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A REVIEW

The genus *Rhodococcus*

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1. Introduction, 195
2. Classification
   2.1 Taxonomic history of *Rhodococcus*, 195
   2.2 Resolving *Rhodococcus* classification, 196
      2.2.1 Numerical taxonomy, 196
      2.2.2 Chemotaxonomy, 196
      2.2.3 Molecular taxonomy, 196
3. Significance of *Rhodococcus*
   3.1 Introduction, 197
   3.2 *Rhodococcus* and environmental biotechnology, 197
      3.2.1 Bioremediation and biodegradation of pollutants, 197
      3.2.2 Biosurfactants and bioflocculants, 198
      3.2.3 Desulphurization of fossil fuels, 199
      3.2.4 Oil prospecting, 199
      3.2.5 *R. coprophilus* as a faecal indicator organism, 199
      3.2.6 Foam formation in activated sludge plants, 199
   3.3 Other applied aspects of *Rhodococcus*, 199
      3.3.1 Industrial syntheses and transformations, 199
      3.3.2 Biosensors, 200
      3.3.3 Miscellaneous applications, 200
   3.4 *Rhodococcus* and infection, 200
      3.4.1 *R. equi* infections in horses and other animals, 200
      3.4.2 *R. equi* infections in humans, 200
      3.4.3 Virulence of *R. equi*, 201
      3.4.4 Human infections by other rhodococci, 202
      3.4.5 *R. fascians* infections in plants, 202
      3.4.6 *R. rhodnii* and trypanosomiasis, 202
4. Identification of *Rhodococcus*
   4.1 Identification to genus level, 202
   4.2 Identification to species level, 202
      4.2.1 Overview, 202
      4.2.2 Growth characteristics and biochemical testing, 202
      4.2.3 Analysis of cellular components, 203
      4.2.4 Serological tests, 203
      4.2.5 Nucleic acid-based tests, 203
   4.3 Importance of identifying rhodococci, 203
5. Concluding remarks, 204
6. References, 204

1. INTRODUCTION

The actinomycete genus *Rhodococcus* is of interest for a variety of reasons. The metabolic abilities of many species means that they can degrade a range of environmental pollutants and transform or synthesize compounds with possible useful applications. There has been increased interest in infections caused by rhodococci in humans, animals and plants. The classification of the genus has changed dramatically in recent years, with species being reclassified and new species described.

This review summarizes recent research into *Rhodococcus* species (updating the extensive review of Finnerty (1992)), with special reference to taxonomic changes, improvements in identification, their importance in industrial and environmental biotechnologies and their role as pathogens of humans, other animals and plants.

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2. CLASSIFICATION

2.1 Taxonomic history of *Rhodococcus*

The genus name ‘*Rhodococcus*’, first used by Zopf in 1891, was revived and redefined in 1977 to accommodate the ‘*rhodochrous*’ complex which comprised a number of strains that resembled but did not belong to the established genera *Nocardia*, *Corynebacterium* and *Mycobacterium* (Goodfellow and Alderson 1977). Rhodococci are described as aerobic, Gram-positive, non-motile, mycolate-containing, nocardioform

actinomycetes (Goodfellow 1989a). The term ‘nocardioform’ is morphologically descriptive and refers to mycelial growth with fragmentation into rod-shaped or coccoid elements (Lechevalier 1989).

Recent years have seen considerable changes made to the classification of the genus. Since the publication of Bergey’s *Manual of Systematic Bacteriology* which listed 20 species, including four designated *incertae sedis* (Goodfellow 1989a), some species have been combined, transferred to other established genera or reclassified in new genera, and new species have been described. *Rhodococcus obuensis* was determined to be synonymous with *R. sputi,* making the former name obsolete (Zakrzewska-Czerwinska *et al.* 1988). Two new genera were established to accommodate organisms previously regarded as rhodococci: *R. auranticus* was transferred to *Tsukamurella,* while *R. bronchialis*, *R. rubropertinctus, R. sputi* and *R. terrae* were transferred to *Gordona* (Collins *et al.* 1988; Stackebrandt *et al.* 1988). This left 13 species listed in the review of Finnerty (1992) and *R. chubuensis,* not listed in this review but regarded as a species *incertae sedis* by Goodfellow (1989a). Subsequently, *R. chlorophenolicus* was transferred to *Mycobacterium* (Briglia *et al.* 1994a; Häggblom *et al.* 1994); *R. aichiensis* and *R. chubuensis* were transferred to *Gordona* (Klatte *et al.* 1994a; Riegel *et al.* 1994); *R. luteus* was determined to be a synonym of *R. fascians* (Klatte *et al.* 1994b) and *R. maris* was transferred to the new genus *Dietzia* as *D. maris* (Rainey *et al.* 1995a). The new species *R. roseus, R. zopfii, R. opacus* and *R. percolatus* were described (Tsukamura *et al.* 1991; Stoecker *et al.* 1994; Klatte *et al.* 1994c; Briglia *et al.* 1996), but *R. roseus* has more recently been shown to be a member of the species *R. rhodochrous* (Rainey *et al.* 1995b).

Thus, there are currently 12 established *Rhodococcus* species, namely *R. coprophilus*, *R. equi*, *R. fascians, R. erythropolis, R. globerulus, R. marinonascens, R. opacus, R. percolatus, R. rhodnii, R. rhodochrous, R. ruber* and *R. zopfii.* Some strains bearing other generic and specific names, such as *Arthrobacter picolinophilus, Corynebacterium hoagii, Nocardia restricta* and *Nocardia calcarea* have been shown recently to belong to established *Rhodococcus* species, and *Nocardia corynebacteroides* also appears to be a *Rhodococcus* but does not belong to any of the currently valid species (Koch *et al.* 1995; Ruimy *et al.* 1995; Rainey *et al.* 1995c). Other unvalidated species names such as *R. longus* (Ivshina *et al.* 1994), *R. minimus* (Tikhonova *et al.* 1996) and *R. luganensis* (de Clari *et al.* 1992) occur in the literature, while there are many strains not identified to species level (Young and McFarlane 1994).

Rhodococcus has been demonstrated to be phylogenetically grouped in a coherent clade whose members are exclusively mycolate-containing genera. Indeed, these are the only genera to possess mycolic acid in the cell wall (Embley and Stackebrandt 1994). The term ‘the CMN group’ was coined to refer to the genera *Corynebacterium, Mycobacterium* and *Nocardia* but can be expanded to include the genera *Rhodococcus, Gordona, Tsukamurella* and *Dietzia* (Ruimy *et al.* 1994). Two more genera have been proposed which would also lie within this clade: *Turicella,* created for a novel clinical isolate, *Turicella otitidis* (Funke *et al.* 1994), and *Skermania,* created for the organism formerly known as *Nocardia pinensis* (Chun *et al.* 1997). The validity of *Turicella* has been disputed, however, by Ruimy *et al.* (1995) who suggest that *T. otitidis* belongs in *Corynebacterium*. Different classifications at family level have been proposed. It has been suggested that *Rhodococcus, Gordona, Tsukamurella* and *Nocardia* should be grouped in the family Nocardiaceae (Goodfellow 1992) and more recently, that these genera should be grouped with *Mycobacterium* in the family Mycobacteriaceae, while *Corynebacterium, Dietzia* and *Turicella* make up the family Corynebacteriaceae (Chun *et al.* 1996).

2.2 Resolving *Rhodococcus* classification

2.2.1 Numerical taxonomy. The revived genus *Rhodococcus* was originally proposed on the basis of a study of actinomycetes which examined test strains for 92 unit characters and grouped them according to the percentage similarity (Goodfellow and Alderson 1977). The characteristics examined included the following: staining and morphology; nutritional tests; tolerance to inhibitory compounds; resistance to various antibiotics; the ability to grow under various conditions of temperature and pH; and chemical analyses. Later, similar numerical taxonomic studies included additional tests, in particular the use of fluorogenic substrates to detect specific enzyme activities (Goodfellow *et al.* 1990).

2.2.2 Chemotaxonomy. Chemical characteristics that define the rhodococci were described by Goodfellow (1989a) and updated by Finnerty (1992). They have cell walls of chemotype IV, which means the only diamino acid in the peptidoglycan is *meso*-diaminopimelic acid and that the major sugars are arabinose and galactose (Lechavalier and Lechavalier 1970). Although the other genera in the CMN group also have cell wall chemotype IV (Goodfellow 1989b), *Rhodococcus* can be separated from them on the basis of the combination of chemotaxonomic characteristics described by Rainey *et al.* (1995a). Key diagnostic characteristics for *Rhodococcus* are: the possession of tuberculostearic acid; mycolic acids with lengths of between 34 and 64 carbon atoms; and that the major menaquinone type is dihydrogenated menaquinones which have eight isoprenoid units but which lack the cyclic element that is a motif characteristic of *Nocardia*. The relative proportions of various lipids (mycolate and other fatty acids) may be useful to differentiate species within the genus (Klatte *et al.* 1994c).

2.2.3 Molecular taxonomy. The most powerful tool in ana-

lysing and reorganizing *Rhodococcus* taxonomy in recent years has been sequence analysis of the 16S rRNA gene. The degree of variation in the sequence between two given organisms can be taken as a measure of how closely related the organisms are. Bacteria with 16S rDNA similarities of 97% and above may be the same species (Stackebrandt and Goebel 1994). Where there is doubt, DNA–DNA re-association values can be obtained; a homology value of 70% is deemed to be the minimum for species identity (Wayne *et al.* 1987).

16S rDNA sequences are now available for all validly described *Rhodococcus* species and DNA–DNA re-association experiments have been performed where necessary (Rainey *et al.* 1995c; Ruimy *et al.* 1995; Briglia *et al.* 1996). Therefore, it is probable that all the current species are stable although there may be some doubt about the status of *R. percolatus* which has 99·3% 16S rDNA sequence identity and 71% DNA–DNA homology with *R. opacus*, but the two species can be separated on the basis of nutritional phenotype and fatty acid composition (Briglia *et al.* 1996). The study of Rainey *et al.* (1995c) showed *Rhodococcus* to be a relatively divergent genus falling into five distinct groups. One group contains *R. erythropolis* with *R. globerulus, R. marinonascens* and *R. opacus,* a second contains *R. rhodochrous* with *R. ruber* and *R. coprophilus,* while *R. rhodnii*, *R. fascians* and *R. equi* each lie on their own distinct branches of the phylogenetic tree. These workers also concluded that the genus *Nocardia* has evolved from within *Rhodococcus* rather than alongside it. Later, Briglia *et al.* (1996) showed that *R. zopfii* lies in the *R. rhodochrous* group while *R. percolatus* lies in the *R. erythropolis* group. Phylogenies may also be inferred from other molecular sequences. Ochi (1995) analysed the sequence of ribosomal AT-L30 proteins and found generally good agreement with the phylogenies obtained from 16S rDNA analyses and most strikingly, evidence that the genus *Nocardia* forms a clade within *Rhodococcus.*

3. SIGNIFICANCE OF *RHODOCOCCUS*

3.1 Introduction

Rhodococci have been isolated from a large variety of sources including soils, rocks, boreholes, groundwater, marine sediments, animal dung, the guts of insects and from healthy and diseased animals and plants (Goodfellow 1989a; Ivshina *et al.* 1994).

The commercial potential of *Rhodococcus* species is increasingly recognized. The wide range of chemicals transformed or degraded by rhodococci makes them actually or potentially useful in environmental and industrial biotechnology, as does their ability to synthesize several products such as surfactants, flocculants, amides and polymers. This increasing interest is reflected in patenting. Finnerty (1992) lists 10 patents relating to rhodococci up to 1990, Warhurst and Fewson (1994) report that a further 20 patent families were submitted in the following two years, while from 1993 to November 1996, a further 80 patents were submitted to the World Patent Index.

Useful phenotypic traits from *Rhodococcus* species may be transferred to other organisms by genetic manipulation. Recent years have seen the development of improved cloning vectors that allow the transfer of genes between different rhodococci and between rhodococci and *Escherichia coli* (Denome *et al.* (1994); Shao *et al.* 1995).

Rhodococci also cause diseases of animals, plants and humans and the overall significance of human infection is increasing, both in terms of the actual occurrence of infection and in the extent to which it is recognized (Finnerty 1992; McNeil and Brown 1994).

3.2 *Rhodococcus* and environmental biotechnology

3.2.1 Bioremediation and biodegradation of pollutants. The capacity of rhodococci to degrade substituted hydrocarbons and other chemicals means that they may play a significant role both in the natural degradation of such compounds and in bioremediation. Rhodococci may be naturally present in contaminated environments and are promising candidates for inocula for bioremediation for a number of reasons. Warhurst and Fewson (1994) comment that rhodococci can persist in soil, even in starvation conditions, and that breakdown of pollutants may not be adversely affected by the presence of more easily assimilable carbon sources. *Rhodococcus* cells are hydrophobic because of the aliphatic chains of mycolic acids in the cell wall; this may allow degradation of hydrophobic pollutants by allowing cells to adhere to oil/water interphases (Neu 1996). There is a striking partitioning of *Rhodococcus* cells into the oil phase when cultured in a flask with an aqueous mineral medium and a liquid hydrocarbon as the carbon source. Some *Rhodococcus* strains are psychrotrophic which may be important for bioremediation in cold climates (Koronelli 1996; Yagafarova and Skvortsova 1996).

The limited number of field or soil microcosm studies of bioremediation using *Rhodococcus* and related organisms as inocula have shown some promise. Briglia *et al.* (1994b) obtained much increased rates of degradation of pentachlorophenol (PCP) in sandy soil inoculated with *R. chlorophenolicus* (now reclassified as a *Mycobacterium*). Miethling and Karlson (1996) used this organism as an inoculum and found increased rates of degradation of PCP in some conditions, and that high numbers of the bacterium persisted in the soil, even after several months. Christofi *et al.* (1998) found that inoculation with *R. ruber* increased counts of hydrocarbon-oxidizing bacteria persisting in composted soil contaminated with crude oil. Sorkhoh *et al.* (1995) took a naturally occurring bacterial consortium (removed from cyanobacterial mats found floating in oil-polluted waters in the Arabian Gulf) and used this mixture to inoculate oil-

contaminated sand. This increased removal of the oil from the sand and rhodococci appeared to predominate in the microbial population. Koronelli (1996) reported that introduction of a hydrocarbon-degrading strain of *R. erythropolis* into artificially contaminated soil plots resulted in increased counts of hydrocarbon degrading bacteria and an increased rate of hydrocarbon degradation.

Bioreactors to degrade toxic compounds in industrial effluents may also be developed in order to prevent pollution occurring. Indigenous *Rhodococcus* spp. may be important in such systems and indeed, *R. percolatus* was first isolated from a chlorophenol-fed bioreactor (Briglia *et al.* 1996). Rhodococci are also candidate organisms for use as inocula (possibly as immobilized cells) in such treatments, having shown promise in laboratory simulations (Valo *et al.* 1990; Feakin *et al.* 1995; Pai *et al.* 1995; Arnold *et al.* 1996).

Chemical pollutants which can be degraded by rhodococci range from simple hydrocarbons, which are used as sole carbon sources in the isolation of rhodococci (Goodfellow and Minnikin 1981), through chlorinated hydrocarbons, aromatic hydrocarbons and nitroaromatics to chlorinated polycyclic aromatics such as polychlorinated biphenyls (PCBs). The reader is referred to the extensive review of Warhurst and Fewson (1994), but other studies have been made since, as referenced below.

A class of chemicals of particular concern is chlorinated phenols which are recalcitrant to degradation and can be hazardous and persistent pollutants in soil and groundwater (Häggblom *et al.* 1989). A number of studies on the degradation of these compounds by rhodococci have been carried out (Apajalahti *et al.* 1987; Häggblom *et al.* 1989; Middledorp *et al.* 1990; Briglia *et al.* 1996) and although the organism most studied was *R. chlorophenolicus* (now *M. chlorophenolicus*), other *Rhodococcus* strains can also degrade chlorophenols.

Polychlorinated biphenyls have been used industrially for a variety of purposes, partly because of their stability. This means that they are persistent in the environment and they are considered to be particularly hazardous. Various PCBs can be degraded by strains of *R. rhodochrous, R. globerulus*, *R. erythropolis* and other unclassified rhodococci (Boyle *et al.* 1992; Asturias and Timmis 1993; Maeda *et al.* 1995; Seto *et al.* 1995).

A variety of other recalcitrant, toxic pollutants that can be degraded by rhodococci have been studied. These include sulphonated azo dyes (Heiss *et al.* 1992) and pesticides such as metamitron (Parekh *et al.* 1994), s-triazines (Mulbry 1994) and *n*-methyl carbamates (Behki *et al.* 1994).

Another aspect of bioremediation is the bioaccumulation of heavy metal ions including those that are radioactive. Microorganisms may play a significant role in their removal from effluent streams, particularly if immobilized in a polymeric matrix. Two *Rhodococcus* strains were found to accumulate high levels of caesium ions (5% and 9% of the dry biomass) (Tomioka *et al.* 1994).

3.2.2 Biosurfactants and bioflocculants. Associated with the biodegradative abilities of *Rhodococcus* is surfactant production (Finnerty 1992). Surfactant molecules contain both strongly hydrophilic and strongly hydrophobic groups and so migrate to interfaces between oil and aqueous phases. The relevance of biosurfactants to biodegradation of pollutants is threefold. Firstly (as discussed previously), cellular surfactants, such as mycolic acids, cause adherence of rhodococci to hydrophobic phases in two phase systems (Neu 1996). Secondly, surfactants lower the interfacial tension between the phases, making it easier for hydrophobic compounds to enter microbial cells (Fiechter 1992). Thirdly, extracellular surfactants disperse hydrophobic compounds, thus increasing the surface area for microbial attack (Finnerty 1994). It has been reported that some biosurfactants, including some of those from rhodococci, are more effective and efficient than many existing synthetic surfactants and may be more biodegradable and less toxic (Finnerty 1994; Philp and Bell 1995).

Purification of biosurfactants may be expensive so applications in bioremediation or with crude oil (e.g. cleaning oil tanks, recovery of oils for reuse, or enhancing oil recovery from natural deposits) may be more economically favourable because crude preparations could be used. Rhodococci may produce biosurfactants at high enough levels to allow the use of whole broths in some oil industry applications (Abu-Ruwaida *et al.* 1991).

Research has continued into structural analysis of rhodococcal biosurfactants (Neu *et al.* 1992) and determination of optimum conditions for their production (Espuny *et al.* 1996). The most studied species is *R. erythropolis* which produces trehalose-containing glycolipids as well as trehalose tetraesters (Finnerty 1992). *Rhodococcus ruber* produces surfactants different to those from *R. erythropolis* (Philp and Bell 1995) while *R. percolatus* also produces extracellular surfactant (Briglia *et al.* 1996). Surfactants from one *R. ruber* strain greatly enhanced oil removal from soil in laboratory soil washing tests (Christofi *et al.* 1998). Mercade *et al.* (1996) isolated rhodococci able to grow and produce surfactants while using waste oil as a sole carbon source, thus reducing production costs.

Rhodococcus erythropolis can produce bioflocculant material with an efficient activity in causing flocculation of a wide range of suspended solids (Kurane and Tomizuka 1992). The flocculant consists of assemblies of polypeptide and lipid, especially mycolate-containing glycolipids (Kurane *et al.* 1994; Kurane *et al.* 1995). Such materials could aid removal of suspended solids in a waste water or effluent treatment. The biodegradability of such a product would again be important.

3.2.3 Desulphurization of fossil fuels. Microbial desulphurization of coal and petroleum has been suggested as a means of preventing sulphurous emissions from combustion, reducing the associated problem of acid rain and increasing the fuel value (Kilbane 1989; Gray *et al.* 1996). The removal of organic sulphur from fuel is difficult. Research from several groups in the USA, Japan and elsewhere has focused on the potential of *Rhodococcus* spp. in this area (Kayser *et al.* 1993; Denome *et al.* 1994; Izumi *et al.* 1994; Wang and Krawiec 1994; Bozdemir *et al.* 1996). Much of the research into the enzymology and genetics of *Rhodococcus* desulphurization reactions has been carried out by Energy Biosystems Corporation (USA). A particular point of note is that carbon-sulphur bonds in model compounds such as dibenzothiophene are cleaved but carbon–carbon bonds remain intact. This selectivity, apparently so far unique to *Rhodococcus,* is significant because it means that fuel could be desulphurized without affecting the calorific value. However, major economic and technological problems are apparent for the operation of large-scale coal slurry or oil and water bioreactors for desulphurization, especially when using alkanotrophic bacteria (Shennan 1996).

3.2.4 Oil prospecting. Rhodococci are often isolated from environments where hydrocarbons are present. Some strains are able to grow using gaseous hydrocarbons (such as propane, butane and acetylene) as a sole carbon source (Woods and Murrell 1990; Ivshina *et al.* 1994; Young and McFarlane 1994; Rosner *et al.* 1997). Detection or quantification of rhodococci able to oxidize gaseous alkanes in soil or groundwater could be indicative of the presence of subterranean hydrocarbon deposits and thus be of use in oil prospecting (Ashraf *et al.* 1994). One study found large numbers of propane and butane degrading isolates in groundwater associated with an oil-bearing site, but none from a non-productive site (Ivshina *et al.* 1981).

3.2.5 Rhodococcus coprophilus *as a faecal indicator organism.* *Rhodococcus coprophilus* grows in animal dung and it has been suggested that it could be used as an indicator organism to assess pollution of waterways by farm animal effluents (Goodfellow and Minnikin 1981). Recent research has shown that this could be a useful approach (Jagals *et al.* 1995).

3.2.6 Foam formation in activated sludge plants. Rhodococci can contribute to formation of thick surface foams in activated sludge waste-water treatment plants, causing operational problems (Blackall 1994). This phenomenon is apparently increasingly common and was reported in the majority of treatment plants in recent surveys (de los Reyes *et al.* 1997). Surface foams can reduce oxygen transfer, cause higher effluent biochemical oxygen demand, lead to greater amounts of suspended solids (including pathogens) in effluents, and provide a medium for the wind-assisted dispersal of pathogens (Schuppler *et al.* 1995; Tipping 1995; Goodfellow *et al.* 1997). Hydrophobic flocs of rhodococci, nocardiae, gordonae etc. may associate with air bubbles, causing a foam. The importance of the hydrophobic cell surface in foam formation was demonstrated by Sunairi *et al.* (1997) while Foot *et al.* (1993) showed that the filamentous matrix produced by nocardioforms can trap air bubbles. The relative significance of different nocardioforms in foaming varies, but rhodococci seem to be significant in some cases (Blackall 1994; Fougias and Forster 1994) and perhaps especially at low temperatures (Soddell and Seviour 1995).

3.3 Other applied aspects of *Rhodococcus*

3.3.1 Industrial syntheses and transformations. *Rhodococcus* species have been shown to produce a number of commercially interesting and potentially useful products.

Rhodococcus rhodochrous J1 is used by the Nitto Chemistry Industry Company Ltd (Japan) to produce over 30 000 tons of acrylamide annually (Yamada and Kobayashi 1996). This is said to be the first instance of successful industrial production of a commodity chemical using a microbe. An overproduced nitrilase enzyme is used. Research has continued into the application of nitrilases (especially from *Rhodococcus*) to produce a range of other products such as acrylic acid and various amides, including the vitamins nicotinamide and p-aminobenzoic acid and the antimycobacterial agents isonicotinic acid hydrazide and pyrazinamide (Kobayashi and Shimizu 1994; Yamada and Kobayashi 1996). These conversions show high yields and high specificity and have considerable potential for industrial application. Nitrilases in *R. rhodochrous* J1 are genetically coupled with amidases which could be used for further transformations (Kobayashi *et al.* 1993). *Rhodococcus* nitrilase genes have been cloned and expressed in *E. coli* (Kobayashi *et al.* 1993). Genetic probes have been developed to screen for nitrilase genes in other strains (Duran *et al.* 1993).

The production of poly(3-hydroxyalkanoic) acids by *R. ruber* has been studied (Pieper and Steinbuchel 1992). One such compound, a copolyester of 3-hydroxybutyrate and 3-hydroxyvalerate (3HB-co3HV), is produced commercially as a biodegradable plastic – "BIOPOL" – by Zeneca Bio Products using the bacterium *Alcaligenes eutrophus* (Lee 1996). *Rhodococcus ruber* can produce 3HB-co3HV with a different proportion of monomers while growing on cheaper substrates than those used for BIOPOL production.

A range of other potentially useful transformations using *Rhodococcus* cells or enzymes has been described. Woods and Murrell (1990) report the transformation of gaseous alkenes into epoxides by a *Rhodococcus* culture and epoxides are of

use in ferro electric liquid crystals (Kieslich 1991). A novel and efficient biotransformation producing sec-cedrenol, a compound with potential medical value, has been described (Takigawa *et al.* 1993). Ludwig *et al.* (1995) identified a secondary alcohol dehydrogenase with unusual specificity for long-chain alcohols, and this could be used to produce specific stereoisomers of secondary alcohols. Peters *et al.* (1993) report possible synthetic uses of carbonyl reductases from *Rhodococcus* to give a range of compounds that can be used for synthesis of pharmaceuticals and agrochemicals. Muconic acids can be produced by a strain of *R. rhodochrous* (Warhurst *et al.* 1994). Cholesterol oxidases from *Rhodococcus* have been studied and could have applications in the food industry or in steroid drugs' production (Finnerty 1992; Christodoulou *et al.* 1994; Kreit *et al.* 1994). Efficient production of the amino acids L-leucine and L-phenylalanine using *Rhodococcus* enzymes has been reported (Hummel *et al.* 1987; Bhalla *et al.* 1992).

3.3.2 Biosensors. There are various reports on the incorporation of *Rhodococcus* cells or enzymes into biosensors for compounds such as substituted phenols and hydrocarbons (Riedel *et al.* 1993; Beyersdorfradeck *et al.* 1994; Peter *et al.* 1996). Biosensors could allow rapid detection of target compounds and an assessment of their bioavailability (which is relevant for environmental toxicity testing) (Selifonova and Eaton 1996). Given the ability of *Rhodococcus* species to degrade many pollutants and other compounds, they offer a rich source of enzymes that may be of use in biosensors. Biosensors can also have other applications. A heroin esterase from a *Rhodococcus* strain has been studied (Cameron *et al.* 1994) and UK Customs and Excise have funded research into development of a biosensor for heroin detection (Coghlan 1994). The possibility of using a *Rhodococcus* phenylalanine dehydrogenase in a biosensor to screen for phenylketonuria has been discussed (Hummel 1997).

3.3.3 Miscellaneous applications. Suggestions have been made for the use of rhodococci in the food industry. In addition to cholesterol oxidases from rhodococci (see above), *R. fascians* has been investigated for its ability to degrade limonin, a bitter tasting compound found in fruit juice. The possibility of improving the flavour of bitter fruit juices using *R. fascians* in bioreactors has been investigated (Iborra *et al.* 1994; Marwaha *et al.* 1994).

The strong coloration of many *Rhodococcus* colonies has lead to interest in the pigments. A small *Rhodococcus* pigment gene has been expressed in *E. coli* and it has been proposed that the gene could be used as a reporter gene to allow rapid identification of colonies of transformed bacteria (Hart *et al.* 1990).

The *Rhodococcus* strain GIN-1 has been studied for its potential use in purifying titanium-containing components from coal fly ash (Shabati and Fleminger 1994). The process investigated is a biomagnetic extraction where the bacteria are bound to both magnetite and TiO_2 and can therefore be used to purify the latter from the ash. A combined chemical-biotechnological treatment system has been described (Shabati and Mukmenev 1996).

3.4 *Rhodococcus* and infection

3.4.1 Rhodococcus equi *infections in horses and other animals.* *Rhodococcus equi* (formerly *Corynebacterium equi*) has been recognized as a pathogen of foals for more than 70 years (Prescott 1991). Although *R. equi* infections sometimes occur in adult horses, foals are the only animals in which infection is common in otherwise healthy individuals. The organism is widespread in soil and in the guts of herbivores. Herbivore dung provides a good growth medium for the bacterium and so keeping foals in crowded conditions may increase the likelihood of exposure to an infective dose of *R. equi* (McNeil and Brown 1994). Outbreaks of infection in a stud farm can be expensive. This is reflected in the funding of recent research by the Japan Race Horse Association (Takai *et al.* 1996a).

In the foal, *R. equi* infections are normally respiratory (Prescott 1991). The symptoms are fever and general respiratory distress. Usually, chronic pus-filled lung abscesses develop and *R. equi* is one of the most common causes of lung abscesses in foals (Lavoie *et al.* 1994). Untreated lesions can progress and cause death by asphyxiation. Infection can disseminate from the lungs to the gut lining (causing diarrhoea), to other organs and to the joints. Vertebral osteomyelitis can also occur (Prescott 1994). In addition to respiratory infection, ingestion or introduction of the organism into cuts can cause intestinal or wound site ulcers.

Rhodococcus equi can also cause disease, usually respiratory infections, in a wide range of other animals (Prescott 1991). Such infections are generally rare, with submaxillary lymphadentitis in pigs being the most common manifestation.

3.4.2 Rhodococcus equi *infections in humans.* In recent years, the role of the organism as a human pathogen has been noted with increasing frequency. The increase is associated with the rise in the number of immunosuppressed individuals, especially those infected with human immunodeficiency virus (HIV). McNeil and Brown (1994) reported more than 100 documented cases in acquired immune deficiency syndrome (AIDS) patients since the infection was first observed in such individuals in 1986, and they suggested that *Rhodococcus* infections can provide a useful indicator of the onset of AIDS in HIV-positive individuals because these infections may precede other indicator conditions. More

recently, there have been numerous reports of other cases, mostly AIDS-associated (Scott *et al.* 1995; Arlotti *et al.* 1996; Donisi *et al.* 1996; Yuoh *et al.* 1996).

Individuals with other immunosuppressive conditions or undergoing immunosuppressive therapy have also been infected. This category includes patients with conditions such as tumours, leukaemia, lymphoma and alcoholism, those undergoing corticosteroid therapy, and those having had kidney, heart and liver transplants (Harvey and Sunstrum 1991; Prescott 1991; McNeil and Brown 1994; Segovia *et al.* 1994; Sabater *et al.* 1996). There are also rare reports of *R. equi* infections in non-immunocompromized individuals; these may be associated with deep, penetrating wounds (Prescott 1991; Walsh and Cunha 1994).

As with equine infections, human infections are usually in the lung, causing pneumonia and abscesses (McNeil and Brown 1994), and the presenting symptoms are often fever, cough and chest pain (Harvey and Sunstrum 1991). However, the infection can disseminate to cause lesions in other organs or bacteraemia (Prescott 1991; McNeil and Brown 1994). The formation and growth of such lesions means that *R. equi* infections are very serious, and often fatal, both in AIDS and non-AIDS patients (Harvey and Sunstrum 1991). Even with quick diagnosis and appropriate treatment, mortality rates in AIDS patients can be as high as 55% (About *et al.* 1996).

Treatment of *R. equi* infections can be difficult and requires judicious choice of antibiotics, probably in combination, and therapy may be required for prolonged periods to avoid relapse (Mascellino *et al.* 1994). Antibiotics effective against *R. equi in vitro* may not be effective *in vivo* (Colebunders *et al.* 1996). Resistance to beta-lactam antibiotics is apparently common (Prescott 1991).

There is evidence linking *R. equi* infections in humans with exposure to livestock, manure and other animal sources, although in many cases there is no known history of exposure to animals (Prescott 1991; Harvey and Sunstrum 1991). Takai *et al.* (1996b) have found avirulent strains of *R. equi* to be widespread in soil and sand in public areas in Japan.

3.4.3 Virulence of Rhodococcus equi. The pathogenicity of *R. equi* is dependent on its ability to evade the immune response by existing and indeed, multiplying, inside macrophages and it is similar in this respect to some of its more notable pathogenic relatives in the genera *Corynebacterium* and *Mycobacterium* (Prescott 1991). The bacterium can apparently inhibit phagosome–lysosome fusion. Virulence, however, is not well understood as the significance of possible virulence factors is not clear and it is not known why virulence and host-range vary between strains. Significant progress is, however, being made to elucidate these matters.

Prescott (1991) suggested that polysaccharide capsules might allow the organism to evade phagocytosis and that extracellular cholesterol oxidase and phospholipase C might allow lysis of host cells. Recent research has suggested that cholesterol oxidase is not significant but phospholipase C and lecithinase are (Smola *et al.* 1994). The lipid composition of the bacteria is also likely to be important. Certain mycolic acids are associated with virulence in *Nocardia* and *Mycobacterium* and there is some evidence that varying mycolate patterns in *R. equi* are related to varying virulence while purified *R. equi* glycolipid can cause lesions in mice (McNeil and Brown 1994).

Research by both Takai *et al.* (1991) and Tkachuk-Saad and Prescott (1991) indicates a connection between virulence associated antigens and the presence of plasmids in strains of *R. equi* virulent for horses and pigs. It has been shown that almost all isolates from infected foals carry an 85 kb virulence plasmid that encodes for externally displayed virulence antigens of size 15 kDa and 17·5 kDa, and that these strains are virulent for mice (Takai *et al.* 1992; Takai *et al.* 1993). Tan *et al.* (1995) investigated three antigens (15 kDa, 17·5 kDa and 18–22 kDa) and showed that all were encoded for by the same plasmid-borne gene (designated the *vapA* gene) and that the two larger antigens were lipid-modified forms of the smaller, protein-only antigen. However, virulence plasmids do not seem to be essential for the organism to cause infection in (immunosuppressed) humans. Takai *et al.* (1994a) found that most isolates (31 out of 35) of *R. equi* from immunocompromized humans did not contain the 85 kb virulence plasmid and were not virulent for mice. Strains isolated from some AIDS patients, and found to be of intermediate virulence for mice, carried one of several plasmids expressing only a 20 kDa antigen (Takai *et al.* 1995a). Takai *et al.* (1996c) suggested that these strains are associated with porcine rather than equine infections. Vullo *et al.* (1996) found that humans infected with *R. equi* did not produce antibodies to the 15 and 17 kDa antigens but did produce antibodies against a 60 kDa protein.

There has been some progress made in the further understanding of the role of these virulence plasmids in infection. Hondalus and Mosser (1994) found that a plasmid-containing strain could multiply readily in murine macrophages, whereas a strain without the plasmid could not. Similarly, Takai *et al.* (1995b) showed that small doses of a plasmid-bearing strain could multiply in mice but equivalent doses of a plasmid-cured derivative could not. However, the latter study also found that larger doses of killed cells caused granulomatous lesions irrespective of the presence of the virulence plasmid. This would seem to indicate that the cell envelope has a pathological effect but that the plasmid is necessary for intracellular survival in the immunocompetent host. Delapenamoctezuma *et al.* (1996) were unable to find any further phenotypic traits encoded for by the 85 kb virulence plasmid.

3.4.4 Human infections caused by other rhodococci. It has

been suggested that other *Rhodococcus* species may be of more importance in human disease than previously thought. Osoagbaka (1989) described the isolation of a number of rhodococci and related bacteria from the sputum of patients with respiratory illnesses. Schaal and Lee (1992) also reported the isolation of various rhodococcal species from clinical samples and Martin *et al.* (1991) suggested that rhodococcal skin infections may be more common than is recognized. There have been reports of *R. erythropolis* causing infections in the immunosuppressed; one case was in an HIV-positive individual (Vernazza *et al.* 1991) while another was in a peritoneal dialysis patient (Brown and Hendler 1989). *Rhodococcus rhodochrous* has been isolated from a chronic corneal ulcer (Gopaul *et al.* 1988). Meningitis caused by a *Rhodococcus* strain (not *R. equi*) in an otherwise healthy host was reported by Demarais and Kocka (1995). A fatal infection by a *Rhodococcus* strain apparently not belonging to any of the recognized species has been described (Spark *et al.* 1993). Reports of such cases are rare in the literature, although it could be that this is due to some extent to difficulties in identifying rhodococci and a lack of recognition of their pathogenic potential.

Many of the organisms recently reclassified as *Gordona* or *Tsukamurella* are also associated with infection in humans; these include *G. terrae*, *G. bronchialis*, *G. sputi* and *T. paurometabolum* (McNeil and Brown 1994).

3.4.5 Rhodococcus fascians *infections in plants.* *Rhodococcus fascians* (formerly *Corynebacterium facians*) causes fasciation in plants (Crespi *et al.* 1992). The infection results in growth abnormalities such as loss of apical dominance and growth of lateral shoots that form leafy galls. This is caused by cytkinins produced by the bacteria and there is evidence that essential genes for this are borne on a large linear plasmid (Crespi *et al.* 1992) or alternatively on a circular plasmid (Stange *et al.* 1996). Further work has been done to characterize the genes involved (Crespi *et al.* 1994). No similar work has been done on any other similar interactions between a Gram-positive bacterium and plants, and this infection has several features which are so far unique. The virulence of different strains varies considerably, as does the susceptibility of different plant hosts, even those that are closely related (Eason *et al.* 1995).

The economic significance of *R. fascians* infections in plants appears to be limited although the bacterium has a broad host range and can infect various crop plants such as peas and tobacco (Eason *et al.* 1995; Stange *et al.* 1996; Vereecke *et al.* 1997).

3.4.6 Rhodococcus rhodnii *and trypanosomiasis.* *Rhodococcus rhodnii* exists as a symbiont in the gut of the insect *Rhodnius prolixus* which is an important vector of American trypanosomiasis (Chagas' disease) (Ben-Yakir 1987). It has been suggested that biological control of the disease could be exerted by targeting the insect via *R. rhodnii* as the growth and development of the insect is apparently dependent on the bacterium. *Beard et al.* (1992) described the infection of *Rhodnius prolixus* by genetically altered strains of *R. rhodnii* and suggested that introducing foreign genes into the insect in this manner could be used to target Chagas' disease.

4. IDENTIFICATION OF *RHODOCOCCUS*

4.1 Identification to genus level

Identification of rhodococci to genus level can be made on the basis of the chemical characteristics by which the taxon is defined, i.e. by analysis of the relevant cell wall components by various chromatographic techniques, normally combined with observation of nocardioform growth (Goodfellow 1989a). However, there is no single chemical or morphological feature which clearly separates all of the rhodococci from the other mycolate-containing nocardioforms (Klatte *et al.* 1994c).

4.2 Differentiation of species

4.2.1 Overview. Goodfellow (1989a) states that for rhodococci, "identification to species level is difficult" by traditional methods. More recently described techniques, involving simplified biochemical testing, analysis of cellular components, serological tests and nucleic acid-based tests have sought to address the problem.

4.2.2 Growth characteristics and biochemical testing. The precise identification of the rhodococci by biochemical and physiological tests is a challenging and laborious task, even with improved enzyme assays using fluorogenic substrates. The large number of tests used by Goodfellow *et al.* (1990) is inappropriate for routine use, while use of a smaller number of tests may be inadequate (McNeil and Brown 1994). The API Coryne system (Biomerieux, Marcy-l'Etoile, France) includes *R. equi* in its database and successful use of this system to identify *R. equi* isolates has been reported (Bern and Lammler 1994), but Soto *et al.* (1994) found the system be unreliable as it incorrectly identified strains of *R. rhodochrous* as *R. equi*. The Biolog system (Biolog Inc., Hayward, CA, USA) has been used to identify rhodococci, although it is not yet clear how accurate and reliable it is for this purpose (Wang and Krawiec 1994; Harrisbaldwin and Gudmestad 1996).

4.2.3 Analysis of cellular components. Various modern methods for microbial identification by automated analysis of

cellular components to provide a 'fingerprint' for a specimen have been applied to nocardioform actinomycetes and could be useful for identifying rhodococci.

Glickman *et al.* (1994) developed a computer-linked HPLC system to analyse mycolic acid profiles. It has proved successful for differentiating *Mycobacterium* species from other mycolate-containing species (including rhodococci) and could be adapted to identify rhodococci. However, an earlier study that investigated the use of HPLC to identify nocardioform species did not produce good fingerprints for all the species concerned (Butler *et al.* 1987).

Analysis of fatty acid methyl esters (FAME) by gas chromatography has proved useful for the differentiation of *Rhodococcus* species in taxonomic studies (Klatte *et al.* 1994c). The Microbial Identification System (MIS; Microbial Identification Inc., Newark, DE, USA) is a standardized system based on FAME analysis designed for bacteriology. Although the data presented in papers such as that of Klatte *et al.* (1994c) show that the different species of *Rhodococcus* have varying FAME patterns, only average values for several strains are quoted, so it is not clear how consistent these values are amongst strains within a species. This makes it difficult to assess the reliability of the system for identifying *Rhodococcus* strains. McNabb *et al.* (1997) investigated use of the MIS for identifying various actinomycetes. While the system was able to differentiate those *Rhodococcus* species tested, heterogeneity within species was observed, e.g. the four strains of *R. rhodochrous* tested fell into four distinct groups.

Bacteria may be identified by pyrolysis of whole cell samples, with subsequent mass spectrometric analysis of the products (Curie-point pyrolysis mass spectrometry or PyMS). This technique has been applied successfully to identification of mycolate-containing nocardioforms, and gives good agreement with the results obtained by numerical taxonomy (Goodfellow *et al.* 1997). However, very few laboratories have the equipment required to perform this procedure.

4.2.4 Serological tests. Serological tests for rhodococci have been described (Ivshina *et al.* 1982; Ivshina *et al.* 1986; Goodfellow 1989a) but these have limitations with regard to specificity and there are practical difficulties in the use of experimental animals. Some immunoassays have been described specifically for virulent strains of *R. equi* (Takai *et al.* 1994b; Lammler 1995; Prescott *et al.* 1996). An immunofluorescence microscopy technique for the quantification of '*R. chlorophenolicus*' has been used to study the organism in activated sludge (Jacobsen 1995).

4.2.5 Nucleic-acid based techniques. A number of nucleic acid techniques for identifying rhodococci have been developed in recent years. Two ribotyping methods have been described (Lasker *et al.* 1992; Jorks 1996). These involve hybridization of a labelled ribosomal operon from *E. coli* to *Rhodococcus* genomes followed by endonuclease cleavage of the hybrids. This gives rise to characteristic DNA electrophoresis banding patterns which allow species to be differentiated. Steingrube *et al.* (1997) have described a rapid identification system for *Nocardia* and related organisms that relies on polymerase chain reaction (PCR) amplification of a segment of a gene coding for a 65 kDa heat-shock protein. Species were distinguished by varying patterns of restriction endonuclease cutting of the amplicon. The technique distinguished *R. equi* from species of *Nocardia, Gordona, Tsukamurella* and other genera. However, no other species of *Rhodococcus* were tested. Recently, techniques that specifically amplify target regions of DNA by PCR have been described for identifying virulent plasmid-bearing strains of *R. equi* and *R. fascians* (Takai *et al.* 1995c; Stange *et al.* 1996). A PCR-based technique has also been described to identify *R. equi* by targeting species-specific regions in the 16S rRNA gene (Bell *et al.* 1996). Similar methods for identifying some other *Rhodococcus* species have also been developed (Bell 1997). Another method used for identification of bacterial isolates or members of natural communities uses the amplification and sequencing of the whole 16S rRNA gene and comparison with other sequences in the GenBank or other databases. This approach has been adopted for identification of nocardioforms (Blackall 1994; Schuppler *et al.* 1995). It has the advantage that isolation and preliminary assessment of the possible identity of the organism are not required, although the requirement for DNA sequencing facilities is restrictive.

4.3 Importance of identifying rhodococci

A number of problems have been identified by various workers in the diagnosis of *R. equi* infections in humans. The organism is not well recognized as a pathogen. If it is observed in a clinical sample, it may be dismissed as a commensal diphtheroid rather than the infecting agent, and its possible appearance as an acid-fast bacillus could lead to confusion with *Mycobacterium tuberculosis* or other mycobacteria (McGowan and Mangano 1991; McNeil and Brown 1994; Leechiong *et al.* 1995; Scott *et al.* 1995). Colony appearance, cellular morphology and acid-fast stain reaction can vary according to such factors as strain, culture age, growth medium or sample type and staining technique (Prescott 1991). Single strains can produce different colony types on solid media. Furthermore, a range of microbes (including *M. tuberculosis*) cause similar lung lesions in AIDS patients (Gallant and Ko 1996) or in horses (Lavoie *et al.* 1994). Delay or failure to diagnose infections correctly may lead to treatment with inappropriate antibiotics with serious consequences.

The identification of other *Rhodococcus* species if found in clinical samples is likely to be far more problematic and it is possible that some cases of non-*R. equi* rhodococcal infection go unrecognized.

It is also desirable to be able to identify bacteria with possible biotechnological applications. This may give an insight into the physiological and biochemical properties of the organisms and allow selection of the most appropriate cloning vectors for genetic manipulation.

Techniques for rapid identification of *Rhodococcu*s spp. may also be useful in environmental biotechnologies, e.g. monitoring bacterial populations during bioremediation studies, using *R. coprophilus* as an indicator of animal faecal pollution of waterways, and using *R. ruber* or *R. rhodochrous* strains as indicators in oil prospecting and identification of nocardioforms involved in foaming in activated sludge systems.

5. CONCLUDING REMARKS

It is clear from the number and range of publications relating to *Rhodococcus* in recent years that the genus is of considerable interest in a wide variety of fields. Much of the biotechnological interest in *Rhodococcus* stems from the diverse range of reactions, sometimes novel, which their enzymes can catalyse. Their hydrophobic, mycolate-containing cell envelope structure also seems to be relevant to many potential applications (bioremediation, biodegradation, biotransformations and biosurfactants) and also to the problems which rhodococci can cause (pathogenicity and activated sludge foaming).

Despite the failure of many microbial processes to yield the fully fledged industrial biotechnologies which were promised, acrylamide production using *Rhodococcus* is a notable success and continuing industrial interest is reflected in the increased rate of patenting relating to *Rhodococcus*. It seems likely that the metabolic abilities of the genus will continue to attract industrial attention and that novel abilities will be identified and applications suggested.

Legislation and taxation aimed at curbing pollution are likely to be the driving force to fund preventative and remediative biotechnologies. *Rhodococcus* species clearly have considerable potential for bioremediation, degradation of toxins in effluents and as biosensors to detect xenobiotics.

The medical importance of *R. equi* and perhaps other rhodococci will continue to rise as the number of immunosuppressed individuals rises. Considerable progress has been made in understanding the virulence and epidemiology of *R. equi* infections.

Improved methods for identification of *Rhodococcus* species are required to yield further information about their environmental and medical significance and to aid in the characterization of commercially useful isolates.

The polyphasic approach taken to resolving *Rhodococcus* taxonomy means that the genus should now be a relatively stable entity, despite its diversity. However, it is probable that many strains in culture collections, and some described in published studies, are mislabelled and that further new species remain to be described.

REFERENCES

About, I., Castan, B., Capdeville, J. and Vanche, J. (1996) Cavitary pneumonia caused by *Rhodococcus equi* in HIV infection. *Revue de Medecine Interne* **17**, 410–414.

Abu-Ruwaida, A.S., Banat, I.M., Haditirto, S. and Khamis, A. (1991) Nutritional requirements and growth characteristics of a biosurfactant producing *Rhodococcus* bacterium. *World Journal of Microbiology and Biotechnology* **7**, 53–61.

Apajalahti, J.H.A. and Salkinoja-Salonen, M.S. (1987) Dechlorination and para-hydroxylation of polychlorinated phenols by *Rhodococcus chlorophenolicus*. *Journal of Bacteriology* **169**, 675–581.

Arlotti, M., Zoboli, G., Moscatelli, G.L. *et al.* (1996) *Rhodococcus equi* infection in HIV-positive subjects – a retrospective analysis of 24 cases. *Scandinavian Journal of Infectious Diseases* **28**, 463–467.

Arnold, S.M., Hickey, W.J., Harris, R.F. and Talaat, R.E. (1996) Integrating chemical and biological remediation of atrazine and S-triazine-containing pesticide wastes. *Environmental Toxicology and Chemistry* **15**, 1255–1262.

Ashraf, W., Mihdir, A. and Murrell, J.C. (1994) Bacterial oxidation of propane. *FEMS Microbiology Letters* **122**, 1–6.

Asturias, J.A. and Timmis, K.N. (1993) Three different 2,3-dihydroxybiphenyl-1, 2-dioxygenase genes in the Gram-positive polychlorobiphenyl-degrading bacterium *Rhodococcus globerulus* P6. *Journal of Bacteriology* **175**, 4631– 4640.

Beard, C.B., Mason, P.W., Aksoy, S., Tesh, R.B. and Richards, F.F. (1992) Transformation of an insect symbiont and expression of a foreign gene in the Chagas Disease vector *Rhodnius prolixus*. *American Journal of Tropical Medicine and Hygiene* **46**, 195–200.

Behki, R.M., Topp, E.E. and Blackwell, B.A. (1994) Ring hydroxylation of N-methylcarbamate insecticides by *Rhodococcus* TE1. *Journal of Agricultural and Food Chemistry* **42**, 1375–1378.

Bell, K.S. (1997) Development of Methods for the Identification and Environmental Detection of *Rhodococcus* Bacteria by Specific DNA Amplification. PhD Thesis. Napier University, Edinburgh.

Bell, K.S., Philp, J.C., Christofi, N. and Aw, D.W.J. (1996) Identification of *Rhodococcus equi* using the polymerase chain reaction. *Letters in Applied Microbiology* **23**, 72–74.

Ben-Yakir, D. (1987) Growth retardation of *Rhodnius prolixus* symbionts by immunizing host against *Nocardia* (*Rhodococcus*) *rhodnii*. *Journal of Insect Physiology* **33**, 379–383.

Bern, D. and Lammler, C. (1994) Biochemical and serological characteristics of *Rhodococcus equi* isolates from animals and humans. *Journal of Veterinary Medicine* **41**, 161–165.

Beyersdorfradeck, B., Karlson, U. and Schmid, R.D. (1994) A microbial sensor for 2-ethoxyphenol. *Analytical Letters* **27**, 285–298.

Bhalla, T.C., Miura, A., Wakamoto, A., Ohba, Y. and Furuhashi, K. (1992) Asymmetric hydrolysis of alpha-aminonitriles to optically

active amino acids by a nitrilase of *Rhodococcus rhodochrous. Applied Microbiology and Biotechnology* **37**, 184–190.

Blackall, L.L. (1994) Molecular identification of activated-sludge foaming bacteria. *Water Science and Technology* **29**, 35–42.

Boyle, A.W., Silvin, C.J., Hassett, J.P., Nakas, J.P. and Tanenbaum, S.W. (1992) Bacterial PCB degradation. *Biodegradation* **3**, 285–298.

Bozdemir, T.O., Dursoy, T., Erincin, E. and Yurum, Y. (1996) Biodesulfurization of Turkish lignites.1. Optimization of the growth parameters of *Rhodococcus rhodochrous*, a sulfur-removing bacterium. *Fuel* **75**, 1596–1600.

Briglia, M., Eggen, R.I.L., van Elsas, D.J. and De Vos, W.M. (1994a) Phylogenetic evidence for transfer of pentachlorophenol-mineralizing *Rhodococcus chlorophenolicus* PCP-1 to the genus *Mycobacterium. International Journal of Systematic Bacteriology* **44**, 494–498.

Briglia, M., Middeldorp, P.J.M. and Salkinoja-Salonen, M.S. (1994b) Mineralization performance of *Rhodococcus chlorophenolicus* strain PCP-1 in contaminated soil simulating on site conditions. *Soil Biology and Biochemistry* **26**, 377–385.

Briglia, M., Rainey, F.A., Stackebrandt, E., Schraa, G. and Salkinoja-Salonen, M.S. (1996) *Rhodococcus percolatus* sp. nov., a bacterium degrading 2,4,6-trichlorophenol. *International Journal of Systematic Bacteriology* **46**, 23–30.

Brown, E. and Hendler, E. (1989) *Rhodococcus* peritonitis in a patient treated with peritoneal dialysis. *American Journal of Kidney Disease* **14**, 417–418.

Butler, W.R., Kilburn, J.O. and Kubica, G.P. (1987) High-performance liquid chromatography analysis of mycolic acids as an aid in laboratory identification of *Rhodococcus* and *Nocardia* species. *Journal of Clinical Microbiology* **25**, 2126–2131.

Cameron, G.W.W., Jordan, K.N., Holt, P.-J., Baker, P.B., Lowe, C.R. and Bruce, N.C. (1994) Identification of a heroin esterase in *Rhodococcus* sp. strain H1. *Applied and Environmental Microbiology* **60**, 3881–3883.

Christodoulou, S., Hung, T.V., Trewhell, M.A. and Black, R.G. (1994) Enzymatic degradation of egg-yolk cholesterol. *Journal of Food Protection* **57**, 908–912.

Christofi, N., Ivshina, I.B., Kuyukina, M.S. and Philp, J.C. (1998) Biological treatment of crude oil contaminated soil in Russia. In *Contaminated Land and Groundwater: Future Directions* ed. Lerner, D.N. and Walton, N.R.G. pp. 45–51. Engineering Geology Special Publication 14. London: Geological Society.

Chun, J., Blackall, L.L., Kang, S.O., Hah, Y.C., Goodfellow, M. (1997) A proposal to reclassify *Nocardia pinensis* Blackall *et al.* as *Skermania piniformis* gen. nov., comb. nov. *International Journal of Systematic Bacteriology* **47**, 127–131.

Chun, J., Kang, S.O., Hah, Y.C. and Goodfellow, M. (1996) Phylogeny of mycolic acid-containing actinomycetes. *Journal of Industrial Microbiology* **17**, 205– 213.

Coghlan, A. (1994) Drug users leave an afterglow. *New Scientist* **1956**, 21.

Colebunders, R., deRoo, A., Versraeten, T. *et al.* (1996) *Rhodococcus* infections in 3 AIDS patients. *Acta Clinica Belgica* **15**, 101–105.

Collins, M.D., Smida, J., Dorsch, M. and Stackebrandt, E. (1988) *Tsukamurella* gen. nov. harboring *Corynebacterium parometabolum* and *Rhodococcus auranticus. International Journal of Systematic Bacteriology* **38**, 385–391.

Crespi, M., Messens, E., Caplan, A.B., van Montagu, M. and Desomer, J. (1992) Fasciation induction by the phytopathogen *Rhodococcus fascians* depends upon a linear plasmid encoding a cytokinin synthase gene. *The EMBO Journal* **11**, 795–804.

Crespi, M., Vereecke, D., Temmerman, W., van Montagu, M. and Desomer, J. (1994) The *Fas* operon of *Rhodococcus fascians* encodes new genes required for efficient fasciation of host plants. *Journal of Bacteriology* **176**, 2492–2501.

de' Clari, F., Menghini, T., Biaggi, G. and Magnoli, P. (1992) Septicaemia due to a new species of *Rhodococcus* that contaminated closed system packed red blood cells – cure with imipenem monotherapy. *Journal of Antimicrobial Chemotherapy* **30**, 729–730.

Delapenamoctezuma, A., Prescott, J.F. and Goodfellow, M. (1996) Attempts to find phenotypic markers of the virulence plasmid of *Rhodococcus equi. Canadian Journal of Veterinary Research* **60**, 29–33.

de los Reyes, F.L., Ritter, W. and Raskin, L. (1997) Group-specific small-subunit rRNA hybridization probes to characterize filamentous foaming in activated sludge systems. *Applied and Environmental Microbiology* **63**, 1107–1117.

Demarais, P.L. and Kocka, F.E. (1995) *Rhodococcus* meningitis in an immunocompetent host. *Clinical Infectious Diseases* **20**, 167–169.

Denome, S.A., Oldfield, C., Nash, L.J. and Young, K.D. (1994) Characterization of the desulfurization genes from *Rhodococcus* sp. *strain IGTS8. Journal of Bacteriology* **176**, 6707–6716.

Donisi, A., Suardi, M.G., Casari, S., Longo, M., Cadeo, G.P. and Carosi, G. (1996) *Rhodococcus equi* infection in HIV-infected patients. *AIDS* **10**, 359–362.

Duran, R., Nishiyama, M., Horinouchi, S. and Beppu, T. (1993) Characterization of nitrile hydratase genes cloned by DNA screening from *Rhodococcus erythropolis. Bioscience, Biotechnology and Biochemistry* **57**, 1323–1328.

Eason, J.R., Jameson, P.E. and Bannister, P. (1995) Virulence assessment of *Rhodococcus fascians* strains on pea cultivars. *Plant Pathology* **44**, 141–147.

Embley, T.M. and Stackebrandt, E. (1994) The molecular phylogeny and systematics of the actinomycetes. *Annual Review of Microbiology* **48**, 257–289.

Espuny, M.J., Egido, S., Rodon, I., Manresa, A. and Mercade, M.E. (1996) Nutritional requirements of a biosurfactant producing strain *Rhodococcus* sp 51T7. *Biotechnology Letters* **18**, 521–526.

Feakin, S.J., Blackburn, E. and Burns, R.G. (1995) Inoculation of granular activated carbon in a fixed-bed with S-triazine degrading bacteria as a water treatment process. *Water Research* **29**, 819–825.

Fiechter, A. (1992) Biosurfactants: moving towards industrial application. *Trends in Biotechnology* **10**, 208–217.

Finnerty, W.M. (1992) The biology and genetics of the genus *Rhodococcus. Annual Review of Microbiology* **46**, 193–218.

Finnerty, W.M. (1994) Biosurfactants in environmental biotechnology. *Current Opinion in Biotechnology* **5**, 291–295.

Foot, R.J., Kocianova, E., Forster, C.F. and Wilson, A.J. (1993) An examination into the structure of stable foams formed on activated sludge plants. *Journal of Chemical Technology and Biotechnology* **56**, 21–25.

Fougias, E. and Forster, C.F. (1994) *Rhodococcus rubra* in relation

to stable foams in activated sludge. *Process Biochemistry* **29**, 553–557.

Funke, G., Stubbs, S., Altweigg, M., Carlotti, A. and Collins, M.D. (1994) *Turicella otitidis* gen. nov., sp. nov., a coryneform bacterium isolated from patients with otitis media. *International Journal of Systematic Bacteriology* **44**, 270–273.

Gallant, J.E. and Ko, A.H. (1996) Cavitary pulmonary lesions in patients infected with human immunodeficiency virus. *Clinical Infectious Diseases* **22**, 671–682.

Glickman, S.E., Kilburn, J.O., Butler, W.R. and Ramos, L.S. (1994) Rapid identification of mycolic acid patterns of mycobacteria by high performance liquid chromatography using pattern-recognition software and a *Mycobacterium* library. *Journal of Clinical Microbiology* **32**, 740–745.

Goodfellow, M. (1989a) Genus *Rhodococcus*. In *Bergey's Manual of Systematic Bacteriology*, *Vol. 4* ed. Williams, S.T., Sharpe, M.E. and Holt, J.G. pp. 2362- 2371. Baltimore: Williams and Wilkins.

Goodfellow, M. (1989b) Supragenic classification of actinomycetes. In *Bergey's Manual of Systematic Bacteriology*, *Vol. 4* ed. Williams, S.T., Sharpe, M.E. and Holt, J.G. pp. 2333–2339. Baltimore: Williams and Wilkins.

Goodfellow, M. (1992) The family *Nocardiaceae*. In *The Prokaryotes* ed. Balows, A., Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. pp. 1188–1213. Berlin: Springer-Verlag.

Goodfellow, M. and Alderson, G. (1977) The actinomycete-genus *Rhodococcus*: a home for the '*rhodochrous*' complex. *Journal of General Microbiology* **100**, 99–122.

Goodfellow, M., Davenport, R., Stainsby, F.M. and Curtis, T.P. (1997) Actinomycete diversity associated with foaming in activated sludge plants. *Journal of Industrial Microbiology* **17**, 268–280.

Goodfellow, M. and Minnikin, D.E. (1981) The genera *Nocardia* and *Rhodococcus*. In *The Prokaryotes*, *Vol. II* ed. Starr, M.P., Stolp, H., Trüper, H.G., Balows, A. and Schlegel, H.G. pp. 2016–2026. Berlin: Springer-Verlag.

Goodfellow, M., Thomas, E.G., Ward, A.C. and James, A.L. (1990) Classification and identification of the rhodococci. *Zentralblatt für Bakteriologie* **274**, 299–315.

Gopaul, D., Ellis, C., Maki, A. and Joseph, M.G. (1988) Isolation of *Rhodococcus rhodochrous* from a chronic corneal ulcer. *Diagnostic Microbiology and Infectious Disease* **10**, 185–190.

Gray, K.A., Pogrebinsky, O.S., Mrachko, G.T., Xi, L., Monticello, D.J. and Squires, C.H. (1996) Molecular mechanisms of biocatalytic desulfurization of fossil fuels. *Nature Biotechnology* **14**, 1705–1709.

Häggblom, M.M., Janke, D. and Salkinoja-Salonen, M.S. (1989) Transformation of chlorinated phenolic compounds in the genus *Rhodococcus*. *Microbial Ecology* **18**, 147–159.

Häggblom, M.M., Nohyek, L.J., Palleroni, N.J. *et al.* (1994) Transfer of polychlorophenol-degrading *Rhodococcus chlorophenolicus* (Apajalahti *et al.* 1986) to the genus *Mycobacterium* as *Mycobacterium chlorophenolicum* comb. nov. *International Journal of Systematic Bacteriology* **44**, 485–493.

Harrisbaldwin, A. and Gudmestad, N.C. (1996) Identification of phytopathogenic coryneform bacteria using the Biolog automated identification system. *Plant Disease* **80**, 874–878.

Hart, S., Kirby, R. and Woods, D.R. (1990) Structure of a *Rhodococcus* gene encoding pigment production in *Escherichia coli*. *Journal of General Microbiology* **136**, 1357–1363.

Harvey, R.L. and Sunstrum, J.C. (1991) *Rhodococcus equi* infection in patients with and without human immunodeficiency virus infection. *Reviews of Infectious Diseases* **13**, 139–145.

Heiss, G.S., Gowan, B. and Dabbs, E.R. (1992) Cloning of DNA from a *Rhodococcus* strain conferring the ability to decolorize sulfonated azo dyes. *FEMS Microbiology Letters* **99**, 221–226.

Hondalus, M.K. and Mosser, D.M. (1994) Survival and replication of *Rhodococcus equi* in macrophages. *Infection and Immunity* **62**, 4167–4175.

Hummel, W. (1997) Screening and characterization of new enzymes for biosensing and analytics. In *Frontiers in Biosensorics I, Fundamental Aspects* ed. Scheller, F.W., Scubert, F. and Fedrowitz, J. pp. 49–61. Basel: Birkhauser Verlag.

Hummel, W., Schutte, H., Schmidt, E., Wandrey, C. and Kula, M.-R. (1987) Isolation of l-phenylalanine dehydrogenase from *Rhodococcus* sp. M4 and its application for the production of l-phenylalanine. *Applied Microbiology and Biotechnology* **26**, 409–416.

Iborra, J.L., Manjon, A., Canovas, M., Lozano, P. and Martinez, C. (1994) Continous limonin degradation by immobilized *Rhodococcus fascians* cells in kappa-carrageenan. *Applied Microbiology and Biotechnology* **41**, 487–493.

Ivshina, I.B., Kamenskikh, T.N. and Liapunov, Y.E. (1994) *IEGM Catalogue of Strains of Regional Specialised Collection of Alkanotrophic Microorganisms*. Moscow: Russian Academy of Sciences.

Ivshina, I.B., Kevorkov, N.N., Koblova, I.V., Nesterenko, O.A., Kvasnikov, E.I. and Kasumova, S.A. (1982) Identification of the bacteria belonging to the genus *Rhodococcus* using the technique of immunodiffusion. *Mikrobiologia* **51**, 636–641.

Ivshina, I.B., Koblova, I.V., Bezmaternykh, G.I., Nesterenko, O.A., Kvasnikov, E.I. and Shkaruba, V.V. (1986) Identification of the genus *Rhodococcus* bacteria by the immunodiffusion method. *Mikrobiologicheskiy* **48**, 3–8.

Ivshina, I.B., Oborin, A.A., Nesterenko, O.A. and Kasumova, S.A. (1981) Bacteria of the *Rhodococcus* genus from the ground water of oil-bearing deposits in the Perm region near the Urals. *Mikrobiolgiya* **50**, 709–717.

Izumi, Y., Ohshiro, T., Ogino, H., Hine, Y. and Shimao, M. (1994) Selective desulfurization of dibenzothiophene by *Rhodococcus erythropolis* D1. *Applied and Environmental Microbiology* **60**, 223–226.

Jacobsen, B.N. (1995) A method for quantification of *Rhodococcus chlorophenolicus* in activated sludge by immunofluoresence microscopy. *Water Research* **29**, 279–283.

Jagals, P., Grabow, W.O.K. and Devilliers, J.C. (1995) Evaluation of indicators for assessment of human and animal fecal pollution of surface run-off. *Water Science and Technology* **31**, 235–241.

Jorks, S. (1996) Differentiation of *Rhodococcus* species by ribotyping. *Journal of Basic Microbiology* **36**, 399–406.

Kayser, K.J., Bielaga-Jones, B.A., Jackowski, K., Odusan, O. and Kilbane, J.J. (1993) Utilization of organosulphur compounds by axenic and mixed cultures of *Rhodococcus rhodochrous* IGTS8. *Journal of General Microbiology* **139**, 3123–3129.

Kieslich, K. (1991) Biotransformations of industrial use. *Acta Biotechnologica* **11**, 559–570.

Kilbane, J.J. (1989) Desulfurization of coal: the microbial solution. *Trends in Biotechnology* **7**, 97–101.

Klatte, S., Jahnke, K.-D., Kroppenstedt, R.M., Rainey, F.A. and Stackebrandt, E. (1994b) *Rhodococcus luteus* is a later subjective synonym of *Rhodococcus fascians. International Journal of Systematic Bacteriology* **44**, 627–630.

Klatte, S., Kroppenstedt, R.M. and Rainey, F.A. (1994c) *Rhodococcus opacus* sp. nov., an unusually nutritionally versatile *Rhodococcus* species. *Systematic and Applied Microbiology* **17**, 355–360.

Klatte, S., Rainey, F.A. and Kroppenstedt, R.M. (1994a) Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordona* as *Gordona aichiensis* comb. nov. & *Gordona amarae comb. nov. International Journal of Systematic Bacteriology* **44**, 769– 773.

Kobayashi, M., Komeda, H., Nagasawa, T. *et al.* (1993) Amidase coupled with low-molecular-mass nitrile hydratase from *Rhodococcus rhodochrous* J1. Sequencing and expression of the gene and purification and characterization of the gene product. *European Journal of Biochemistry* **217**, 327–336.

Kobayashi, M. and Shimizu, S. (1994) Versatile nitrilases: nitrile-hydrolysing enzymes. *FEMS Microbiology Letters* **120**, 217–214.

Koch, C., Klatte, S., Schumann, P., Burghardt, J., Kroppenstedt, R.M. and Stackebrandt, E. (1995) Transfer of *Arthrobacter picolinophus* Tate and Ensign 1974 to *Rhodococcus erythropolis. International Journal of Systematic Bacteriology* **45**, 576–577.

Koronelli, T.V. (1996) Principles and methods for raising the efficiency of biological degradation of hydrocarbons in the environment: review. *Applied Biochemistry and Microbiology* **32,** 519–525.

Kreit, J., Lefebvre, G. and Germain, P. (1994) Membrane-bound cholesterol oxidase from *Rhodococcus* sp. cells – production and extraction. *Journal of Biotechnology* **33**, 271–282.

Kurane, R., Hatamochi, K., Kakuno, T. *et al.* (1994) Purification and characterization of lipid bioflocculant produced by *Rhodococcus erythropolis. Bioscience, Biotechnology and Biochemistry* **58**, 1977–1982.

Kurane, R., Hatamochi, K., Kakuno, T., Kiyohara, M., Tajima, T. and Taniguchi, Y. (1995) Chemical structure of lipid bioflocculant produced by *Rhodococcus erythropolis. Bioscience, Biotechnology and Biochemistry* **59**, 1652–1656.

Kurane, R. and Tomizuka, N. (1992) Towards new biomaterial produced by microorganism – bioflocculant and bioabsorbent. *Nippon Kagaku Kaishi* **5**, 453–463.

Lammler, C. (1995) A rapid slide agglutination test for the serological identification of *Rhodococcus equi. Medical Science Research* **23**, 637–638.

Lasker, B.A., Brown, J.M. and McNeil, M.M. (1992) Identification and epidemiological typing of clinical and environmental isolates of the genus *Rhodococcus* with use of a digoxigenin-labelled rDNA gene probe. *Clinical Infectious Diseases* **15**, 223–233.

Lavoie, J.P., Fiset, L. and Laverty, S. (1994) Review of 40 cases of lung abscesses in foals and adult horses. *Equine Veterinary Journal* **26**, 348–352.

Lechavalier, M.P. and Lechavalier, H.A. (1970) Chemical composition as criterion in the classification of aerobic actinomycetes. *International Journal of Systematic Bacteriology* **20**, 435–443.

Lechevalier, H.A. (1989) Nocardioform actinomycetes. In *Bergey's Manual of Systematic Bacteriology*, *Vol. 4* ed. Williams, S.T., Sharpe, M.E. and Holt, J.G. pp. 2348–2350. Baltimore: Williams and Wilkins.

Lee, S.Y. (1996) Bacterial polyhydroxyalkanoates. *Biotechnology and Bioengineering* **49**, 1–14.

Leechiong, T., Sadigh, M., Simms, M. and Buller, G. (1995) Case reports – pericarditis and lymphadenitis due to *Rhodococcus equi. American Journal of the Medical Sciences* **310**, 31–33.

Ludwig, B., Akundi, A. and Kendall, K. (1995) A long-chain secondary alcohol dehydrogenase from *Rhodococcus erythropolis* ATCC 4277. *Applied and Environmental Microbiology* **61,** 3729–3733.

Maeda, M., Chung, S.Y., Song, E. and Kudo, T. (1995) Multiple genes encoding 2,3-dihydroxybiphenyl 1, 2-dioxygenase in the Gram–positive polychlorinated biphenyl–degrading bacterium *Rhodococcus erythropolis* TA421, isolated from a termite ecosystem. *Applied and Environmental Microbiology* **61**, 549–555.

Martin, T., Hogan, D.J., Murphy, F., Natyshak, I. and Ewan, E.P. (1991) *Rhodococcus* infection of the skin with lymphadenitis in a non-immunocompromised girl. *Journal of the American Academy of Dermatology* **24**, 328–332.

Marwaha, S.S., Puri, M., Bhullar, M.K. and Kothari, R.M. (1994) Optimization of parameters for hydrolysis of limonin for debittering of kinnow mandarine juice by *Rhodococcus fascians. Enzyme and Microbial Technology* **16**, 723– 725.

Mascellino, M.T., Iona, E., Ponzo, R., Mastroianni, C.M. and Delia, S. (1994) Infections due to *Rhodococcus equi* in 3 HIV-infected patients – microbiological findings and antibiotic susceptibility. *International Journal of Clinical Pharmacology Research* **14**, 157–163.

McGowan, K.L. and Mangano, M.F. (1991) Infections with *Rhodococcus equi* in children. *Diagnostic Microbiology and Infectious Disease* **14**, 347–352.

McNabb, A., Shuttleworth, R., Behme, R. and Colby, W.D. (1997) Fatty acid characterization of rapidly growing aerobic actinomycetes as a means of identification. *Journal of Clinical Microbiology* **35**, 1361–1368.

McNeil, M.M. and Brown, J.M. (1994) The medically important aerobic actinomycetes: epidemiology and microbiology. *Clinical Microbiology Reviews* **7**, 357–417.

Mercade, M.E., Monleon, L., Deandres, C. *et al.* (1996) Screening and selection of surfactant-producing bacteria from waste lubricating oil. *Journal of Applied Bacteriology* **81**, 161–166.

Middeldorp, P.J.M., Briglia, M. and Salkinoja-Salonen, M.S. (1990) Biodegradation of pentachlorophenol in natural soil by inoculated *Rhodococcus chlorophenolicus. Microbial Ecology* **20,** 123–139.

Miethling, R. and Karlson, U. (1996) Accelerated mineralization of pentachlorophenol in soil upon inoculation with *Mycobacterium chlorophenolicum* PCP-1 and *Sphingomonas chlorophenolica* RA2. *Applied and Environmental Microbiology* **62**, 4361–4366.

Mulbry, W.W. (1994) Purification and characterization of an inducible S-triazine hydrolase from *Rhodococcus corralinus* NRRL B-1554R. *Applied and Environmental Microbiology* **60**, 613–618.

Neu, T.R. (1996) Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. *Microbiological Reviews* **60**, 151–166.

Neu, T.R., Dengler, T., Jann, B. and Pollara, K. (1992) Structural studies of an emulsion-stabilizing exopolysaccharide produced by

an adhesive, hydrophobic *Rhodococcus* strain. *Journal of General Microbiology* **138**, 2531–2537.

Ochi, K. (1995) Phylogenetic analysis of mycolic acid-containing wall-chemotype IV actinomycetes and allied taxa by partial sequencing of ribosomal protein AT-L30. *International Journal of Systematic Bacteriology* **45**, 653–660.

Osoagbaka, O.U. (1989) Evidence for the role of *Rhodococcus* species in pulmonary diseases. *Journal of Applied Bacteriology* **66**, 497–506.

Pai, S.L., Hsu, Y.L., Chong, N.M., Sheu, C.S. and Chen, C.H. (1995) Continuous degradation of phenol by *Rhodococcus* sp. immobilized on granular activated carbon and in calcium alginate. *Bioresource Technology* **51**, 37–42.

Parekh, N.R., Walker, A., Roberts, S.J. and Welch, S.J. (1994) Rapid degradation of the triazinone herbicide metamitron by a *Rhodococcus* sp. isolated from treated soil. *Journal of Applied Bacteriology* **77**, 467–475.

Peter, J., Hutter, W., Stollnberger, W. and Hampel, W. (1996) Detection of chlorinated and brominated hydrocarbons by an ion sensitive whole-cell biosensor. *Biosensors and Bioelectronics* **11**, 1215–1219.

Peters, J., Zelinski, T., Minuth, T. and Kula, M.R. (1993) Synthetic applications of the carbonyl reductases isolated from *Candida parapsilosis* and *Rhodococcus erythropolis. Tetrahedron-Asymmetry* **4**, 1683–1692.

Philp, J.C. and Bell, K.S. (1995) Applied studies with bacteria of the genus *Rhodococcus* relevant to treatment of land and water contaminated by industrial processes. *Proceedings of the International Symposium on Industrialisation and the Environment; Taejon National University of Technology, Seoul, Korea*, pp. 105–126. Seoul: Taejon University.

Pieper, U. and Steinbuchel, A. (1992) Identification, cloning and sequence analysis of the poly (3-hydroxyalkanoic acid) synthase gene of the Gram-positive bacterium *Rhodococcus ruber. FEMS Microbiology Letters* **96**, 73–79.

Prescott, J.F. (1991) *Rhodococcus equi*: an animal and human pathogen. *Clinical Microbiology* **4**, 20–34.

Prescott, J.F. (1994) *Rhodococcus equi* vertebral osteomyelitis in foals. *Equine Veterinary Journal* **26**, 1–2.

Prescott, J.F., Fernandez, A.S., Nicholson, V.M. *et al.* (1996) Use of a virulence-associated protein-based enzyme-linked immunosorbent-assay for *Rhodococcus equi* serology in horses. *Equine Veterinary Journal* **28**, 344–349.

Rainey, F.A., Burghardt, J., Kroppenstedt, R.M., Klatte, S. and Stackebrandt, E. (1995b) Polyphasic evidence for the transfer of *Rhodococcus roseus* to *Rhodococcus rhodochrous. International Journal of Systematic Bacteriology* **45**, 101–103.

Rainey, F.A., Burghardt, J., Kroppenstedt, R.M., Klatte, S. and Stackebrandt, E. (1995c) Phylogenetic analysis of the genera *Rhodococcus* and *Nocardia* and evidence for the evolutionary origin of the genus *Nocardia* from within the radiation of *Rhodococcus* species. *Microbiology* **141**, 523–528.

Rainey, F.A., Klatte, S., Kroppenstedt, R.M. and Stackebrandt, E. (1995a) *Dietzia*, a new genus including *Dietzia maris* comb. nov., formerly *Rhodococcus maris. International Journal of Systematic Bacteriology* **45**, 32–36.

Riedel, K., Hensel, J., Rothe, S., Neumann, B. and Scheller, F. (1993) Microbial sensors for determination of aromatics and their chloroderivatives.2. determination of chlorinated phenols using a *Rhodococcus* containing biosensor. *Applied Microbiology and Biotechnology* **38**, 556–559.

Riegel, P., Kamne-Fotso, M.V., de Briel, D. *et al.* (1994) *Rhodococcus chubuensis* Tsukamura 1982 is a later subjective synonym of *Gordona sputi* (Tsukamura 1978) Stackebrandt 1989 comb. nov. *International Journal of Systematic Bacteriology* **44**, 764–768.

Rosner, B.M., Rainey, F.A., Kroppenstedt, R.M. and Schink, B. (1997) Acetylene degradation by new isolates of aerobic bacteria and comparison of acetylene hydratase enzymes. *FEMS Microbiology Letters* **148**, 175–180.

Ruimy, R., Boiron, P., Boivin, V. and Christen, R. (1994) A phylogeny of the genus *Nocardia* deduced from the analysis of small subunit ribosomal DNA sequences, including the transfer of *Nocardia amarae* to the genus *Gordona* as *Gordona amarae* comb. nov. *FEMS Microbiology Letters* **123**, 261–268.

Ruimy, R., Riegel, P., Boiron, P., Monteil, H. and Christen, R. (1995) Phylogeny of the genus *Corynebacterium* deduced from analyses of small-subunit ribosomal DNA sequences. *International Journal of Systematic Bacteriology* **45**, 740– 746.

Sabater, L., Andreu, H., Garciavaldecasa, J.C. *et al.* (1996) *Rhodococcus equi* infection after liver transplantation. *Transplantation* **61**, 980–982.

Schaal, K.P. and Lee, H.-J. (1992) Actinomycete infections in humans – a review. *Gene* **115**, 201–211.

Schuppler, M., Mertens, F., Schon, G. and Göbel, U.B. (1995) Molecular characterization of nocardioform actinomycetes in activated sludge by 16S rRNA analysis. *Microbiology* **141**, 513–521.

Scott, M.A., Graham, B.S., Verrall, R., Dixon, R., Schaffner, W. and Tham, K.T. (1995) *Rhodococcus equi* – an increasingly recognized opportunistic pathogen – report of 12 cases and review of 65 cases in the literature. *American Journal of Clinical Pathology* **103**, 649–655.

Segovia, J., Pulpon, L.A., Crespo, M.G. *et al.* (1994) *Rhodococcus equi* – 1st case in a heart-transplant patient. *Journal of Heart and Lung Transplantation* **13**, 332–335.

Selifonova, O.V. and Eaton, R.W. (1996) Use of an *ipb-lux* fusion to study regulation of the isopropylbenzene catabolism operon of *Pseudomonas putida* RE204 and to detect hydrophobic pollutants in the environment. *Applied and Environmental Microbiology* **62**, 778–783.

Seto, M., Kimbara, K., Shimura, M., Hatta, T., Fukuda, M. and Yado, K. (1995) A novel transformation of polychlorinated biphenyls by *Rhodococcus* sp. strain RHA1. *Applied and Environmental Microbiology* **61**, 3353–3358.

Shabati, Y. and Fleminger, G. (1994) Adsorption of *Rhodococcus* Strain GIN-1 (NCIMB 40340) on titanium dioxide and coal fly ash particles. *Applied and Environmental Microbiology* **60**, 3079–3088.

Shabati, Y. and Mukmenev, I. (1996) A combined chemical-biotechnological treatment of coal fly ash (CFA). *Journal of Biotechnology* **51**, 209–217.

Shao, Z., Dick, W.A. and Behki, R.M. (1995) An improved *Escherichia coli–Rhodococcus* shuttle vector and plasmid transformation in *Rhodococcus* spp. using electroporation. *Letters in Applied Microbiology* **21**, 261–266.

Shennan, J.L. (1996) Microbial attack on sulphur-containing hydro-

carbons: implications for the biodesulphurisation of oils and coals. *Journal of Chemical Technology and Biotechnology* **67**, 109–123.

Smola, J., Katerov, V. and Schalen, C. (1994) Hemolytic and phospholipase-C (PLC) activities of *Rhodococcus equi*. *Journal of Applied Bacteriology* **77**, 325–333.

Soddell, J.A. and Seviour, R.J. (1995) Relationship between temperature and growth of organisms causing *Nocardia* foams in activated sludge plants. *Water Research* **29**, 1555–1558.

Sorkhoh, N.A., Alhasan, R.H., Khanafer, M. and Radwan, S.S. (1995) Establishment of oil-degrading bacteria associated with cyanobacteria in oil-polluted soil. *Journal of Applied Bacteriology* **78**, 194–199.

Soto, A., Zapardiel, J. and Soriano, F. (1994) Evaluation of API Coryne System for identifying coryneform bacteria. *Journal of Clinical Pathology* **47**, 756–759.

Spark, R.P., McNeil, M.M., Brown, J.M., Lasker, B.A., Montano, M.A. and Garfield, M.D. (1993) *Rhodococcus* species fatal infection in an immunocompetent host. *Archives of Pathology and Laboratory Medicine* **117**, 515–520.

Stackebrandt, E. and Goebel, B.M. (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology* **44**, 846–849.

Stackebrandt, E., Smida, J. and Collins, M.D. (1988) Evidence of phylogenetic heterogeneity within the genus *Rhodococcus*: revival of the genus *Gordona* (Tsukamura). *Journal of General and Applied Microbiology* **34**, 341–348.

Stange, R.R., Jeffares, D., Young, C., Scott, D.B., Eason, J.R. and Jameson, P.E. (1996) PCR amplification of the *Fas*-1 gene for the detection of virulent strains of *Rhodococcus fascians*. *Plant Pathology* **45**, 407–417.

Steingrube, V.A., Wilson, R.W., Brown, B.A. *et al.* (1997) Rapid identification of clinically significant species and taxa of aerobic actinomycetes, including *Actinomadura*, *Gordona*, *Nocardia*, *Rhodococcus*, *Streptomyces* and *Tsukamurella* isolates, by DNA amplification and restriction endonuclease analysis. *Journal of Clinical Microbiology* **35**, 817–822.

Stoecker, M.A., Herwig, R.P. and Staley, J.T. (1994) *Rhodococcus zopfii* sp. nov., a toxicant-degrading bacterium. *International Journal of Systematic Bacteriology* **44**, 106–110.

Sunairi, M., Iwabuchi, Y., Yoshiwara, Y., Murooka, H., Moriaski, H. and Nakajima, M. (1997) Cell-surface hydrophobicity and scum formation of *Rhodococcus rhodochrous* strains with different colonial morphologies. *Journal of Applied Microbiology* **82**, 204–210.

Takai, S., Fukunaga, N., Ochiai, S. *et al.* (1996c) Identification of intermediately virulent *Rhodococcus equi* isolates from pigs. *Journal of Clinical Microbiology* **34**, 1034–1037.

Takai, S., Fukunaga, N., Ochiai, S., Sakai, T., Sasaki, Y. and Tsubaki, S. (1996b) Isolation of virulent and intermediately virulent *Rhodococcus equi* from soil and sand on parks and yards in Japan. *Journal of Veterinary Medical Science* **58**, 669–672.

Takai, S., Hidak, D., Fujii, M. *et al.* (1996a) Serum antibody responses of foals to virulence-associated 15-kiloDalton to 17-kiloDalton antigens of *Rhodococcus equi*. *Veterinary Microbiology* **52**, 63–71.

Takai, S., Iie, M., Watanabe, S., Tsubaki, S. and Sekizaki, T. (1992) Virulence-associated 15- to 17-kiloDalton antigens in *Rhodococcus equi*: temperature dependant expression and location of the antigens. *Infection and Immunity* **60**, 2995–2997.

Takai, S., Ikeda, T., Sasaki, Y. *et al.* (1995c) Identification of virulent *Rhodococcus equi* by amplification of gene coding for 15-kiloDalton to 17-kiloDalton antigens. *Journal of Clinical Microbiology* **33**, 1624–1627.

Takai, S., Imai, Y., Fukunaga, N., Uchida, Y., Kamisawa, K. and Sasaki, Y. (1995a) Identification of virulence associated antigens and plasmids in *Rhodococcus equi* from patients with AIDS. *Journal of Infectious Diseases* **172**, 1306–1311.

Takai, S., Madarame, H., Matsumoto, C. *et al.* (1995b) Pathogenesis of *Rhodococcus equi* infections in mice – roles of virulence plasmids and granulomagenic activity of bacteria. *FEMS Immunology and Medical Microbiology* **11**, 181–190.

Takai, S., Morishta, T., Nishio, Y. *et al.*1994b) Evaluation of a monoclonal antibody-based colony blot test for rapid identification of virulent *Rhodococcus equi*. *Journal of Veterinary Medical Science* **56**, 681– 684.

Takai, S., Sasaki, Y., Ikeda, T., Uchida, Y., Tsubaki, S. and Sekizaki, T. (1994a) Virulence of *Rhodococcus equi* isolates from patients with and without AIDS. *Journal of Clinical Microbiology* **32**, 457–460.

Takai, S., Sekizaki, T., Ozawa, T., Sugawara, Y., Watanabe, Y. and Tsubaki, S. (1991) Association between a large plasmid and 15- to 17-kiloDalton antigens associated with virulent *Rhodococcus equi*. *Journal of Clinical Microbiology* **29**, 439–443.

Takai, S., Watanabe, Y., Ikeda, T. *et al.* (1993) Virulence associated plasmids in *Rhodococcus equi*. *Journal of Clinical Microbiology* **31**, 1726–1729.

Takigawa, H., Kubota, H., Sonohara, H. *et al.* (1993) Novel allylic oxidation of α-cedrene to sec-cedrenol by a *Rhodococcus* strain. *Applied and Environmental Microbiology* **59**, 1336– 1341.

Tan, C., Prescott, J.F., Patterson, M.C. and Nicholson, V.M. (1995) Molecular characterization of a lipid modified virulence associated protein of *Rhodococcus equi* and its potential in protective immunity. *Canadian Journal of Veterinary Research* **59**, 51–59.

Tikhonova, E.B., Ermakova, I.T. and Golovlev, E.L. (1996) Response of *Rhodococcus minimus* cultures to changes in the concentration of the carbon source – macromolecular composition and energy metabolism. *Mikrobiologia* **65**, 395–399.

Tipping, P.J. (1995) Foaming in activated-sludge processes – an operators overview. *Journal of the Chartered Institution of Water and Environmental Management* **9**, 281–289.

Tkachuk-Saad, O. and Prescott, J.F. (1991) *Rhodococcus equi* plasmids: isolation and partial characterisation. *Journal of Clinical Microbiology* **29**, 2696–2700.

Tomioka, N., Uchiyama, H. and Yagi, O. (1994) Cesium accumulation and growth characteristics of *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. strain CS402. *Applied and Environmental Microbiology* **60**, 2227–2231.

Tsukamura, M., Yano, I., Kudo, T. and Miyama, A. (1991) *Rhodococcus roseus* sp. nov., nom. rev. *International Journal of Systematic Bacteriology* **41**, 385– 389.

Valo, R.J., Haggblom, M.H. and Salkinoja-Salonen, M.S. (1990) Bioremediation of chlorophenol-containing simulated ground water by immobilized bacteria. *Water Research* **24**, 253–258.

Vereecke, D., Messens, E., Klarskov, DeBruyn, A., VanMontagu,

M. and Goethals, K. (1997) Patterns of phenolic compounds in leafy galls of tobacco. *Planta* **201**, 342–348.

Vernazza, P.L., Bodmer, T. and Galeazzi, R.L. (1991) *Rhodococcus erythropolis* infection in HIV-associated immunodeficiency. *Schweizerische Medizinische Wochenschrift* **121**, 1095–1098.

Vullo, V., Mastroianni, C.M., Lichtner, M. *et al.* (1996) Serological responses to *Rhodococcus equi* in individuals with and without human immunodeficiency virus infection. *European Journal of Clinical Microbiology and Infectious Diseases* **15**, 588–594.

Walsh, R.D. and Cunha, B.A. (1994) *Rhodococcus equi* – fatal pneumonia in a patient without AIDS. *Heart and Lung* **23**, 519–520.

Wang, P. and Krawiec, S. (1994) Desulfurization of dibenzothiophene of 2- hydroxybiphenyl by some newly isolated bacterial strains. *Archives of Microbiology* **161**, 266–271.

Warhurst, A.W., Clarke, K.F., Hill, R.A., Holt, R.A. and Fewson, C.A. (1994) Production of catechols and muconic acids from various aromatics by the styrene-degrader *Rhodococcus rhodochrous* NCIMB 13259. *Biotechnology Letters* **16**, 513–516.

Warhurst, A.W. and Fewson, C.A. (1994) Biotransformations catalyzed by the genus *Rhodococcus. Critical Reviews in Biochemistry* **14,** 29–73.

Wayne, L.G., Brenner, D.J., Colwell, R.R. *et al.* (1987) Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. *International Journal of Systematic Bacteriology* **37,** 463–464.

Woods, N.R. and Murrell, J.C. (1990) Epoxidation of gaseous alkenes by a *Rhodococcus* sp. *Biotechnology Letters* **12,** 409–414.

Yagafarova, G.G. and Skvortsova, I.N. (1996) A new oil-oxidizing strain of *Rhodococcus erythropolis. Applied Biochemistry and Microbiology* **32,** 207– 209.

Yamada, H. and Kobayashi, M. (1996) Nitrile hydratase and its application to industrial production of acrylamide. *Bioscience, Biotechnology and Biochemistry* **60,** 1391–1400.

Young, J.E.P. and McFarlane, G. (1994) *The National Collection of Industrial and Marine Bacteria Catalogue of Strains*. Aberdeen: NCIMB.

Yuoh, G., Hove, M.G.M., Wen, J. and Haque, A.K. (1996) Pulmonary malacoplakia in acquired immunodeficiency syndrome – an ultrastructural study of morphogenesis of Michaelis Gutman Bodies. *Modern Pathology* **9**, 476–483.

Zakrzewska-Czerwinska, J., Mordarski, M. and Goodfellow, M. (1988) DNA Base composition and homology values for some *Rhodococcus* species. *Journal of General Microbiology* **134,** 2807–2813.

Zopf, W. (1891) Uber Ausscheidung von Fettfarbstoffen (Lipochromen) seitens gewisser Spaltpilze. *Berichte der Deutschen Botanischen Gesellschaft* **9,** 22–28.