

Pulmonary Nocardiosis; Similarity to Tuberculosis (A Bacteriological and Proteomics Study)

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ABSTRACT

Objective: The aims of the present study were to decide the occurrence of nocardia spp. among Sudanese patients suspected with tuberculosis and to investigate all proteins expressed by the genome of *Nocardia africana* (formerly isolated from patients with pulmonary infection misdiagnosed as MDR and their structures and functions compared to *Mycobacterium tuberculosis*.

Materials and Methods: Three hundred and twenty-nine patients, presented with pulmonary infection were included in this study. Those patients were examined for the presence of acid- fast bacilli. Two tubes of Lownstein- Jensen (L.J) medium were inoculated with 20 µl of the neutralized sputum sample. All cultures were incubated at 37 °C for 8 weeks before being discarded. Phenotypic characterizations were performed. For nocardia proteom poly acrylamide gele lectrophoresis (PAGE)-based analyses of the four nocardia strains *N. farcinica* SD1828, *N. africana* SD 925, and *N. asteroides* N317 are discussed. In-gel tryptic digestion of these isolates was also performed, then the resulting peptides were introduced to MALDI-TOF peptide mass fingerprints were searched using MASCOT software.

Results: Ten isolates showed rapid growth pattern within 2-3 days after inoculation, further conventional methods suggested that all these isolates were belonging to the family nocardia. Two Dimensional 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.

Conclusions: Nocardia revealed considerable occurrence among patients with pulmonary infections (3.3%) giving clinical symptoms similar to those occur by *M. tuberculosis* infection, this may be due to similarities in functional proteins expressed by their genomes. This finding suggested that pulmonary nocardiosis might occur in patients who suffer from chronic lung disease in Sudan. It is important, therefore, that clinicians in Chest Units should consider this condition, especially when patients with respiratory infections fail to respond to antitubercular therapy.

Key words: Proteomics, Nocardia, 2D-PAGE

BACKGROUND

Nocardiae are Gram-positive aerobic actinomycetes, which are predominantly saprophytic (Orchard, 1981) but also include species forming parasitic association with animals and plants (Goodfellow, 1992). They are in the same family as clinically and industrially important genera such as *Mycobacterium*, *Streptomyces*, *Corynebacterium* and *Rhodococcus* and they are known to cause a variety of infections in humans and animals. Nocardiae cause a variety of suppurative infections of humans and animals (Ishikawa, 2004; Mogahid *et al.*, 2007). Human infections may be distinguished clinically into cutaneous, subcutaneous, and lymphocutaneous nocardiosis; extrapulmonary nocardiosis; pulmonary nocardiosis; and systemic nocardiosis involving two or more body sites. The incidence of such infections is not known, although nocardiosis has been reported in most regions of the world. Nocardial infections of the internal organs in nontropical countries are mainly caused by *Nocardia asteroides*, *N. farcinica*, and *N. nova*; relatively few are caused by *N. brasiliensis*, *N. otitidiscaviarum*, *N. pseudobrasiliensis*, and *N. transvalensis*. There have been isolated reports of pulmonary nocardiosis from tropical countries caused by *N. asteroides*, *N. brasiliensis*, *N. farcinica*, *N. otitidiscaviarum*, and *N. transvalensis*, (Koltzsch *et al.*, 2003; Bauer *et al.*, 1966; Gang *et al.*, 2005). According to information from the Center of Disease Control and prevention 80% of cases present as invasive pulmonary infection, disseminated disease, or brain abscess, and 20% present as cellulitis. *Nocardia asteroides* causes at least 50% of invasive infections. In the United States, estimated 500-1,000 new cases of nocardia infection occur annually. The number of cases has increased with the overall rise in the number of severely immunocompromised persons. Diagnosis is a major challenge. *N. farcinica* frequently is resistant to antimicrobial agents, including the drug of choice trimethoprim-sulfamethoxazole, and has been demonstrated to be more virulent in an animal model. A new combination drug therapy (sulfonamide, ceftriaxone, and amikacin) has shown promise for infections difficult to treat. The hope is that the application of newer molecular diagnostic and subtyping methods may assist in earlier diagnosis and outbreak investigation (www.cd.gov).

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. By proteomics, many new proteins have been identified in different microorganisms and their functions have been

well understood (Cutler *et al.*, 1999, Gorg *et al.*, 2000).

Two-dimensional (2-D) gel electrophoresis of pulse-labeled proteins revealed a global analysis of protein synthesis and turnover in *Escherichia coli* (Weichart *et al.*, 2003). Comparative proteomics of the human pathogen *Campylobacter jejuni* revealed an important first step in characterizing strain differences potentially responsible for different disease outcomes associated with this organism (Brubacher *et al.*, 2003). Several studies subjected *Mycobacterium tuberculosis* to comprehensive proteomic analysis. Culture supernatant proteins of virulent *Mycobacterium tuberculosis* H₃₇Rv and attenuated *Mycobacterium bovis* BCG were comprehensively analyzed using proteomics techniques (Mattow *et al.*, 2001). In another study, proteomics revealed open reading frames in *Mycobacterium tuberculosis* H₃₇Rv not predicted by genomics (Peter *et al.*, 2003). This clearly showed that, analysis of proteins directly may come up with new knowledge which may completely alter the approaches to disease diagnosis, control and treatment. Definitive diagnosis of nocardiosis depends on the isolation and identification of the causative organism from clinical materials. These procedures are not straightforward; hence the true incidence of the disease is masked, a problem which is compounded by poor documentation. Generally, nocardia cases are difficult to diagnose (Garcia-Benitez *et al.*, 2001).

MATERIALS AND METHODS

Collection of the Samples

Three hundred and twenty- nine patients, who were attending Abu-Anga Teaching Hospital, El-Shaab Teaching Hospital and the National Health Laboratory, in the Sudan during the period from October 2004 to January 2006, were examined for the presence of acid-fast bacilli. They were suspected of having tuberculosis infection according to the symptoms. Most of the patients had either not responded to treatment with antitubercular drugs or had responded and then relapsed. Sputum samples were collected according to WHO criteria in sterile, plastic wide-mouthed, strong leak-proof containers. Following treatment with the digestion-decontamination procedure of Roberts *et al.*, 1991, the sputum samples were concentrated by centrifugation and the resultant preparations were used to inoculate Lowenstein-Jensen (LJ) slopes, which were incubated at 37°C for 14 days and then used to make smears, which were examined with a standard Ziehl-Neelsen acid-fast stain.

Phenotypic Characterization

Ten of the LJ slopes supported the growth of small orange filamentous colonies, which were considered to be typical of nocardiae. The isolates, which were designated SD1001, SD1002, SD1002, SD1003, SD1004, SD1005, SD1006, SD1007, SD1008, SD1009 and SD1010, were subcultured and maintained on glucose-yeast extract agar (GYEA) slopes at room temperature. The ten isolates were examined for a range of phenotypic properties described by Isik *et al.*, 1999. Standard procedures were also used for the extraction and analysis of mycolic acids as described by Minnikin, *et al.*, 1975 and *Nocardia africana* strains used as controls.

Preparation on Nocardia Lysate

The test strains were cultivated on GYEA medium (Appendix II) for 2-3 days at 37°C then checked for purity. *Nocardia* species were then harvested, inactivated in the water path at 90°C for one hour and the cells were washed with sterile phosphate buffer saline (P.B.S) and then centrifuged at 15000 rpm for 5 minutes. Supernatants were discarded and the pellet was washed by resuspending in MilliQ water and centrifuged again at 10000 rpm for 10 minutes, and the supernatant was discarded.

Then, 1ml of urea lysis buffer 8 M (Appendix IV) was added to each sample in eppendorf tubes in addition to 100 µl of protease

inhibitor (CALBIOCHEM, Cat No 539134), 20 µl of (500 U) benzonase (Novagen Lot N 62211-1) and 0.5gm of silica beads (0.1mm, Cat No 11079101 Z-Biospec products, Inc). The mixture was then vortexed for one minute (Vortex Genie 2 Model G.560E Bohemia USA).

Samples were then lysed by freezing in liquid nitrogen and thawing in 37°C in waterpath, this step was repeated for 5 times. Samples were then put on a shaker (Type MM 300 cat No 85720 GmbH and co KG, Germany) with a frequency of 3000 per minute for 30 minutes. Step of freeze-thawing was repeated again for other 5 times and the shaking step was also repeated as previously described. Samples were then spin down at 15000 rpm for 5 minutes, supernatant (which contain the soluble proteins) was collected and protein concentration was measured with spectrophotometer (Model 8024-0600, From Pharmacia Biotech, England).

Two Dimensional Gel Electrophoresis

Seventy (70) µg of protein were Loaded on immobilized pH gradients (I PG) dry Strip (pH 4-7, 7 cm) together with IPG buffer (pH 3- 10) and 20 mM DTT), overnight passive rehydration was performed for the dry strip then Ettan I PG phor (Amersham Bioscience) was used to achieve First dimension Iso Electrical Focusing. A second dimension SDS – PAGE was done by using Hofer miniv E8 ×9cm gels device according to Laemmli, 1970

Tryptic Digestion and Mass Spectrometric Sequencing

In-solution tryptic digestion of nocardia isolates lysate was also performed; a total of 4µl (containing approximately 250 µg) of the lysate was dry with vacuum centrifuge, the dry protein was redissolved in 250 µl of 8 M urea, 0.4 M N₄HCO₃ followed by 25µl of 45 mM dithiothreitol the mixture was incubated at 50 °C for 15 minutes, 25 µL of 100 mM Iodoacetamide were added and the mixture was incubated at ambient temperature in darkness for additional 15 minutes, 700 µL of deionized water was added together with 5% (w/w) trypsin (12.5 µg). The digestion was performed at 37° C overnight in darkness. Resulting peptides were then desalted by using Zip Tip column (microbed C₁₈; Millipore, USA), then the peptides were introduced to mass spectrophotometer (QSTAR from Applied Biosystem), peptide mass

fingerprints were searched using MASCOT software.

RESULTS

Bacteriology Results

All positive smears showed positive growth on LJ medium after 2-21 days post inoculation. 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) with ZN staining procedure for more confirmation. The 319 isolates were initially identified as members of the *Mycobacterium tuberculosis* complex.

Ten (3%) of the LJ slopes revealed the growth of small orange filamentous colonies, which were tentatively considered to be nocardiae (Fig. 1).

The 10 nocardia isolates, which were then designated SD1001, SD1002, SD1003, SD1004, SD1005, SD1006, SD1007, SD1008, SD1009 and SD10010 were subcultured and maintained on glucose-yeast extract agar (GYEA) slopes at room temperature and as suspensions of mycelial fragments in glycerol (20% [vol/ vol]) at -70°C . The 10 isolates were studied phenotypically.

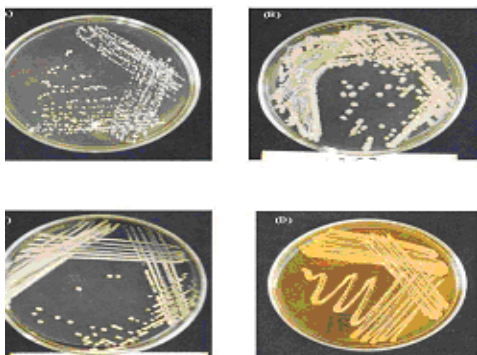


Fig. 1: Growth of *N. africana* SD 1002 (A); SD 1003 (B); SD 925 (C); *N. farcinica* SD 1819 (D); on glucose yeast extract agar medium. 7 days old cultures.

Biochemical tests for nocardia isolates

Selected biochemical tests were performed. The result for these tests showed that all the strains utilize glucose by oxidation pathway and that they were all catalase positive and also positive for urea. Concerning growth at 45°C , 7 out of the ten were positive (70%). Only 2 (20%) were positive for manitol and rhaminose as well as starch whilst all the isolates were negative for xanthine, casein, tyrosine, sorbitol, arabinose and citrate. Regarding mycolic acids, all tested isolates showed the standard patterns of mycolic acid components using thin layer chromatographic technique. The tested strains were found to have phenotypic properties typical of members of the genus nocardia (Figs 1 and 2).

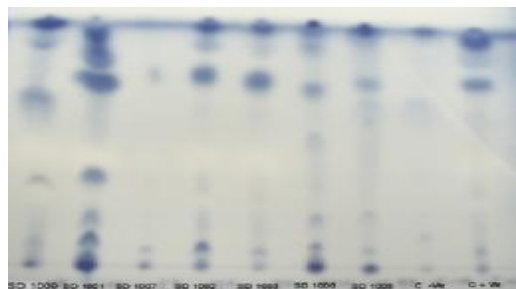


Fig. 2: Thin layer chromatography (TLC) (10x10cm), run twice in a solvent containing Toluene-Aceton (97:3, V/V), C +Ve: *N. africana* SD 925, C -Ve: *S. aurues*.

Proteomic Results

The results of 2-D gel electrophoresis revealed a total of hundreds of protein spots for nocardia lysate. The molecular weights range between 25 -75 kDa and the Iso-electric point (pI) of these range between 4-7 (Figs 3 and 4).

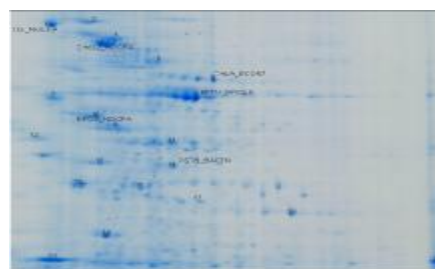


Fig. 3: Representative spot assignments for *N. farcinica* SD1828, pH 4-7, MW 25-75 kDa.

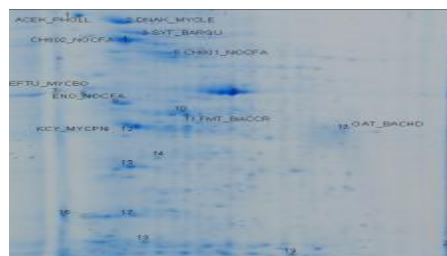


Fig. 4: Representative spot assignments for *N. africana*. SD 925, pH 4-7, MW 25-75 kDa.

On the other hand, different peptides were detected by mass spectrometer. Alignment of those peptides was performed on Swiss-Prot. The resulting data revealed highly significant homology with *Mycobacterium tuberculosis* (set of Table 1).

CONCLUSION

A relatively large sample size (329) was analyzed in this study compared with previous studies in Sudan conducted in the same field and nocardia species has constituted 3% of all isolate. A significant resistant pattern (40%) were observed through these ten (10) isolates, hence more attention should be drawn towards patients who did not respond to anti-tuberculosis therapy, as other pathogens, including *Nocardia* spp. may be the cause of the infection.

Hundreds of nocardia protein spots were captured on stained gel. Some proteins were identified in a gel digest using Q-STAR instrument analysis of the results showed significant similarities between nocardia and *Mycobacterium tuberculosis*; this may provide evidences for best understanding of the pathogenesis and increase the knowledge with respect to disease treatment and vaccination.

Table 1/1: Elongation factor Tu of *Mycobacterium leprae* EFTU_MYCLE (P30768)

Description	SwissProt Accession #	Organism
_MYCPA	Q73SD1	<i>Mycobacterium paratuberculosis</i>
EFTU_MYCTU	P0A558	<i>Mycobacterium tuberculosis</i>
EFTU_MYCBO	P0A559	<i>Mycobacterium bovis</i>
_MYCSS	Q1BDD3	<i>Mycobacterium sp. (strain MCS)</i>
_9MYCO	Q1T9Z3	<i>Mycobacterium sp. KMS</i>
_9MYCO	Q1TV69	<i>Mycobacterium sp. JLS</i>
_MYCVN	Q262Y2	<i>Mycobacterium vanbaalenii</i> PYR-1
_MYCFV	Q27DQ3	<i>Mycobacterium flavescence</i>
_NOCPA	Q5YPG4	<i>Nocardia farcinica</i>

Sequence comparison EFTU_MYCLE and Q5YPG4_NOCPA

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 Sbjct: 20 VAKAKFERTKPHVNIGTIGHVDHGKTTLTAAITKVLADKY PDLNQSFAFDQIDKAPEEKA 79

Query: 61 RGITINISHVEYQTEKRHYAHVDAPGHADYIKNMITGAAQMDGAILVV AATDGPMPQTRE 120
 RGITINISHVEYQTEKRHYAHVDAPGHADYIKNMITGAAQMDGAILVV AATDGPMPQTRE
 Sbjct: 80 RGITINISHVEYQTEKRHYAHVDAPGHADYIKNMITGAAQMDGAILVVAATDGPMPQTRE 139

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 HVLLARQVGVPYILVALNK+ QEFDE+APVVRVS LKAL
 Sbjct: 140 HVLLARQVGVPYILVALNKADMVDDEEILEL VEMEVRELLAAQEFDEEAPVVRV SGLKAL 199

Query: 181 EGDAKWVESVTQLMDA VDESIPAPVRETDPFLMPVEDVFXXXXXXXXXXXXXXXXXXXX
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Query: 361 ISVTLIQPVAMDEGLRFAIREGGRTVGAGR VVKIHK 396
 +SV LIQPVAM+EGLRFAIREGGRTVGAGR V KIIK
 Sbjct: 380 MSVKLIQPVAMEEGLRFAIREGGRTVGAGR VTKIHK 41

Table 1/2: Chaperone protein dnaK, HSP70 of *Mycobacterium paratuberculosis* DNAK_MYCPA (Q00488)

Description	SwissProt Accession #	Organism
DNAK_MYCLE	P19993	<i>Mycobacterium leprae</i>
DNAK_MYCTU	POA5B9	<i>Mycobacterium tuberculosis</i>
DNAK_MYCBO	POA5C0	<i>Mycobacterium bovis</i>
_MYCVN	Q25TL7	<i>Mycobacterium vanbaalenii</i> PYR-1
_MYCFV	Q27CE8	<i>Mycobacterium flavescence</i> PYR-GCK
_MYCSS	Q1BEV1	<i>Mycobacterium</i> sp. strain MCS
_9MYCO	Q1TWF2	<i>Mycobacterium</i> sp. JLS
_9MYCO	Q1TDC6	<i>Mycobacterium</i> sp. KMS
DNAK_NOCF A	Q5YNI0	<i>Nocardia farcinica</i>

Sequence comparison DNAL_MYCPA and DNAK_NOCF A

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Table 1/3: 60 kDa chaperonin 1 of *N. farcinica* CH601_NOCF A (Q5Z1F9)

Description	SwissProt Accession #	Organism
_RHOSR	Q0S3B9	<i>Rhodococcus sp. strain RHA1</i>
_MYCSS	Q1BCW7	<i>Mycobacterium sp. strain MCS</i>
_9MYCO	Q1TR57	<i>Mycobacterium sp. JLS</i>
_9MYCO	Q1TCR3	<i>Mycobacterium sp. KMS</i>
_MYCVN	Q263S8	<i>Mycobacterium vanbaalenii</i> PYR-1
_MYCFV	Q27E56	<i>Mycobacterium flavescence</i> PYR-GCK
CH601_MYCPA	P60545	<i>Mycobacterium paratuberculosis</i>
CH601_MYCTU	P0A518	<i>Mycobacterium tuberculosis</i>
CH601_MYCBO	P0A519	<i>Mycobacterium bovis</i>
CH601_MYCLE	P37578	<i>Mycobacterium leprae</i>

Sequence comparison CH601_NOCF A and Q0S3B9_RHOSR and Q1BCW7_MYCSS

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 Sbjct: 421 STELAD-NLGLTGDEATGVKVVREALQAPLYWIASNAGLDGSVVTSKVAEQPKGHGFNAA 479
 L LR S+SGDEA+GVEVAL APL+WIA+NAGLDG+VVV+KV+E +GFNAA
 Sbjct: 421 RKALDSLRSVSGDEALGVEVFNSALSAPLYWIATNAGLDGSVVVNVKSELPAQGQGFNAA 480
 Query: 479 TLSYGDLLTDGVVDPVKXXXXXXXXXXXXXXXXXVMVLTTEASAVVDKPAEEAEDHSHHGHHA
 536
 TL+YGDLL DGVVDPVKM+LTTEASAVV+KPAEE E + HGH+H
 Sbjct: 480 TLTYGDLLADGVVDPVKVTRSAVVNAASVARMILTTEASAVVEKPAEENEQQTGHGHSH 537
 TL +GDLL DGVVDPVKMVLTE+A+VDKPAEE EDH HGHHA
 Sbjct: 481 TLEFGDLLADGVVDPVKVTRSAVLNAASVARMVLTTEAIVDKPAEE-EDHGHGHHHGHHA
 539
 Query: 536 H 536
 Sbjct: 540 540
 H
 Sbjct: 540 H 540

Table 1/4: 60 kDa chaperonin 2 of *N. farcinica* CH602_NOCPA (Q9AFA6)

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

Sequence comparison CH602_NOCPA and CH60_NOCAS and CH602_MYCPA

Query: 1MAKTIAAYDEEARRGLERGLNSLADAVKVTLGPKGRNVVLEKKWGAAPTITNDGVSIAKEIE 60
 MAKTIAAYDEEARRGLERGLNSLADAVKVTLGPKGRNVVLEKKWGAAPTITNDGVSIAKEIE
 Sbjct: 1MAKTIAAYDEEARRGLERGLNSLADAVKVTLGPKGRNVVLEKKWGAAPTITNDGVSIAKEIE 60
 Query: 2 AKTIAAYDEEARRGLERGLNSLADAVKVTLGPKGRNVVLEKKWGAAPTITNDGVSIAKEIE 61
 AKTIAAYDEEARRGLERGLN+LADAVKVTLGPKGRNVVLEKKWGAAPTITNDGVSIAKEIE
 Sbjct: 1 AKTIAAYDEEARRGLERGLNALADAVKVTLGPKGRNVVLEKKWGAAPTITNDGVSIAKEIE 60
 Query: 61 LEDPYEKIGAELVKEVAKKXXXXXXXXXXXXXXXXXXXXXXXXXREGLRNVAAGANPLGLKRGIE
 120
 LEDPYEKIGAELVKEVAKK REGLRNVAAGANPLG KRGIE
 Sbjct: 61 LEDPYEKIGAELVKEVAKKTTDDVAGDGTATVLAQALVREGLRNVAAGANPLGRKRGIE 120
 Query: 62 EDPYEKIGAELVKEVAKKXXXXXXXXXXXXXXXXXXXXXXXXXREGLRNVAAGANPLGLKRGIEK
 121
 EDPYEKIGAELVKEVAKKREGLRNVAAGANPLGLKRGIEK
 Sbjct: 61 EDPYEKIGAELVKEVAKKTTDDVAGDGTATVLAQALVREGLRNVAAGANPLGLKRGIEK
 120
 Query: 121 KAVEAVTAKLLDTAKEVETKEQIAATAGISAGDASIGELIAEAMDKVKGEGVITVEESNT 180
 KAVEAVTAKLLDTAKEVETKEQIAATAGISAGDA+IGELIAEAMDKVKGEGVITVEESNT
 Sbjct: 121 KAVEAVTAKLLDTAKEVETKEQIAATAGISAGDAAIGELIAEAMDKVKGEGVITVEESNT 180
 Query: 122 AVEAVTAKLLDTAKEVETKEQIAATAGISAGDASIGELIAEAMDKVKGEGVITVEESNTF 181
 AVE VT LL +AKEVETK+QIAATA ISAGD SIG+LIAEAMDKVG EGVITVEESNTF

Sbjct: 121 AVEKVTETLLKSAKEVETKDQIAATAAISAGDQSIGDLIAEAMDKVGNEGVIITVEESNTF 180

Query: 181 FGLQLELTEGMRFDKGYISGYFVTDPERQEAVLEDPYILLVGSKVSTVKDLLPPEKVIQ 240
 FGLQLELTEGMRFDKGYISGYF TDPERQEAVLEDPYILLVGSKVSTVKDLLPPEKVIQ

Sbjct: 181 FGLQLELTEGMRFDKGYISGYFATDPERQEAVLEDPYILLVGSKVSTVKDLLPPEKVIQ 240

Query: 182 GLQLELTEGMRFDKGYISGYFVTDPERQEAVLEDPYILLVGSKVSTVKDLLPPEKVIQA 241
 GLQLELTEGMRFDKGYISGYFVTD ERQEAVLEDP+ILLV SKVSTVKDLLPPEKVIQA

Sbjct: 181 GLQLELTEGMRFDKGYISGYFVTD AERQEAVLEDPFILLVSSKVSTVKDLLPPEKVIQA 240

Query: 241 AGKPLLI AEDVEGEALSTLVVNKIRGTFKSVAVKAPGFGDRRKAQLADIAILTGGEVIS 300
 AGKPLLI AEDVEGEALSTLVV KI GTFKSVAVKAPG GDRRKAQLADIAILTGG+VIS

Sbjct: 241 AGKPLLI AEDVEGEALSTLVVKKILGTFKSVAVKAPGGGDRRKAQLADIAILTGGQVIS 300

Query: 242 GKPLLI AEDVEGEALSTLVVNKIRGTFKSVAVKAPGFGDRRKAQLADIAILTGGEVISE 301
 GKPLLI AEDVEGEALSTLVVNKIRGTFKSVAVKAPGFGDRRKA L D+AILTGG+VISE

Sbjct: 241 GKPLLI AEDVEGEALSTLVVNKIRGTFKSVAVKAPGFGDRRKAMLQDMAILTGGQVISE 300

Query: 301 EEVGLSLETAGIELLGQARKVVVTKDETTIVEGAGDAEAIKGRVAQIRTEIENS DSDYDR 360
 EEVGLSLETAGIELLGQARKVVVTKDETTIVEGAGDAEAI GRV+QIR EIENS DSDYDR

Sbjct: 301 EEVGLSLETAGIELLGQARKVVVTKDETTIVEGAGDAEAIAGRVSQIRAEIENS DSDYDR 360

Query: 302 EVGLSLETAGIELLGQARKVVVTKDETTIVEGAGDAEAIKGRVAQIRTEIENS DSDYDRE 361
 EVGLSLE+A ILLG+ARKVVVTKDETTIVEGAGD++AI GRVAQIRTEIENS DSDYDRE

Sbjct: 301 EVGLSLESADISLLGKARKVVVTKDETTIVEGAGDSDAIAGRVAQIRTEIENS DSDYDRE 360

Query: 361
 EKLQERLXXXXXXXXXXXXXXXXXTEVELKERKHRIEDAVRNXXXXXXXXXXXXXXXXXXXXXXXXXXXX 420
 EKLQERL TEVELKERKHRIEDAVRN

Sbjct: 361 EKLQERLAKLAGGVAVIKAGAATEVELKERKHRIEDAVRNAKAAVEEGIVAGGGVAF LQS 420

Query: 362
 KLQERLXXXXXXXXXXXXXXXXXTEVELKERKHRIEDAVRNXXXXXXXXXXXXXXXXXXXXXXXXXXXX 421
 KLQERL TEVELKERKHRIEDAVRN

Sbjct: 361 KLQERLAKLAGGVAVIKAGAATEVELKERKHRIEDAVRNAKAAVEEGIVAGGGVALLHAI 420

Query: 421 XXXXDELKLTGDEATGANIVRVALSAPLKQIAFNAGLEPGVVAEKVS NLEAGHGLNADSG 480
 D+ KL GDEATGANIVRVALSAPLKQIAFNAGLEPGV+AEKVS NL AG GLNA +

Sbjct: 421 VPALDDFKLEGDEATGANIVRVALSAPLKQIAFNAGLEPGVLAEKVSNLPAGQGLNAQT N 480

Query: 422 XXXDELKLTGDEATGANIVRVALSAPLKQIAFNAGLEPGVVAEKVS NLEAGHGLNADSGE 481
 DELKL G+EATGANIVRVAL APLKQIAFN GLEPGVVAEKV N AG GLNA +GE

Sbjct: 421 PALDELKLEGEEATGANIVRVALEAPLKQIAFNAGLEPGVVAEKVRNSPAGTGLNAATGE 480

Query: 481 EYEDLLAAGVADPVKVTR SALQNAASIAALFLTTEAVVADKPEKA-AAPAGDPTGGMGGM 539
 E EDLLAAGVADPVKVTR SALQNAASIAALFLTTEAVVADKPEKA AAPA MGGM

Sbjct: 481 EDEDLLAAGVADPVKVTR SALQNAASIAALFLTTEAVVADKPEKASAAPATGHRFKMGGM 540

Query: 482 YEDLLAAGVADPVKVTR SALQNAASIAALFLTTEAVVADKPEKAAAPAGDPTGGMGGMDF 541
 YEDLL AG+ADPVKVTR SALQNAASIA LFLTTEAVVADKPEKAAAPAGDPTGGMGGMDF

Sbjct: 481 YEDLLKAGIADPVKVTR SALQNAASIAAGLFLTTEAVVADKPEKAAAPAGDPTGGMGGMDF 540

Query: 540 DF 541
 DF

Sbjct: 541 DF 542

Table 1/5: Transkriptase alpha chain of *N. farcinica* RPOA_NOCF A (Q5Z1K9)

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

Table 1/6: Enolase of *N. farcinica* ENO_NOCF A (Q5YQ30)

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

REFERENCES

- Bauer AW., Kirby WM., Sherris JC. and Turck M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J. Clin Pathol* 45: 493-496.
- Brubacher JL., Dewitte-Orr SJ., Zorzitto JR., Playle RC., Bols NC. (2003). Redox-active metals in commercial preparations of lipopolysaccharide: implications for studies of cellular responses to bacterial products. *Cell Microbiol.* 5(4): 233-243
- Gang Wu., Lei Nie and Weiwen Z. (2005). Predicted highly expressed genes in *Nocardia farcinica* and the implication for its primary metabolism and nocardial virulence. *Antonie van Leeuwenhoek* 89(1): 135-146.
- Garcia BV., Garcia H. L., Archer DC., and OrozcoTR. (2001). Acute primary superficial nocardiosis due to *Nocardia brasiliensis*: a case report in an immunocompromised patient. *Eur J Epidemiol.* 17(11): 1019-1022.
- Goodfellow M. (1992). The family Nocardaceae. The prokaryotes; Vol 2. 2nd Ed., Eds. Balows, A., Truper, H. G. *et al.*, Springer-Verlag New York, 1188-213.
- Hamid ME., Maldonado L., Sharaf Eldin GS., Mohammed MF., Saeed NS., Goodfellow M. (2001) *Nocardia africana* sp. nov, a new pathogen isolated from patients with pulmonary infections. *Clin. Microbiol.* 39(2) : 625-30.
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/nocardiosis_t.htm
- Isik KJ., Chun YC. and Goodfellow M. (1999). *Nocardia salmonicida* nom. rev., a fish pathogen. *Int. J. Syst. Bacteriol.* 49:833-837.
- Ishikawa Jun., Atsushi Y., Yuzuru M., Yasutaka H., Haruyo K., Kunimoto H., Tadayoshi S. and Masahiara H. (2004) The complete genomic sequence of *Nocardia farcinica* IFM 10152. *PNAS* 101(41):14925-14930.
- Koltzsch M., Claudia N., Simone K. and Volker G. (2003). Ca²⁺-dependent binding and activation of dormant ezrin by dimeric S100P. *Mol Biol of the Cell* 14(6): 2372-2384.
- Mattow J., Jungblut PR., Schaible UE., Mollenkopf HJ., Lamer S., Zimny-Arndt U., Hagens K., Müller EC., Kaufmann, SH. (2001). Identification of proteins from *Mycobacterium tuberculosis* missing in attenuated *Mycobacterium bovis* BCG strains. *Electrophoresis* 22(14):2936-46.
- Minnikin DE, Alshamaony L. and M. Goodfellow. 1975. Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analyses of whole-cell methanolsates. *J. Gen. Microbiol.* 88:200-204.
- Mogahid EE, Kanury VS., Zaved S., Dinesh SK., Rashmi T., Nageeb SS., Moawia MM. and Mohamed H. (2007) Proteomics of *Nocardia africana* (SD769) recently isolated from patients with pulmonary infection in Sudan. *Biomacromol Mass Spectrom* 1(3): 171-177.
- Orchard, V. A. (1981). The ecology of *Nocardia* and related taxa. *Zentralbl. Bakteriol. Suppl.* 11: 167-80.
- Peter R., Jungblut ECM., Jens M. and Stefan HEK. (2003) Proteomics Reveals Open Reading Frames in *Mycobacterium tuberculosis* H37Rv Not Predicted by
- Roberts GD., Koneman EW. and Kim YK. (1991). *Mycobacterium*, p.304-339. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
- Weichert D., Querfurth N., Dreger M., Hengge-Aronis R. (2003). Global role for ClpP-containing proteases in stationary-phase adaptation of *Escherichia coli*. *J. Bacteriol.* 185(1): 115-125.