academicJournals

Vol. 7(29), pp. 3853-3857, 19 July, 2013
DOI: 10.5897/AJMR2013.5907
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Dietzia species as a cause of mastitis: Isolation and identification of five cases from dairy cattle

Mohamed E. Hamid

Department of Clinical Microbiology and Parasitology, College of Medicine, King Khalid University, P. O. Box 641, Abha, Kingdom of Saudi Arabia.

Accepted 5 July, 2013

Many infective agents are implicated as causal agents of mastitis. Miscellaneous causes of mastitis in bovine due to actinomycetes such as nocardiae have been described but neither *Rhodococcus* nor *Dietzia* species have been reported. The aim of this study was to report the isolation and characterization of rhodococcus-like actinomycetes as causal agents of mastitis in cattle. Milk samples (n = 100) collected from cattle suffering from mastitis in Sudan were subjected to microscopic examination using modified Ziehl Neelsen stains and cultured on Trpytic Soya agar medium. Out of the 100 milk samples collected from cattle suffering from mastitis in Sudan, five (5%) revealed rhodococcus-like growth which showed gram-positive coccobacilli. Two of the five samples showed partially acid-fast coccobacilli when milk samples were directly stained with modified Ziehl Neelsen method. The isolates were tentatively identified as members of the genus *Rhodococcus* on the basis of morphological, biochemical and mycolic acids pattern. Comparative analysis of the 16S rDNA gene sequence confirmed that the strains fall within the phylogenetic branch which accommodates members of the genus *Dietzia*, but well separated from the validly described *Dietzia* spp. The isolation of 5% *Dietzia* sp. could represent a new significant cause of clinical mastitis in cattle.

Key words: Actinomycetes, nocardioform, *Dietzia*, Sudan, mastitis, cattle.

INTRODUCTION

Mastitis is an inflammation of the udder tissue caused by microorganisms notably bacteria and can be clinical with visible signs or subclinical with no visible signs. The main pathogens associated with mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae* and *E. coli* (Radostits et al., 2007). Bovine mastitis is the most costly disease affecting dairy cattle throughout the world in terms of economic loss due to its major effect on milk production (Petrovski et al., 2006). The prevalence of mastitis among dairy cattle in Sudan as in many other African countries is increasing during the past 50 years. This could be attributed to the increasing in the intesive herding and importation of the high breeds namely the Friesian breed and the selection high yielding local breeds (El Zubeir and EL Owni, 2006). A number of other aetiological agents of mastitis in dairy cattle have been reported in the literature including nocardiae (Pier et al., 1961; Manninen et al., 1993; Beaman et al., 1995; Goodfellow and Maldonado, 2007; Hamid et al., 2007), but neither *Rhodococcus* nor *Dietzia* species have been

*Corresponding author.E-mail: mehamid2@yahoo.com. Tel:+966509773687.

described. However, *Corynebacterium pyogenes*, a close bacteria to *Dietzia* species, is a well known cause of mastitis in cattle (Radostits et al., 2007). Some species of *Rhodococcus* and *Dietzia* have been found to cause variety of pyogranulomatous infections in a human and animals (Koerner et al., 2009; Von Bargen and Haas, 2009).

The objective of this study was to report the isolation and characterization of rhodococcus-like actinomycetes as causal agents of mastitis in cattle.

MATERIALS AND METHODS

Animals and area of investigation

Specimens considered in this study were encountered during a survey for mastitis caused by *Nocardia* spp. from 100 dairy cattle in Khartoum State, Sudan, suffering from mastitis. Thorough clinical examination with special attention to udders was conducted.

Milk samples

Milk samples (duplicate taken two days apart) were collected in sterile 50 ml containers and transported within 2 h to the laboratory for bacteriological investigations. Samples were processed for bacteriological analysis according to standard methods (Quinn et al., 1999). California mastitis test was used for the detection of mastitis. The 100 positive cases recognized during the survey were considered for microscopy and culture of actinomycetes.

Smear and culture methods

Smear from each suspected milk sample was stained with modified Ziehl Neelsen method (Quinn et al., 1999). The slide was flooded with dilute carbol fuchsin for 15 min, then washed thoroughly under running water, decolorized with 5% acetic acid for 15 s, and counter-stained with methylene blue for 1 min. Tryptic Soya Agar (TSA; Oxoid) was used for the isolation of the causative agent and for subsequent sub-culturing. About 0.1 ml milk sample was inoculated onto TSA plates, incubated aerobically for up to 7 days. Growth of actinomycetes was suspected on morphological basis and confirmed using selected biochemical tests, mycolic acids and 16S rDNA gene sequence analysis (Goodfellow, 1998). Detailed phenotypic characterization methods are described by Isik et al. (1999). The phenotypic tests undertaken included colony morphology and microscopic properties; catalase, hydrolysos of arbutin, esculin, urea and nitrate reduction. API (RAPID) Coryne test was done as descrined by the manufacturer (API-Coryne, BioMèrieux, France).

Mycolic acid analysis

Rhodococcus-like strains and other actinomycete-like strains were examined for the presence of mycolic acids by using thin-layer chromatography (TLC). Extraction of mycolic acids and TLC analysis of extracted mycolates were performed as previously described (Hamid et al., 1993). The presence of single-spot co-chromatographs with reference *Rhodococcus rhodochrous* strain (N54; ATCC 13808) confirmed the presence of mycolates of *Dietzia* species (Nishiuchi et al., 2000).

16S rDNA sequencing and phylogenetic analysis

Isolation of chromosomal DNA and PCR amplification of the 16S rDNA gene were carried out following the methods described by Kim et al. (1998). Obtained 16S rDNA nucleotide sequences data were tested on the BLAST electronic system (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to establish a quick phylogenetic position. Following an assignment of the isolate with *Nocardia* spp. in the BLAST system, the sequences were aligned by computer and corrected manually using PHYDIT for Windows (Version 3.1., J. Chun) and in comparison to all known sequences of *Nocardia* spp. obtained from GenBank database (http://www.ncbi.nlm.nih.gov/nucleotides). Distance estimation and tree topology was done using the neighbor-joining algorithm with the aid of TREECON for windows software (Version 3.1b, University of Antwerp, Belgium). To test the significance of the resulting tree topology, bootstrap analysis in 100 re-sampling using the TREECON program was performed.

RESULTS AND DISCUSSION

Of the 100 milk samples, five (5%) revealed rhodococcus-like red to salmon and cream yellow color colonies. The isolates (then labeled: SD1715, SD1716, SD1732, SD1743, SD1744) were gram positive cocco-bacilli, two of the five samples showed partially acid-fast coccobacilli when milk samples were directly stained with modified Ziehl Neelsen method (Figure 1). The isolates were tentatively identified as members of the genus *Rhodococcus* based on morphological, bioche-mical tests. In the present study, TLC analysis of whole cell acid methanolysates, the test strains showed major single spots mycolates which were indistinguishable on the *Rf* values from references *R. rhodochrous* (Figure 2). Therefore, the strains were considered rhodococci. Sequencing of the 16S rDNA gene of two strains indicated that the strain fall within the phylogentic branch that accommodates members of the genus *Dietzia* (Figure 3), but was well separated from the validly described *Dietzia maris* and from *D. psychralcaliphila. Rhodococcus equi* and *Dietzia* spp. are closely related actinomycetes that show similar phenotypic properties. In humans, *R. equi* is an opportunistic pathogen associated with severe immunodeficiency (Pilares et al., 2010). API (RAPID) Coryne system 2.0 was found useful for identifying the diverse group of coryneform bacteria encountered in the routine clinical laboratory (Funke et al., 1997).

Dietzia bacteria appear to be widely distributed in the environment, and reports of isolates from clinical material are increasing (Koerner et al., 2009; Von Bargen and Haas, 2009). The pathogenic role for *Dietzia* species, an

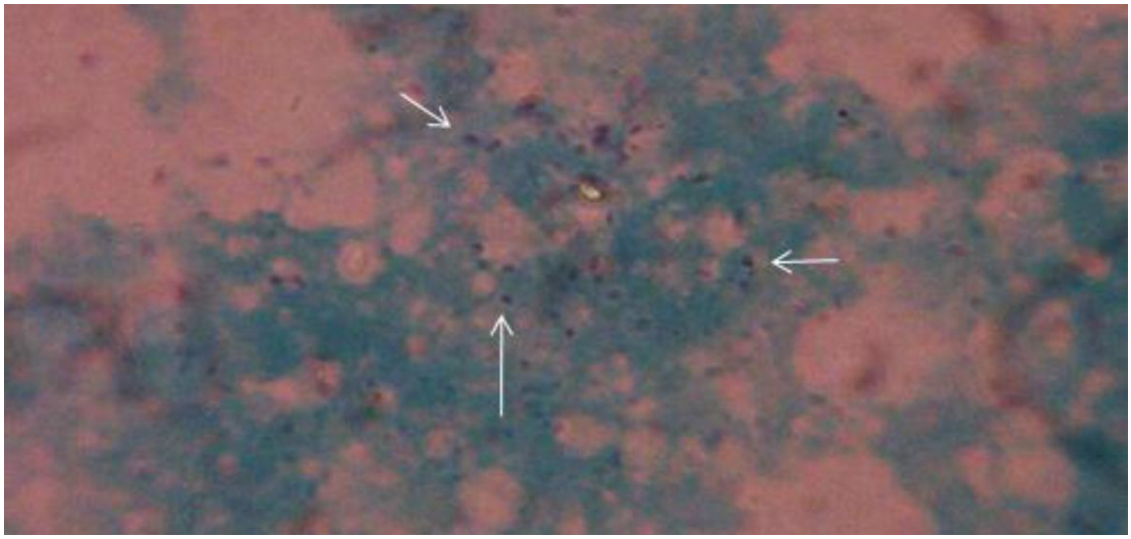

Figure 1. Modified Ziehl Neelsen stained smear from a cow milk isolates (SD1744) suffering from mastitis. Notice the presence of acid-fast short rods and coccobacilli (arrows).

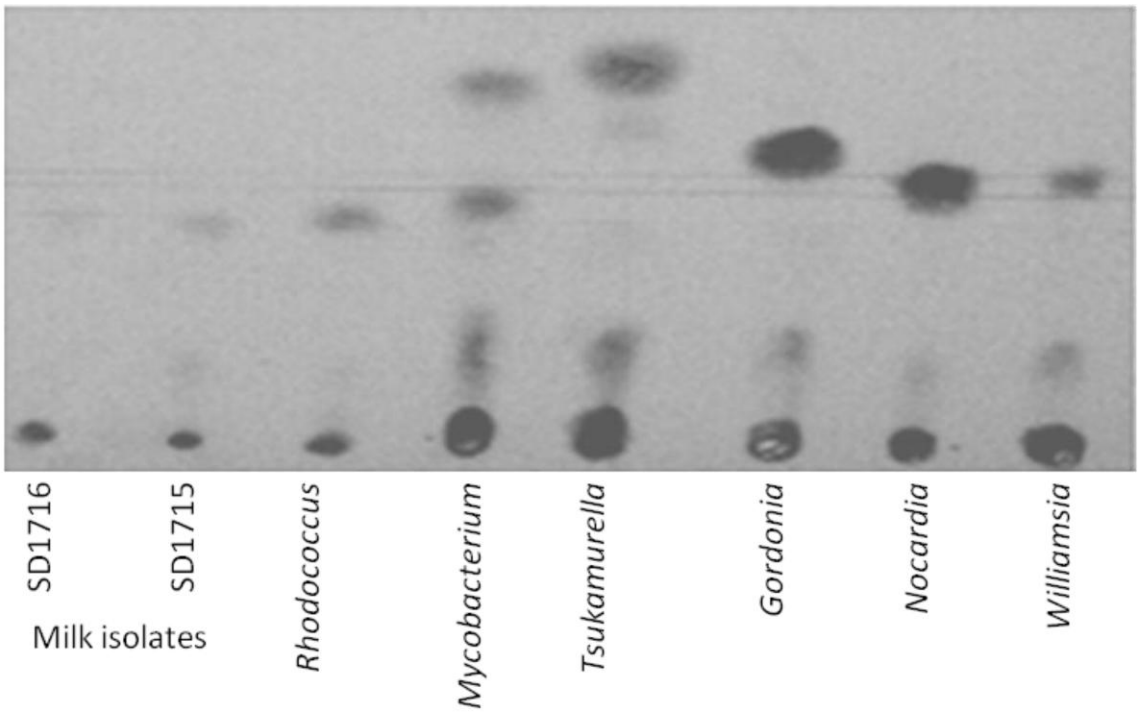

Figure 2. Thin layer chromatography of whole cell acid methanolysates of bovine mastitis isolates (SD1715, SD1716). The test strains showed major single spots mycolates that are indistinguishable on the Rf values from references *R. rhodochrous*. Developing solvent: petroleum ether: diethyl ether (95:5 v/v) run twice, dried, stained with 5% ethanolic molybdophosphoric acid and heated at 100°C for 15 min.

environmental microorganism, was supported by its presence, in the present study, on direct smears and its isolation in pure culture from milk specimens. Isolation of five *Dietzia* strains in the present research denotes a new

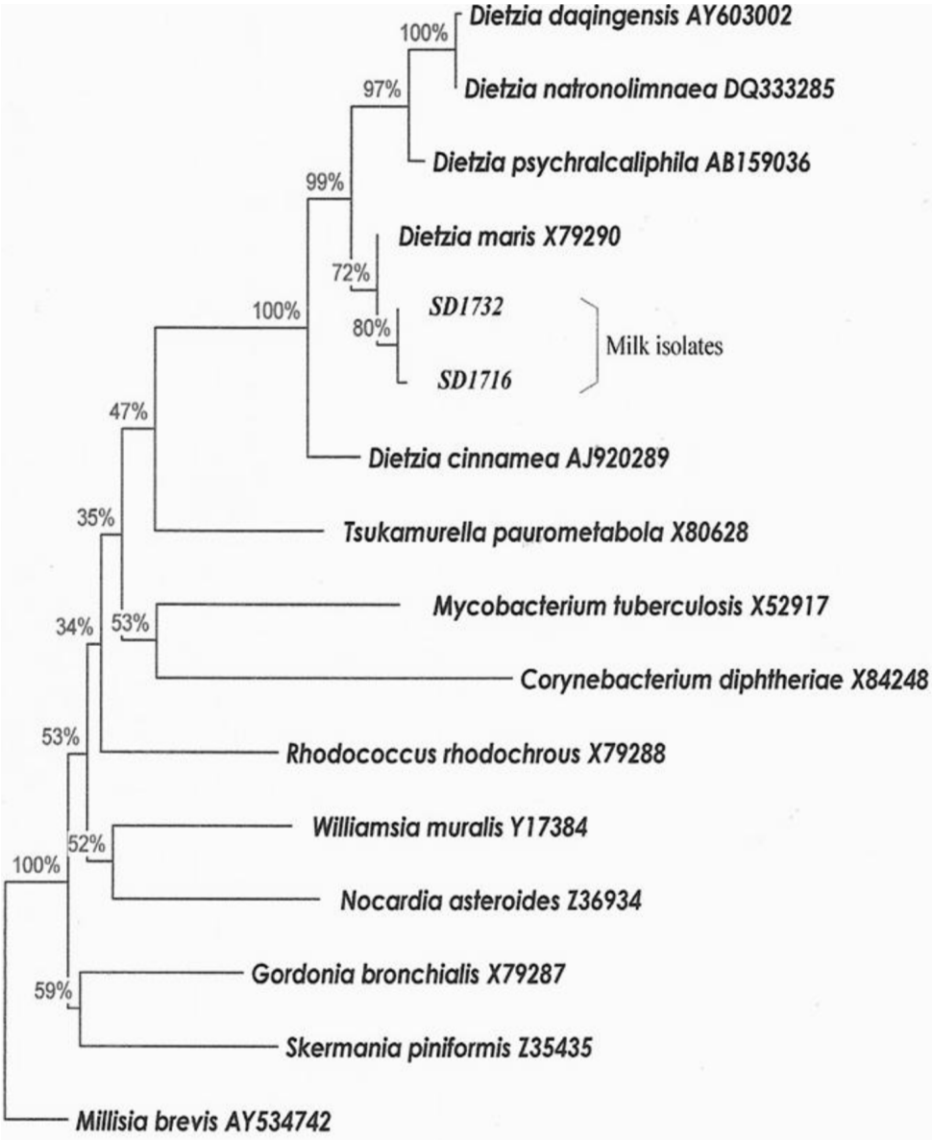

Figure 3. Phylogenetic tree based on sequences derived from 16S rDNA gene showing the relationship of two milk isolates (SD1716, SD1732) to related mycolic acids containing actinomycetes. The isolates fall within the phylogenetic branch which accommodates members of the genus *Dietzia* but are distinct from *D. maris* and from other *Dietzia* spp.

causal agent of bovine mastitis. Such results need further investigation to determine the true prevalence and risk factor leading to this type of infection. *Dietzia* spp. have emerged as an opportunistic pathogens which can cause localized as well as systemic infection in human and animals (Koerner et al., 2009; Von Bargen and Haas, 2009; Bemer-Melchior et al., 1998; Pidoux et al., 2001; Yassin et al., 2006; Jones et al., 2008; Pilares et al., 2010). None of the isolates examined in the preset study were found to belong to the validly described species so far. Their phenotypic and genotypic distinctiveness might merits independent species. Studies are underway to elucidate and complete the description of the isolates.

The genus *Dietzia* has only been established fairly recently. The Gram morphology and colony appearance of the species of this genus is remarkably similar to *R. equi.* In the absence of simple, accurate methods for their identification, *Dietzia* spp. might have been misidentified as a *Rhodococcus* spp. and/or considered contaminants only (Koerner et al., 2009). The association of coryneform

bacteria with many infections in the past might include cases due to *Dietzia* species since characterization depended on few morphological and biochemical tests. Currently, reliance on these characteristic alone could not be conclusive. The shortage of the morphological and biochemical tests in the identification of actinomycetes has been previously debated (Goodfellow, 1998; Goodfellow et al., 1998; Goodfellow and Maldonado, 2007; Beaman et al., 1995).

Conclusions

The recovery of five isolates belonging to the genus *Dietzia* in the present study represents a new significant cause of clinical mastitis in cattle. Efforts are needed to validate the detection methods and to conclude on their true prevalence in comparison to other pathogens associated with bovine mastitis.

ACKNOWLEDGEMENTS

The author is grateful to Prof. M. Goodfellow (University of Newcastle) for help with sequencing and to Mrs Safa Abdelrahman and Mr Adil Mahgoub (University of Khartoum) for help with field work. The project received supports from The British Council (KHT/991/21/Vet).

REFERENCES

Beaman BL, Saubolle MA, Wallace RJ (1995). *Nocardia, Rhodococcus, Streptomyces, Oerskovia* and other Aerobic *Actinomycetales* of Medical Importance. In: Manual of Clinical Microbiology, Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (Eds.). 6th Edn., ASM Prees, Washington D.C.

Bemer-Melchior P, Haloun A, Riegel P, Drugeon HB (1998). Bacteremia due to *Dietzia maris* in an immunocompromised patient. Clin. Infect. Dis. 29:1338-1340.

El Zubeir IEM, EL Owni OAO (2006). Seasonal variation of incidences and etiological agents of bovine mastitis in Friesian cattle in Sudan. Res. J. Anim. Vet. Sci. 1:25- 29.

Funke G, Renaud FN, Freney J, Riegel P (1997). Multicenter evaluation of the updated and extended API (RAPID) Coryne database 2.0. J. Clin. Microbiol. 35:3122-31226.

Goodfellow M, Maldonado L (2007). The Families *Dietziaceae, Gordoniaceae, Nocardiaceae* and *Tsukamurellaceae* in the Prokaryotes. In: A Handbook on the Biology of Bacteria, 3rd Edn., Vol. 3. Dworkin, M., E. Rosenberg, K.H. Schleifer and E. Stackebrandt (Eds.). Springer, New York. pp. 843-889.

Goodfellow M (1998). The Genus Nocardia Trevisan 1889, 9^{AL}. In: Topley and Wilson's Microbiology and Microbial Infections: Volume 2: Systematic Bacteriology, Balows, A. and B.I. Duerden (Eds.). 9th Edn., Edward Arnold, London, UK., ISBN-13: 9780340663172. pp. 464-489

Goodfellow M, Alderson G, Chun J (1998). Rhodococcal systematics: problems and developments. Antonie Van Leeuwenhoek 7:3-20.

Hamid ME, Minnikin DE, Goodfellow M, Ridell M (1993. Thin layer chromatographic analysis of glycolipids and mycolic acids from *Mycobacterium farcinogenes, Mycobacterium senegalense* and related taxa. Zentralbl. Bakteriology 279:354-367.

Hamid ME, Osman A, Khamis SA, Goodfellow M (2007). Bovine mastitis caused by Nocardia farcinica. Bull. Anim. Health Prod. Afr. 55:203-206.

Isik K, Chun J, Hah YH, Goodfellow M (1999). *Nocardia salmonicida* nom. rev., a fish pathogen. Int. J. Syst. Bacteriol. 49: 833-837.

Jones AL, Koerner RJ, Natarajan S, Perry JD, Goodfellow M (2008). *Dietzia papillomatosis* sp. nov., a novel actinomycete isolated from the skin of an immunocompetent patient with confluent and reticulated papillomatosis. Int. J. Syst. Evol. Microbiol. 58:68-72.

Kim SB, Falconer C, Williams E, Goodfellow M (1998) *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydotrophic species from soil. Int. J. Syst. Bacteriol. 48: 59-68.

Koerner RJ, Goodfellow M, Jones AL (2009). The genus *Dietzia*: FEMS Immunol. Med. Microbiol. 55:296-305.

Manninen KI, Smith RA, Kim LO (1993). High presumptive identification of bacterial isolates associated with the recent Canada-wide mastitis epizootic as *Nocardia farcinica.* Can. J. Microbiol. 39: 635-641.

Nishiuchi Y, Baba T, Yano I (2000). Mycolic acids from *Rhodococcus*, *Gordonia, and Dietzia*. J. Microbiol. Methods 40:1-9.

Petrovski KR, Trajcev M, Buneski G (2006). A review of the factors affecting the costs of bovine mastitis. J. S. Afr. Vet. Assoc. 77: 52-60.

Pidoux O, Aragenson JN, Jacomo V, Drancourt M (2001). Molecular identification of a *Dietzia maris* hip prosthesis infection isolate. J. Clin. Microbiol. 39:2634-2636.

Pier AC, Willers EH, Mejia MJ (1961). *Nocardia asteroides* as a mammary pathogen of cattle. I. The disease in cattle and the comparative virulence of 5 isolates. Am. J. Vet. Res. 22: 502-517.

Pilares L, Aguero J, Vazquez-Boland JA, Martinez-Martinez L, Navas J (2010). Identification of atypical Rhodococcus-like clinical isolates as *Dietzia* spp. by 16S rDNA gene sequencing. J. Clin. Microbiol. 48:1904-1907.

Quinn PJ, Carter ME, Markey BK, Carter GR (1999). The Actinomycetes. In: Clinical Veterinary Microbiology, Quinn, P.J. (Eds.). Wolfe, Baltimore. pp. 144-155.

Radostits OM, Gay CC, Hinchcliffe KW, Constable PD (2007). Veterinary Medicine, 10th ed., W.B. Saunders Co., Philadelphia, USA, ISBN: 0702029750.

Von Bargen, K. and A. Haas, 2009. Molecular and infection biology of the horse pathogen *Rhodococcus equi*. FEMS Microbiol. Rev. 33:870-891.

Yassin AF, Hupfer H, Schaal KP (2006). *Dietzia cinnamea* sp. nov., a novel species isolated from a perianal swab of a patient with a bone marrow transplant. Int. J. Syst. Evol. Microbiol. 56:641-645.