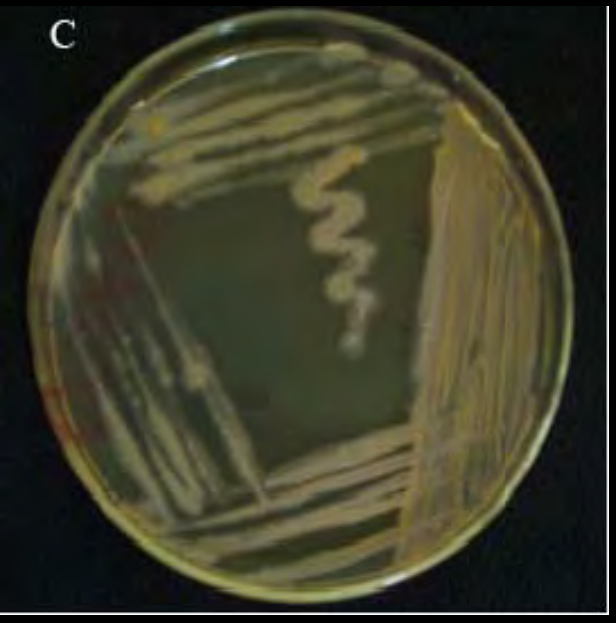
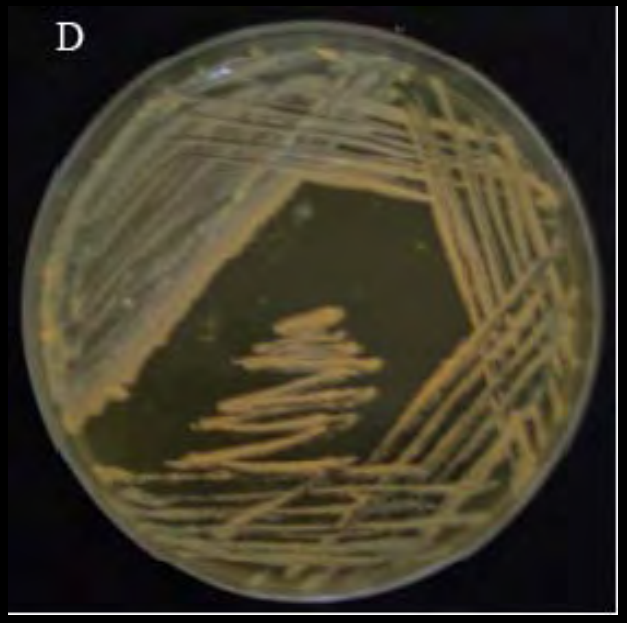
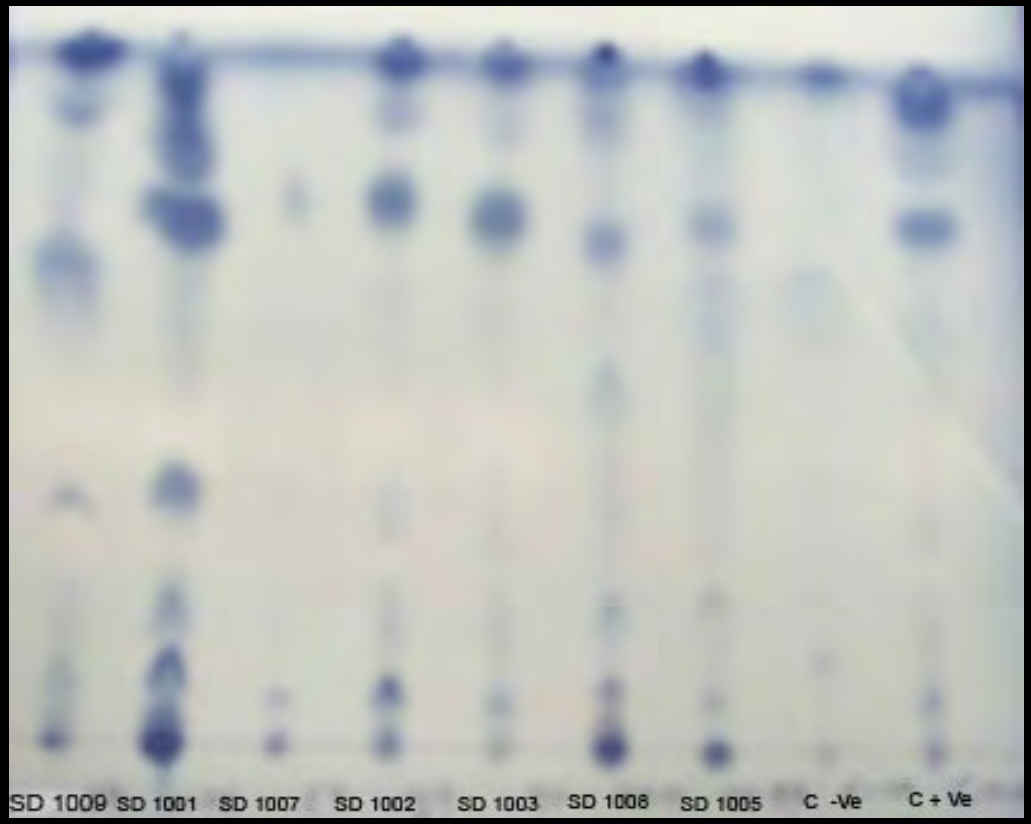
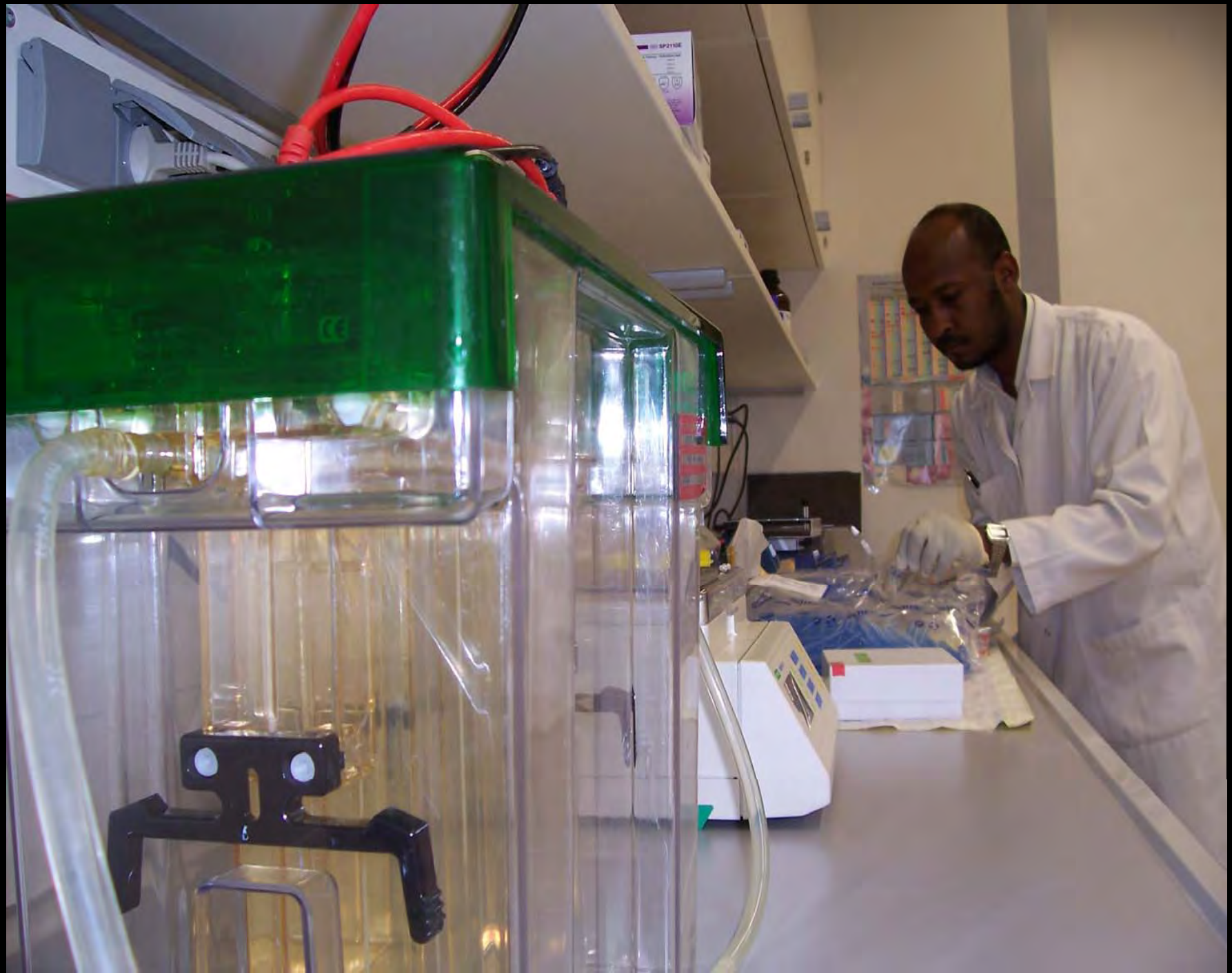

C


D

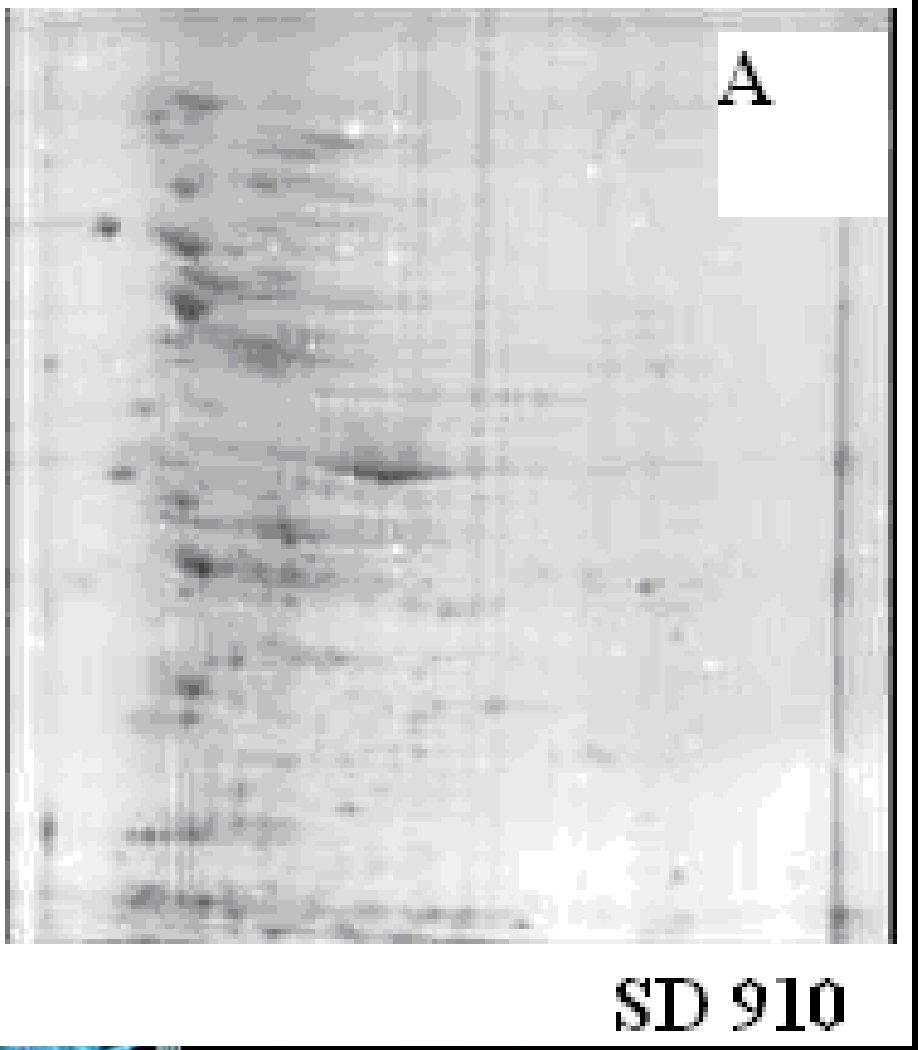

SD 1009
SD 1001
SD 1007
SD 1002
SD 1003
SD 1008
SD 1005
C -Ve
C + Ve

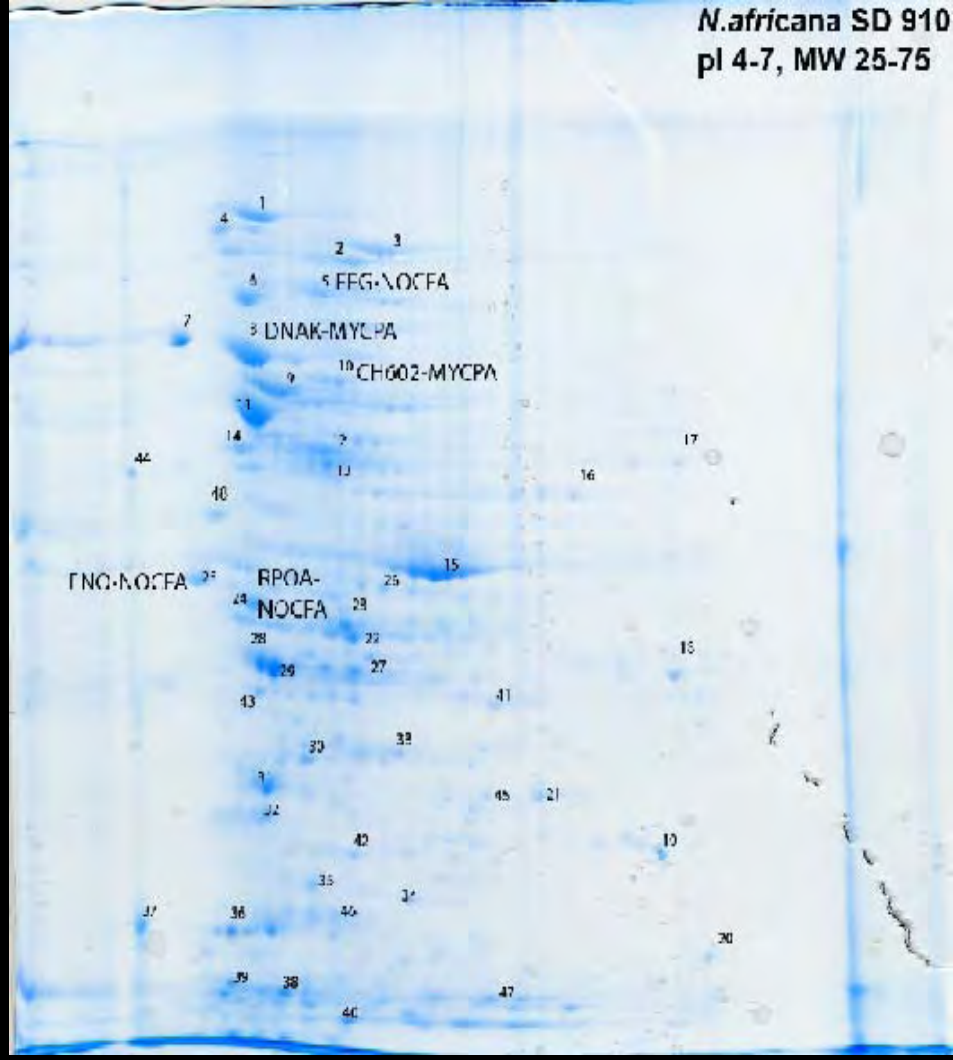
PROTEOMICS RESULTS

- **Two Dimentional Poly Acrylamide Gel Electrophoresis (2D-PAGE) using pH strips 3-10 revealed that the soluble proteins were visible in a much smaller pI range. All strains exhibited similar protein distributions. A similarity analysis revealed that mycobacterium sequences are of high relevance for the investigated strains.**

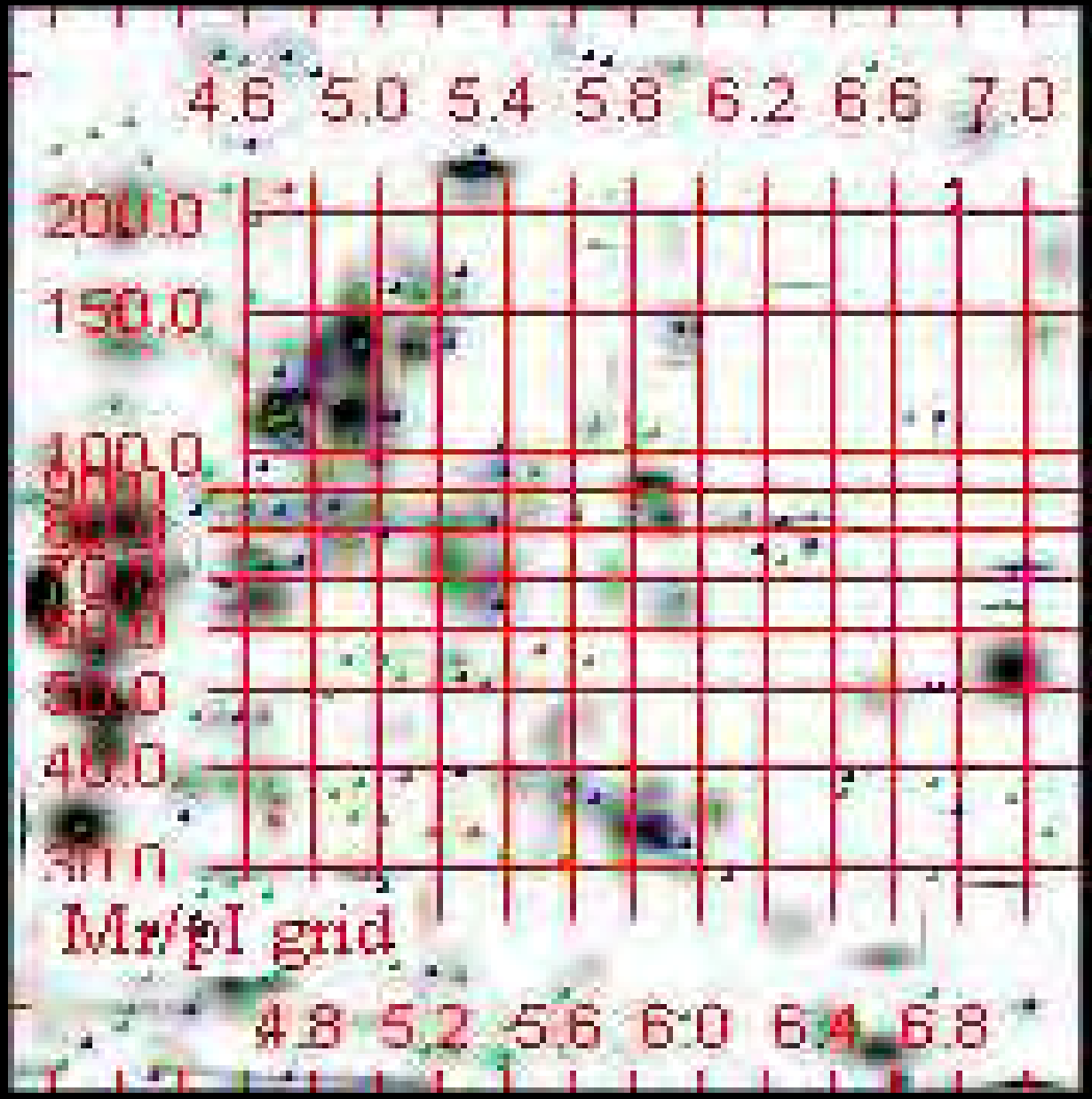


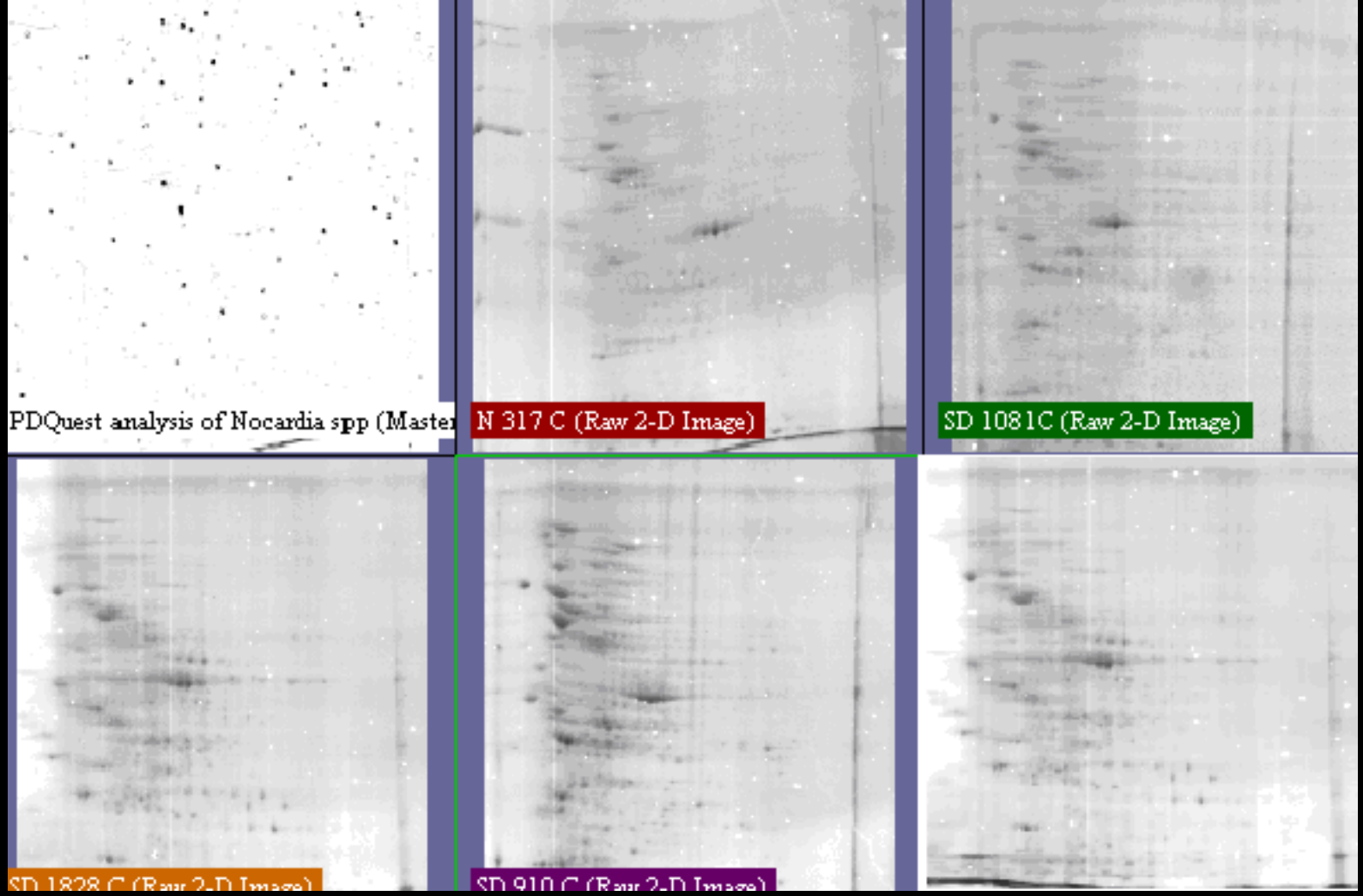

FALCON
352096



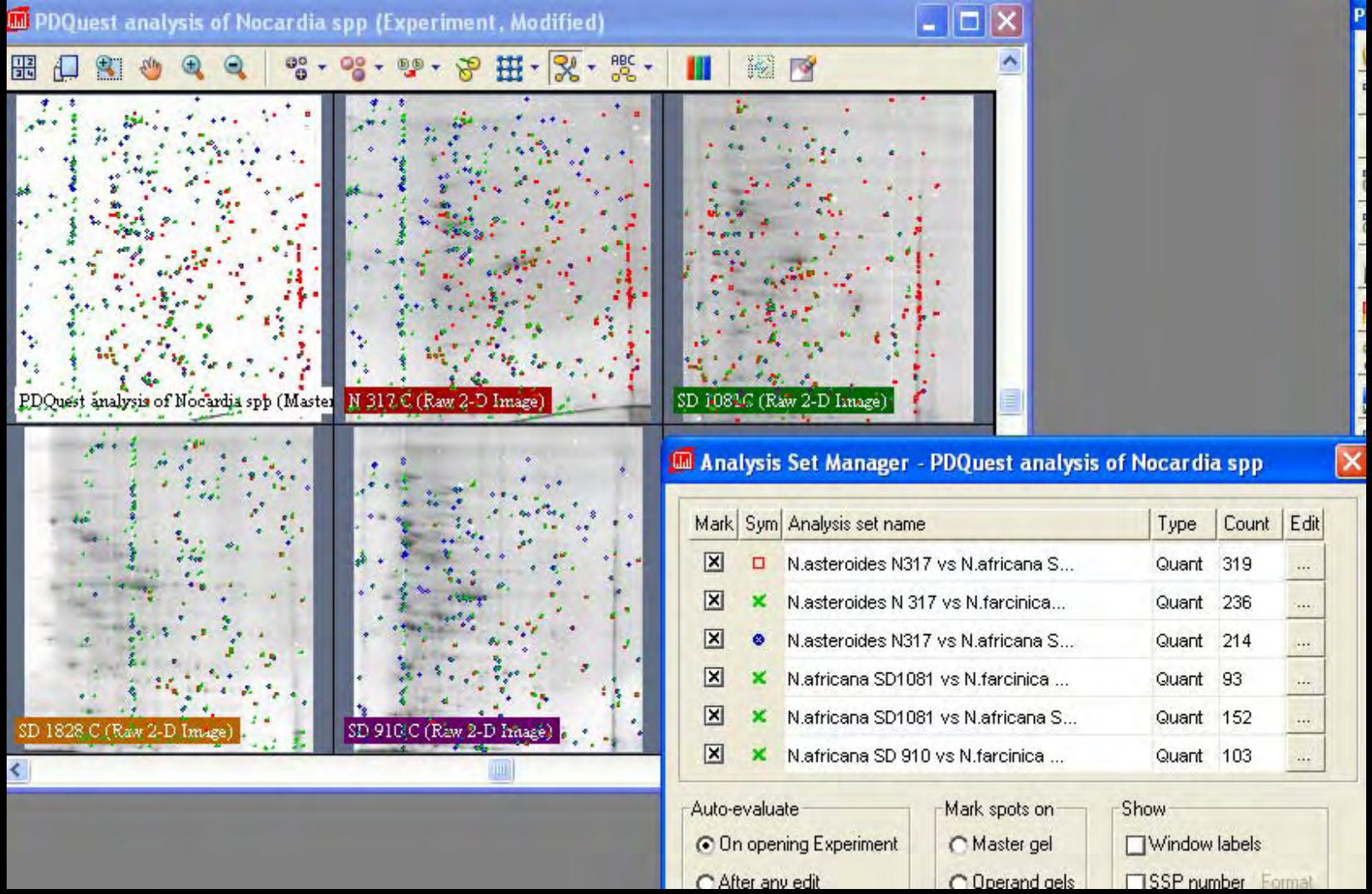


Gel images of *N. africana* SD910

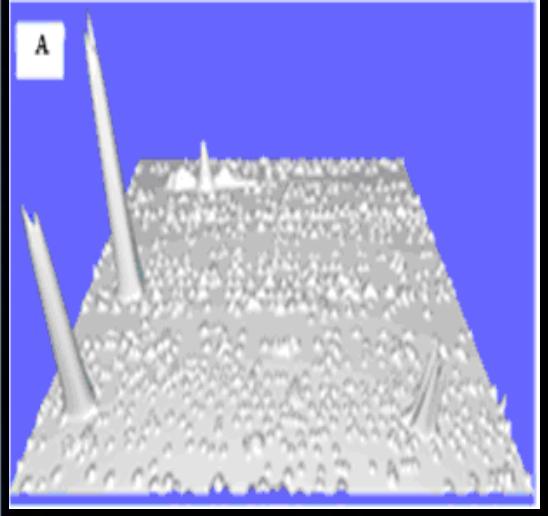

4.6
5.0
5.4
5.8
6.2
6.6
7.0
200.0
150.0
100.0
50.0
0.0
Mr/pI grid
4.8
5.2
5.6
6.0
6.4
6.8

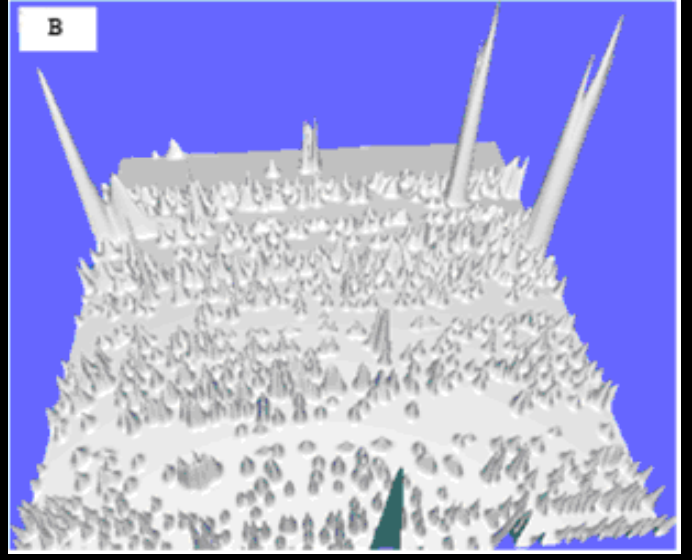


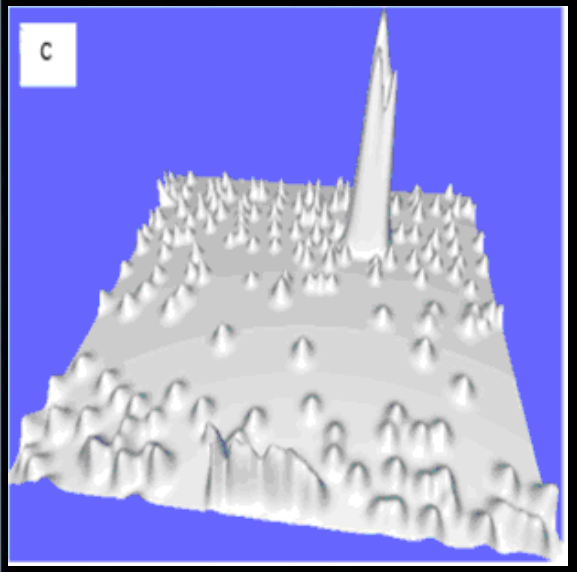
Comparison of filtered Gel images of *N. asteroides* N317, *Nocardia.* sp. N 1086, *N. farcinica* SD1828, *N. africana* SD910 and *N. africana* SD925 with master gel as analyzed with PDQuest.

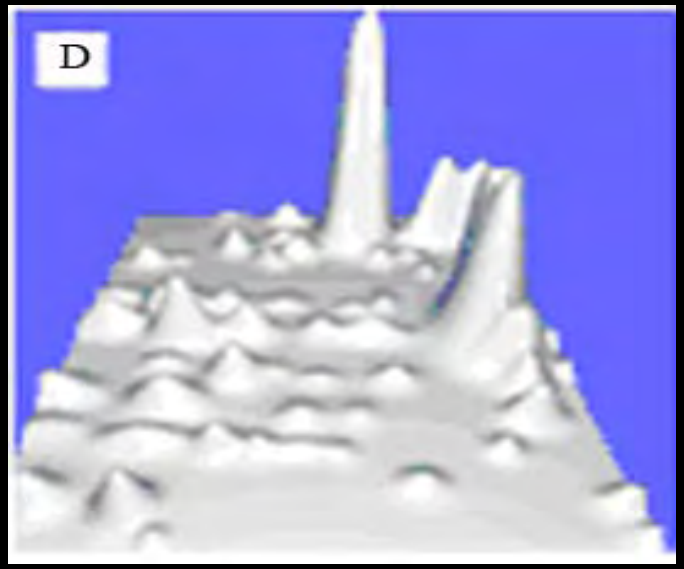


Overlay of gels of analyzed with PDQuest.









3 D view of Spots in the central regions of the gels


MALDI micro MX

Finger print of common peptides detected Elongation factors *of N. africana* SD910 spot5 assigned to elongation factor G (EF-G; Q5YPG3; EFG_NOCFA, 76971).

MW	Start	End	Peptide
1014.57	320	328	K.IAVHPFFGK.L
1090.54	124	132	R.QADKYDVPR.I
1216.65	32	41	R.ILFYTGVNYK.I
1429.77	475	487	K.VEANVGKPQVAYR.E
1442.68	112	123	K.EGVEPQSEQVWR.Q
1772.91	142	156	K.LGADFYFTVQTIKDR.L
1779.92	651	666	K.ALVPLSEMFGYIGDLR.S
1795.92	651	666	K.ALVPLSEMFGYIGDLR.S
1833.97	472	487	R.EFKVEANVGKPQVAYR.E
1893.98	233	249	K.FFGGEELTIDEIKGAIR.K
2022.08	233	250	K.FFGGEELTIDEIKGAIRK.M
2614.30	100	123	R.VLDGAVAVFDGKEGVEPQSEQVWR.Q

Searching homology for the isolated proteins BLAST2 search results

(http://www.expasy.org/cgi-bin/blast.pl)

Elongation factor Tu of Mycobacterium leprae EFTU_MYCLE (P30768

SwissProt Accession #	Organism
Q73SD1	*Mycobacterium paratuberculosis*
P0A558	*Mycobacterium tuberculosis*
P0A559	*Mycobacterium bovis*
Q1BDD3	*Mycobacterium* sp. *(strain MCS)*
Q1T9Z3	*Mycobacterium* sp. *KMS*
Q1TV69	*Mycobacterium* sp. *JLS*
Q262Y2	*Mycobacterium vanbaalenii PYR-1*
Q27DQ3	*Mycobacterium flavescence*
Q5YPG4	*Nocardia farcinica*

Chaperone protein dnaK, HSP70 of *Mycobacterium paratuberculosis* DNAK_MYCPA (Q00488)

Description	SwissProt Accession #	Organism
DNAK_MYCLE	P19993	*Mycobacterium leprae*
DNAK_MYCTU	P0A5B9	*Mycobacterium tuberculosis*
DNAK_MYCBO	P0A5C0	*Mycobacterium bovis*
_MYCVN	Q25TL7	*Mycobacterium vanbaalenii PYR-1*
_MYCFV	Q27CE8	*Mycobacterium flavescence PYR-GCK*
_MYCSS	Q1BEV1	*Mycobacterium* sp. *strain MCS*
_9MYCO	Q1TWF2	*Mycobacterium* sp. *JLS*
_9MYCO	Q1TDC6	*Mycobacterium* sp. *KMS*
DNAK_NOCFA	Q5YNI0	*Nocardia farcinica*

60 kDa chaperonin 2 of *N. farcinica* CH602_NOCFA (Q9AFA6)

Description	SwissProt Accession #	Organism
CH60_COREQ	Q93QI2	*Corynebacterium equii (Rhodococcus equi)*
_RHOSR	Q0SET3	*Rhodococcus* sp. *strain RHA1*
_MYCFV	Q27CR6	*Mycobacterium flavescence PYR-GCK*
_MYCSS	Q1BEF6	*Mycobacterium* sp. *strain MCS*
_9MYCO	Q1TWU6	*Mycobacterium* sp. *JLS*
_MYCVN	Q267S7	*Mycobacterium vanbaalenii PYR-1*
_9MYCO	Q1TDR8	*Mycobacterium* sp. *KMS*
_9MYCO	Q8GAR8	*Mycobacterium* sp. *185-409*
CH60_NOCAS	Q9AFC5	*Nocardia asteroides*
_MYCMR	Q8G8X0	*Mycobacterium marinum*
CH602_MYCPA	P42384	*Mycobacterium paratuberculosis*

Enolase of *N. farcinica* ENO_NOCFA (Q5YQ30)

Description	SwissProt Accession #	Organism
_RHOSR	Q0S4I1	*Rhodococcus sp. strain RHA1*
_MYCSS	Q1B439	*Mycobacterium sp. strain MCS*
_9MYCO	Q1TAN9	*Mycobacterium sp. KMS*
_9MYCO	Q1TX18	*Mycobacterium sp. JLS*
_MYCFV	Q275H2	*Mycobacterium flavescens PYR-GCK*
ENO_MYCPA	Q741U7	*Mycobacterium vanbaalenii PYR-1*
_MYCVN	Q267Y1	*Mycobacterium tuberculosis*
ENO_MYCBO	Q7U0U6	*Mycobacterium bovis*
ENO_MYCTU	P96377	*Mycobacterium tuberculosis*
ENO_MYCLE	Q9CD42	*Mycobacterium leprae*

Transkriptase alpha chain of *N. farcinica* RPOA_NOCFA (Q5Z1K9)

Description	SwissProt Accession #	Organism
_RHOSR	Q0S3E7	*Rhodococcus sp. strain RHA1*
_MYCSS	Q1BD08	*Mycobacterium sp. strain MCS*
_9MYCO	Q1T8C7	*Mycobacterium sp. KMS*
_9MYCO	Q1TR19	*Mycobacterium sp. JLS*
_MYCFV	Q27E10	*Mycobacterium flavescens PYR-GCK*
_MYCVN	Q263M4	*Mycobacterium vanbaalenii PYR-1*
RPOA_MYCTU	P66701	*Mycobacterium tuberculosis*
RPOA_MYCBO	P66702	*Mycobacterium bovis*
RPOA_MYCPA	Q73S43	*Mycobacterium paratuberculosis*
RPOA_MYCLE	Q9X798	*Mycobacterium leprae*

CONCLUSIONS

- **Similarity in clinical manifistations between pulmonary nocardiosis and pulmonary tuberculosis and ZN results needs further investigations by different methods in order to differentiate between the different causative agents and to describe the correct treatment regimen.**

CONCLUSIONS

- **A proteomics study was found useful, a protocol for cell lysis was successfully conducted on nocardia cells.**
- **A comparative analysis using PDQuest soft ware, showed the similarities between different nocardia species investigated as presented by 2D gels.**
- **Peptides finger print using MALDI- MS. of different proteins confirmed the existence of various vital proteins in all *Nocardia* isolates.**

CONCLUSIONS

- **MALDI- MS. analysis showed that there were many proteins which were restricted to a certain *Nocardia* species comparing with others, thus this or that protein can be used to distinguish between different *Nocardia* spp.**

- **Homology of the isolated proteins revealed that *Mycobacterium* and to a lesser extent *Rhodococcus* sequences are of high relevance for the five investigated nocardia strains.**

- **For a successful proteomics protocol, the high quality pure protein preparation is a critical point, which is difficult to achieve in the case of Nocardia as it is rich with mycolic acids.**

- **Thus, more additional steps for protein purification and concentration were necessary. High specialized laboratory facilities are needed as well.**

- **Moreover, the proteome data concerning Nocardiae in the data bases is so limited, which makes the alignment of isolated proteins a difficult task.**

PROSPECTIVE WORK

FUTURE WORK

- A DIGE (Differential Gel Electrophoresis) based approach is recommended for further improvements, because classical 2-D gel electrophoresis has a considerable degree of gel-to-gel variations that can be minimized by DIGE.

- A large body of MS data is available in the NoDaMS database which was created depending on the results obtained from this study, to share peak lists for future investigations using advanced protein databases

- **Further proteomics studies are essential to establish the complete proteome of Nocardia africana as well as other Nocardia spp. For this approach, different types of culture media and variable condition are necessary to investigate the possibility of having other secretory and/or excretory proteins**

AKNOLEDGMENTS

- SUST
- DAAD
- ICGEB
- Mr. Adil Mahgoub
- Dr. Kanory Rao
- Staff of TB Refference lab
- Staff of Abu-Anga Hospital
- Staff of El Shaab Teaching Hospital


THANK YOU