Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 83-92
Received: 05-03-2017
Accepted: 06-04-2017

Yasmin Elsayed
Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt

John Refaat
Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt

Usama R Abdelmohsen
1) Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt
2) Department of Botany II, Julius-von-Sachs Institute for Biological Sciences, University of Würzburg, Julius-von-Sachs-Platz 3, Würzburg, Germany

Mostafa A Fouad
Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt

Correspondence
Yasmin Elsayed
Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt

The Genus *Rhodococcus* as a source of novel bioactive substances: A review

Yasmin Elsayed, John Refaat, Usama R Abdelmohsen and Mostafa A Fouad

Abstract
Actinobacteria were well-known as bioactive compounds producers. This phylum comprises the most widely distributed group of microorganisms in nature. The actinomycete genus *Rhodococcus* has one of the largest known bacterial genomes, so it forms an enormous reservoir of secondary metabolites and enzymes. In this review, estimation is made on the present state of research on the genus *Rhodococcus* and its perspectives. The highlights include the production of metabolites such as antibacterial, antifungal, anti-trypanosomal, anticancer, siderophores, pigments, enzymes and enzyme inhibitors, these diverse bioactivities are mediated by several classes of compounds including polyketides, fatty acids, alkaloids, peptides, flavonoids, aminoglycosides and terpenes by Actinomycetes *Rhodococcus* genus and their application in industry.

Keywords: *Rhodococcus*, Actinomycetes, Secondary metabolites, Biological activities

1. Introduction
Actinomycetes are a diverse group of aerobic Gram-positive microorganisms, and all members of this order are characterized in part by their high "(G+C) guanine-cytosine" DNA content, and most exhibit a highly differentiated developmental life cycle. They are characterized by their diverse shapes and colors, formation of a network of aerial and substrate hyphae as well as production of melanoid pigments of various colors (*i.e.*, greenish brown, brownish black or distinct brown) on the medium [1]. They belong to the phylum *Actinobacteria*, which represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain bacteria, including five subclasses and 14 suborders [2]. Actinomycetes exist abundantly in terrestrial and marine environments, such as soil, sediment from the deep sea [3], plants [4, 5], insects [6, 7] and marine invertebrates [8, 9], as well as extreme environments, such as the cryophilic region [10], and hyper-arid desert soil [11]. Moreover, these microorganisms produce various useful bioactive compounds such as antibiotics [12, 13], anticancer drug [14], immunosuppressive agents [15] and enzyme inhibitors [16]. The activities represented by diverse classes of natural products such as polyketides, alkaloids, fatty acids, peptides andterpenes [17-19]. Large numbers of antibiotics have been isolated from the Actinobacteria, including those from the genera *Streptomyces* and *Nocardia*. Recently, the genus *Rhodococcus* has also been regarded as a prolific antibiotic producer [20]. In addition, *Rhodococci* offer advantages as experimental systems over more familiar actinomycetes. For example, *Rhodococci* grow faster than *M. tuberculosis* and have a simpler developmental cycle than *Streptomycetes*. Despite their importance, *Rhodococci* have not been well characterized [21]. The genus *Rhodococcus* was classified under the family Nocardiaceae which includes Gram-positive aerobic members of the order Actinomycetales and suborder Corynebacterineae within the new class of phylum Actinobacteria, *Rhodococcus* is a genus of aerobic, nonsporulating, mycolate-containing, nocardioform, nonmotile Gram-positive bacteria closely related to *Mycobacterium* and *Corynebacterium* [22]. The term 'nocardioform' is morphologically descriptive and refers to mycelial growth with fragmentation into rod-shaped or coccoid elements [23]. While a few species are pathogenic, most are benign, and have been found to thrive in a broad range of environments, including soil, rock, boreholes, groundwater, animal dung, guts of insects, water, and marine sediments. In this review, the distribution of genus *Rhodococcus* in various biological sources, their metabolite production and their biological activity are outlined in details.

2. Pathogenic *Rhodococcus*
The *Rhodococcus* genus has two pathogenic species: *R. fascians* and *R. equi*. The former, a plant pathogen, causes leafy gall disease in both angiosperm and gymnosperm plants [24].

The species *R. equi* is the causative agent of foal pneumonia (rattles) and mainly infects foals up to three months in age. However, it has a wide host range, sporadically infecting pigs, cattle, and immunocompromised humans, in particular AIDS patients and those undergoing immunosuppressive therapy [25]. Both pathogens are economically significant. *R. fascians* is a major pathogen of tobacco plants. *R. equi*, one of the most important foal pathogens, is endemic on many stud farms around the world. Recently, a novel human adenoviral vector vaccine for *R. equi* was developed and tested in the mouse model. This vaccine could potentially be developed further for use as a vaccine to prevent *R. equi* disease in foals [26].

3. Taxonomic History

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* [27]. Recent years have seen considerable changes made to the classification of the genus. Some species have been combined, transferred to other established genera or reclassified in new genera, and new species have been described. For example, *R. chlorophenolicus* was transferred to *Mycobacterium*; *R. aichiensis* and *R. chubuensis* were transferred to *Gordona* [28]. New species have also been discovered and added to the genus *Rhodococcus*, such as *R. roseus, R. zopfii, R. opacus*, and *R. percolates*, but *R. roseus* has more recently been shown to be a member of the species *R. rhodochrous* [28].

Currently, there are over 40 species classified under the genus *Rhodococcus* [29] such as *R. equi* [27], *R. corynebacterioides* [30], *R. fascians* [31], *R. gordoniae* [32], *R. jostii* [33], *R. marinonascens* [34], *R. opacus* [35], *R. rhodochrous* [36], *R. ruber* [27], *R. triatomae* [37], *R. tukisamuensis* [38], *R. wratislaviensis* [39], *R. yunnanensis* [40], and *R. zopfii* [41].

4. Genome

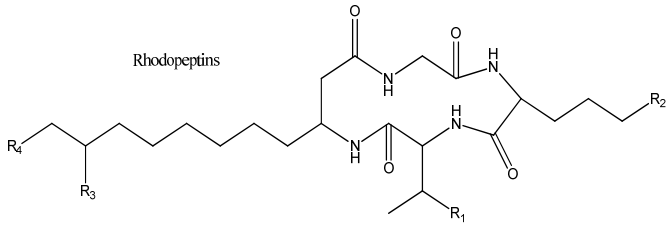
Rhodococci are notable for their ability to degrade a variety of natural and xenobiotic compounds, until 2006 when McLeod *et al*. sequenced the complete genome of *R. jostii* RHA1 for the first time [21]. *Rhodococcus* sp. RHA1 (RHA1) represents a genus of considerable industrial interest. RHA1 has one of the largest bacterial genomes sequenced to date, comprising 9,702,737 bp (67% GC) arranged in a linear chromosome and three linear plasmids (pRHL1, pRHL2, and pRHL3). A very important component of the McLeod *et al*. research was the discovery of a large number of genes for secondary metabolism, such as 24 nonribosomal peptide synthase genes, six of which exceed 25 kbp, and seven polyketide synthase genes, providing evidence that *Rhodococci* harbor an extensive secondary metabolism such as siderophores, cell signaling molecules, pigments, and antibiotics. This discovery was surprising due to the small number of reported secondary metabolites from *Rhodococcus* [21]. In the past, nonribosomal peptides from some organisms have been developed into drugs that include vancomycin, daptomycin, cyclosporine A, β-lactams and teicoplani. Likewise, polyketides have been very important in medicine as well and have given rise to drugs like erythromycin and tetracycline. This discovery makes the researcher to think for the ability of *Rhodococcus* for producing new antibiotics [29].

5. Metabolites production by genus *Rhodococcus*

5.1. Antimicrobial agents

About 23,000 antibiotics have been discovered from microorganisms. It has been estimated that approximately 10,000 of them were isolated from actinomycetes [42]. Actinomycetes, such as the genus *Rhodococcus*, have the ability to produce a wide variety of bioactive secondary metabolites, including antibiotics [29].

The first antimicrobial compounds isolated from *Rhodococcus* made by Chiba *et al*. 1999, their study reported five novel cyclic tetrapeptides exhibiting anti-fungal activity against *Candida albicans* and *Cryptococcus neoformis* but showed no antibacterial activity [43]. Chiba *et al*. named these compounds, rhodopeptin C1, C2, C3, C4, and B5, which was isolated from *Rhodococcus* sp. Mer-N1033, and it was isolated from a soil sample collected at Mt. Hayachine, Iwate Prefecture, Japan [43] (Fig. 1).



Rhodopeptin C1 R_1= CH_3 R_2= NH_2 R_3= CH_3 R_4= CH_3
Rhodopeptin C2 R_1= CH_2-CH_3 R_2= NH_2 R_3= CH_3 R_4= CH_3
Rhodopeptin C3 R_1= CH_3 R_2= NH_2 R_3= H R_4= CH-$(CH_3)_2$
Rhodopeptin C4 R_1= CH_3 R_2= NH_2 R_3= H R_4= CH (CH_3)-C_2H_5
Rhodopeptin B5 R_1= CH_3 R_2= CH_2-NH_2 R_3= H R_4= CH_2-CH $(CH_3)_2$

Fig 1**:** Structures of rhodopeptin C1, C2, C3, C4, and B5

In 2007, Iwatsuki *et al*. discovered two anti-mycobacterial peptides with a lasso structure, named lariatins A and B, produced by *R. jostii* K01-B0171 which was isolated from a soil sample in Yunnan, China [44]. Both lariatins had antimycobacterial properties against *Mycobacterium smegmatis with* MIC values of 3.13 and 6.25 mg/ml in agar dilution method, respectively. Furthermore, lariatin A inhibited the growth of *Mycobacterium tuberculosis* with an MIC of 0.39 mg/ml in liquid microdilution method [44] (Fig. 2).

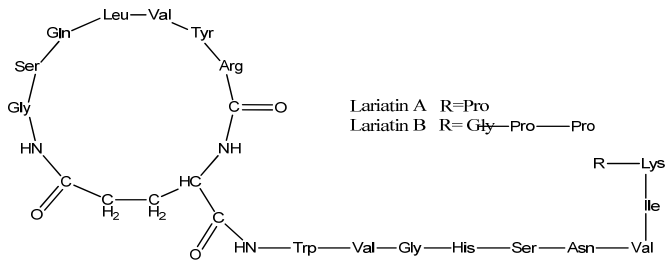


Fig 2: The structures of lariatins A and B.

In 2008, Kitagawa and Tamura [45] screened about eighty *Rhodococcus* strains acquired from Japanese and German culture collections. Fifteen *R. erythropolis* strains were characterized in their study as antibiotic producers and classified into three groups according to their antibiotic spectrum: Group I exhibited antibiotic activity against a broad range of Gram-positives; Group II, mainly against the genus *Rhodococcus* and some other Gram-positives; and Group III, particularly against *R. erythropolis*. Their results demonstrated that the genus comprises diverse antibiotic producers, and is a good source of new antibiotics [45].

Kitagawa and Tamura [20] continued working on the structure and identification of Group I antibiotics producing *Rhodococcus* strains, they isolated a new quinoline antibiotic, aurachin RE from *R. erythropolis* JCM 6824. The aurachin RE has a very similar structure to aurachin C, an antibiotic derived from *Stigmatella aurantiaca* Sga15 (Fig. 3). Both antibiotics were found to inhibit the growth of a wide range of gram positive organisms, though aurachin RE has a much stronger antimicrobial activity [20].

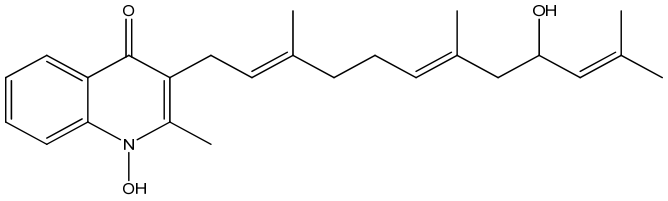


Fig 3: The structure of aurachin RE.

In 2010 another Germane team [46] investigated the freshly isolated actinomycete strains (*Rhodococcus sp.* Acta 2259) from selected terrestrial and limnetic habitats, and isolated four aurachins compound, which showed growth inhibitory activities against numerous Gram-positive bacteria such as *Staphylococcus epidermidis* DSM 20044, *Bacillus subtilis* DSM 347 and *Propionibacterium acnes* DSM 1897. But Gram-negative bacteria, such as *E. coli* K12 DSM 498, *Pseudomonas fluorescens* NCIMB 10586, *P. aeruginosa* DSM 50071 and *Xanthomonas campestris* DSM 2405 were not susceptible to aurachin Q (1), aurachin D (2), aurachin R (3), and aurachin C (4) (Fig. 4) [46].

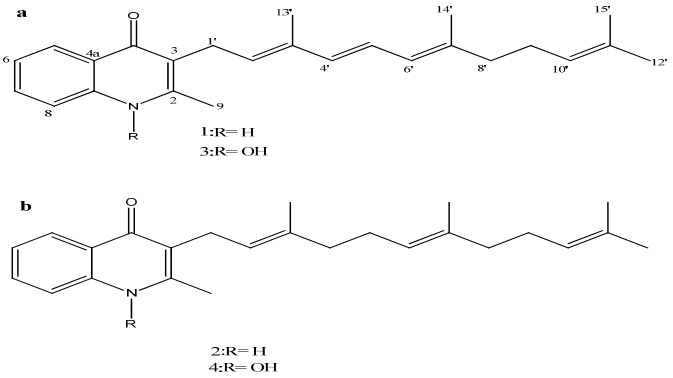


Fig 4: Structures of aurachin Q (1) and R (3) (a), and aurachin D (2) and C (4) (b).

In 2008 Kurosawa *et al*. isolated two antibiotics, named rhodostreptomycinn A and B, from culture broths of *R. fascians* 307CO. These antibiotics biosynthesized in the *Rhodococcus* following horizontal gene transfer from the *Streptomyces,* they are 2 isomers of a new class of aminoglycosides antibiotics and differ widely in the structure from actinomycins, polypeptide antibiotics that are produced by *Streptomyces* [47]. Rhodostreptomycins exhibited good antibiotic activities against an extensive range of Gram-negative and Gram-positive bacteria such as: *Streptomyces padanus, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Helicobacter pylori.* The activity of rhodostreptomycin B is more potent than that of rhodostreptomycin A (Fig. 5), suggesting that the difference in stereochemistry between (A) and (B) influences the biological activity [47].

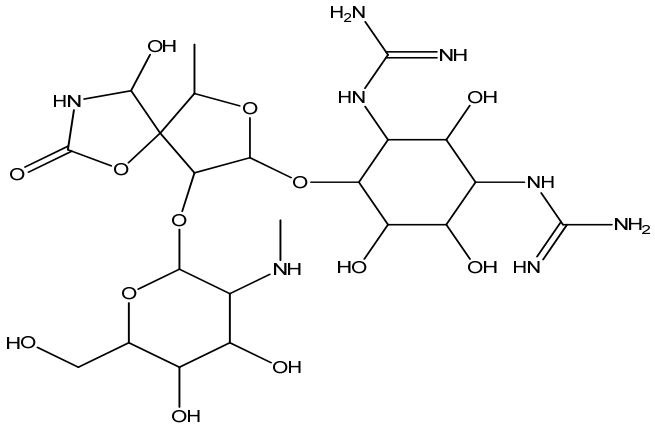
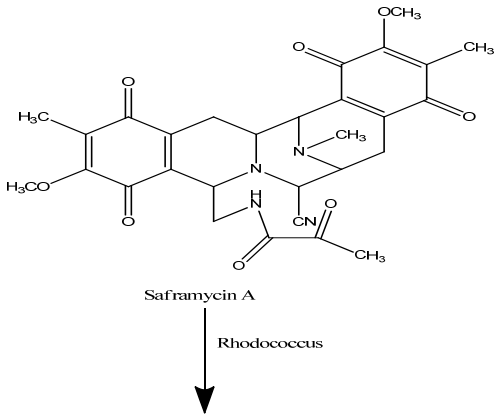


Fig 5: Rhodostreptomycin

In 1982 Yazawa *et al*. studied the microbial conversions of saframycin A (Fig. 6). Saframycins belonging to the tetrahydroisoquinoline family of antibiotics, are a group of microbial natural products isolated from *Streptomyces lavendulae* strain 314 [48]. Saframycin A, has antiproliferative activity against a variety of tumor cell lines at low doses, is one of the most potent members of this class of compounds [48-50]. Yazawa *et al*. worked on reduction of the ketone group of the pyruvoyl amine side chain of saframycin A, the reduction occurred specifically in some genera of actinomycetes: (*Nocardia, Rhodococcus, Mycobacterium*) and they founded that *Rhodococcus* sp. has ability to conversion of saframycin A to three products, saframycins AR, AR2 and AR3 [51].



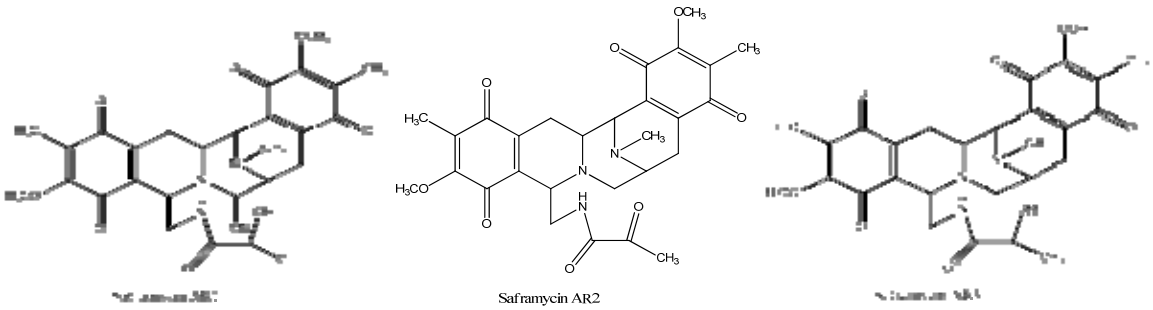


Fig 6: Conversion of saframycin A by *Rhodococcus* to three products, saframycins AR1, AR2 and AR3

In 2011 Borisova had twenty-four new *Rhodococcus* strains, isolated from soils in East Tennessee and screened these strains for antibiotic production. One strain MTM3W5.2 produced a large zone of inhibition against *R. erythropolis*. The antimicrobial compound produced by MTM3W5.2 had a large MW of 911.5452 Da and acts much like a bacteriocin but no amino acids were detected in this molecule based on TLC analysis made by Borisova. Bacteriocins are compounds similar to antibiotics, produced by some organisms to inhibit the growth of other organisms in their environment in competition for nutrients. What distinguishes them from antibiotics is that they are usually peptides synthesized by ribosomes and have a narrow spectrum of activity [52]. Currently, not much is known about bacteriocins produced by *Rhodococcus* [29].

In 2012 Lee *et al*. isolated significant amounts of indole-acetaldehyde and indole-3-acetic acid from the spent media of the plant pathogen *Rhodococcus* sp. BFI 332 (Fig. 7), and the former of which reduced *E.coli, Staphylococcus aureus* and *Staphylococcus epidermidis* biofilm formation. Pathogenic biofilms have been associated with persistent infections due to their high resistance to antimicrobial agents. Overall, the study of Lee *et al*. suggested that indole derivatives are present in the Actinomycetes strains, can be used as biofilm inhibitors against pathogenic bacteria [53].

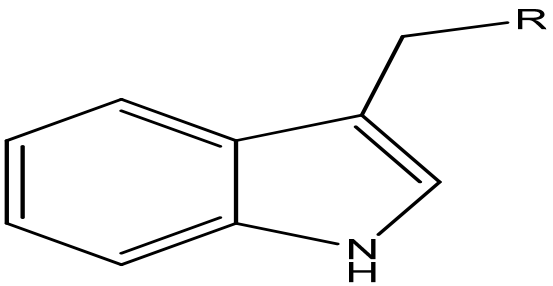


Fig 7: Indole-3-acetaldehyde R=CHO and Indole -3-acetic acid R=COOH

In 2016 Undabarrena *et al*. isolated actinobacteria (10genera) included *Rhodococcus* sp. from marine sediments, these actinobacteria tested against both Gram negative *Pseudomonas aeruginosa, Escherichiacoli* and *Salmonella enterica* and Gram-positive *S.aureus*, *Listeri amonocytogenes*. Crude extract of *Rhodococcus* sp.H-CA8f in Undabarrena *et al*. study showed an antimicrobial effect against all bacteria tested [54].

Cheng *et al*. 2015 isolated 64 actinomycetes from 12 different marine sponge species that had been collected from offshore island, eight of them displayed bioactivities against *Trypanosma brucei brucei* TC221 with half maximal inhibitory concentration (IC_{50}) values <20 µg/ml (Table. 1) [55].

Table 1: Anti-trypanosomal activities of sponge associated actinomycetes

Isolate	IC_{50} (48h) µg/ml	IC_{50} (72h) µg/ml
Streptomyces sp. SBT344	<10	<10
Streptomyces sp. SBT348	16.52	20.50
Modestobacter sp. SBT362	19.34	21.28
Modestobacter sp. SBT363	<10	<10
Nonomuraea sp. SBT364	<10	<10
Rhodococcus sp. SBT367	*19.97*	*22.37*
Geodermatophilus sp. SBT381	18.60	21.36
Micromonospora sp. SBT687	14.87	19.95

5.2 Omega-3 fatty acids

In 2015 Blakie worked on *R. opacus* PD630, which known for their ability to accumulate up to 87% of its cell dry weight as fatty acids. These fatty acid accumulations make *R. opacus* PD630 highly desirable as a biotechnological producer strain of Omega-3 fatty acids (ω-3 FA). However, the primary source of (ω-3 FA) is declining fish stocks. A more sustainable alternative is the cultivation of marine bacteria; nevertheless, the yields to date are less than favorable compared with those of fish. Blakie founded that oleaginous bacteria, when genetically engineered with the 16-27 kb pfaA-E gene cluster that encodes ω-3 FA synthesis, may potentially overcome this yield deficiency [56].

5.3 *Rhodococcus* Pigments

Biological pigments (such as carotenoids) are substances produced by different types of organisms. The crucial roles of carotenoids and their metabolites in photo oxidative protection and photosynthesis, not to mention nutrition, vision, and cellular differentiation, make them an important and complex class of biological pigments [57].

One such non-photosynthetic bacterium producing carotenoids is *Rhodococcus*. In 1989, Ichiyama *et al*. studied carotenoid pigments of the genus *Rhodococcus*. According to carotenes contained, *Rhodococcus* species were divided in Ichiyama *et al*. study into three groups: the first group formed beta-carotene; the second group formed gamma-carotene-like substance; and the third group formed neither carotene [58].

In 2011 Osawa *et al*. [59] isolated and identified a novel antioxidative carotenoids, OH-chlorobactene glucoside (1), OH-γ-carotene glucoside (2), OH-4-keto-γ-carotene glucoside hexadecanoate (3) and OH-chlorobactene glucoside hexadecanoate (4) from *Rhodococcus sp. CIP*, which showed potent antioxidative activities (Fig. 8) [59].

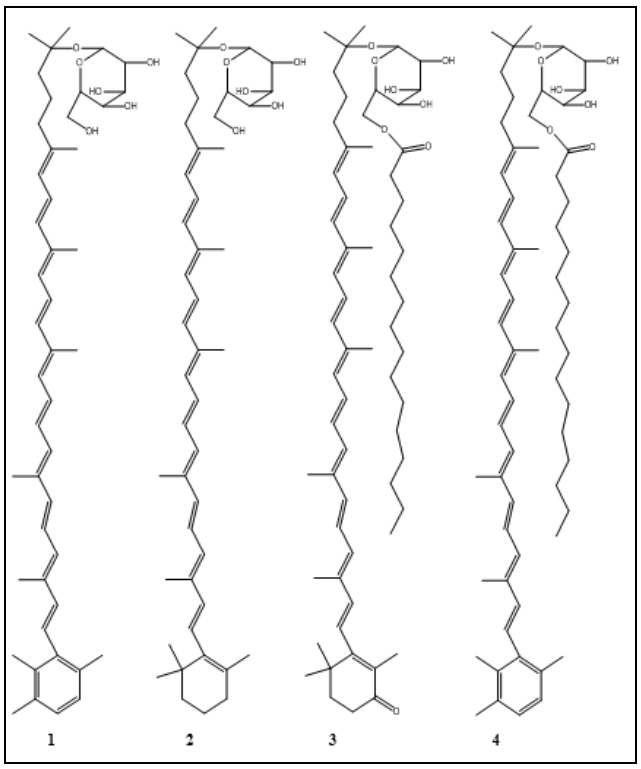


Fig 8: Structures of the carotenoids produced by *Rhodococcus* sp. CIP [59].

5.4 Siderophores

Siderophores (from the Greek: "iron carriers") are defined as relatively low molecular weight, ferric ion specific chelating agents elaborated by bacteria and fungi growing under low iron stress. The role of these compounds is to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cell [60]. In the past, three classes of siderophores have been described based on their chemical components: catecholates, hydroxamates, and hydroxyl carboxylates [61].

In 2001, Carrano *et al*. [62] discovered a new class of siderophores isolated from *R. erythropolis* IGTS8, which named heterobactins (Fig. 9). These siderophores consist of a tripeptide of sequence (N-OH)-L-Orn-Gly-D-Orn-(δ-*N* dihydroyxbenzoate). The alpha amino group of the D-Orn is derivatized either as a 2-hydroxybenzoxazolate in heterobactin A or remains free in heterobactin B [62].

In 2007, Dhungana *et al*. isolated a new siderophore rhodobactin (Fig. 10) from iron-deficient cultures of *R. rhodochrous* strain OFS. Rhodobactin is a mixed ligand hexadentate siderophore containing 1 hydroxamate and 2 nonequivalent catecholate moieties for iron chelation [63].

In 2008 Miranda *et al*. worked on the pathogenic *R. equi*., Miranda team discovered that during growth at low iron concentrations, *R. equi* produce a chromophore that the team hypothesized to be an iron-siderophore complex [64].

Bosello *et al*. 2011, had a unique mixed-type catecholate-hydroxamate siderophore isolated from *R. jostii* RHA1 and named it rhodochelin. Structural analysis of rhodochelin revealed that is a branched tetrapeptide composed of a 2,3-DHB, threonine, and 2 moieties of *δ-N*-formyl-*δ-N*-hydroxyornithine (Fig. 11) [61].

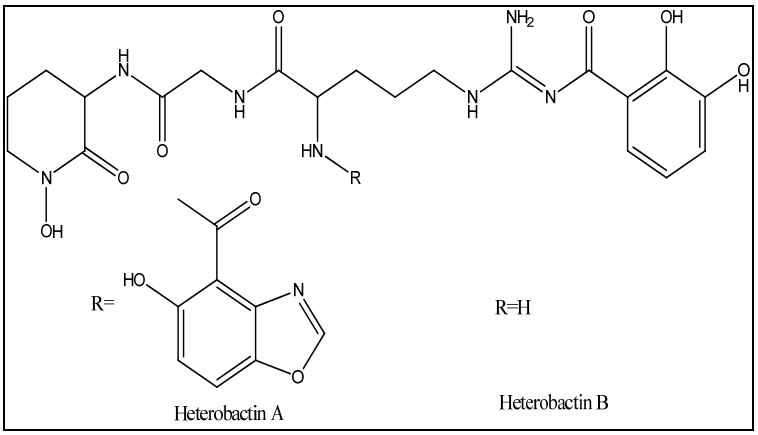


Fig 9: Structure of heterobactin A and B**.**

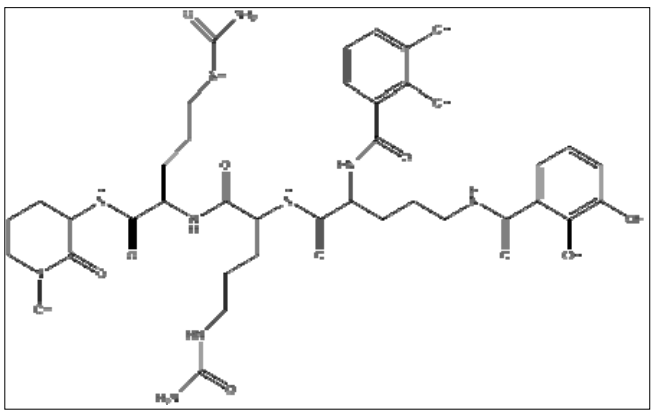


Fig 10: Structure of Rhodobactin.

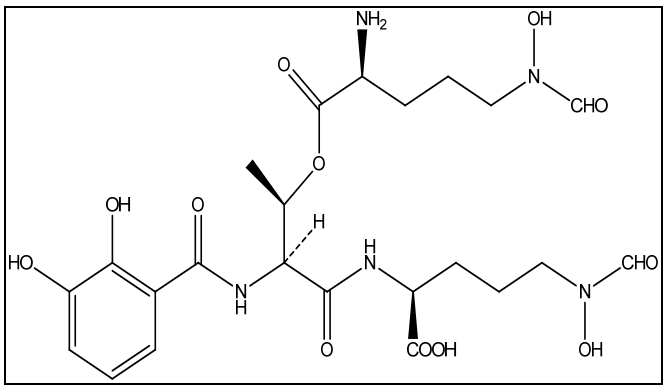


Fig 11: Structure of Rhodochelin.

5.5 Miscellaneous

5.5.1 α-glucosidases inhibitor

In 2008 Wei and Yu on the basis of the structure of 4\,7,8-trihydroxyisoflavone 7- O-α-D-arabinofuranoside (Fig. 12), a *Rhodococcus* metabolite showing potent inhibitory activities against the α-glucosidases of rat liver microsome (IC_{50}= 0.46 ng/mL, that is comparable to the literature value of 14 ng/mL. [65].

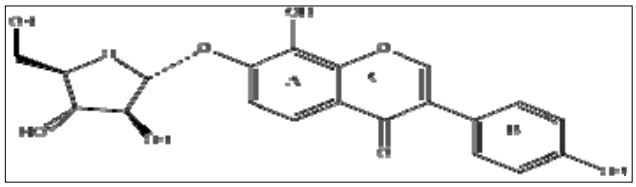


Fig 12: 7- O-α-D-arabinofuranoside

5.5.2 Mycothiol

The pseudodisaccharide mycothiol (Fig. 13) is present in millimolar levels as the dominant thiol in most species of actinomycetales, which include *Rhodococcus*. The primary role of mycothiol is to maintain the intracellular redox homeostasis. As such, it acts as an electron acceptor/donor and serves as a cofactor in detoxification reactions for alkylating agents, free radicals and xenobiotics. In addition, like glutathione, mycothiol may be involved in catabolic processes with an essential role for growth on recalcitrant chemicals such as aromatic compounds [66].

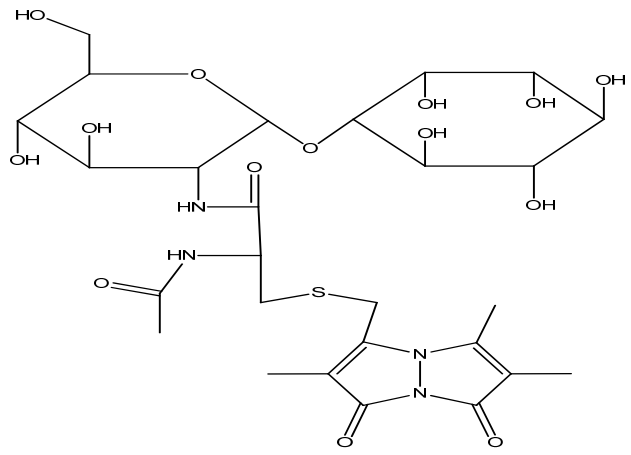


Fig 13: Pseudodisaccharide mycothiol.

5.5.3 Microbial conversion of chalcones

The biological activity of flavonoids and their derivatives has been studied for several years. It was proved that consumption of flavonoids in our daily diet considerably reduces the risk of developing certain types of cancer. Epidemiological observations indicate also that the diet rich in isoflavonoids reduces risk of breast cancer in women and prostate cancer in men. Chalcones themselves show interesting biological properties, including antioxidant, anti-inflammatory and anticancer ones [67, 68], On the other hand, their hydrogenated derivatives dihydrochalcones may be used as substrates for synthesis of propafenone derivatives, which have higher anticancer properties than starting chalcones [69]. Stompor *et al*. 2016 screened the ability of *Rhodococcus* strains to convert chalcones. *Rhodococcus in* Stompor *et al*. study, catalyzed the reduction of C=C bond in the chalcones to give respective more potent anticancer dihydrochalcones [70].

5.5.4 3-hydroxypropionic acid

In 2009 Lee *et al*. isolated a novel microorganism, designated as *R. erythropolis* LG12 from soil based on its ability to use acrylic acid as the sole carbon source. The effect of the culture conditions of *R. erythropolis* LG12 on the production of 3-hydroxypropionic acid (3HP) from acrylic acid was also examined in Lee *et al*. study [71]. 3-Hydroxypropionic acid (3HP) is using as an intermediate for the synthesis of many commercially valuable chemicals, including 1,3-propanediol, malonic acid, acrylic acid, acrylonitrile, and acrylamide, all of which were used in large quantities for the production of adhesives, polymers, plastic packing, fibers, cleaning agents, and resins [72].

Another *Rhodococcus* strain has also special characteristic it is *R. opacus* strains, has a strong innate tolerance to benzene, toluene and lignocellulosic hydrolysates from different sources [73, 74] and can metabolize aromatic compounds such as lignin [75, 76]. Lignin-derived (e.g. phenolic) compounds can compromise the bioconversion of lignocellulosic biomass to fuels and chemicals due to their toxicity and recalcitrance. In 2016 Yoneda *et al*. studied the lipid-accumulating character of bacterium *R. opacus* PD630, which has recently emerged as a promising microbial host for lignocellulose conversion to value-added products due to its natural ability to tolerate and utilize phenolics [77].

6. *Rhodococcus* in industry

The actinomycete genus *Rhodococcus* is of interest for a variety of reasons. The most industrially important genus of actinomycetes is arguably *Rhodococcus.* Applications of *Rhodococci* include bioactive steroid production, fossil fuel biodesulfurization, and the production of acrylamide and acrylic acid, the most commercially successful application of a microbial biocatalyst [21]. 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 [28]. Because of it has many enzymes that allow them to carry out a number of chemical reactions that have been useful in the environmental and industrial biotechnology fields [78]. Consequently, *Rhodococcus* cells have 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 so it great considered *Rhodococcus* is surfactant production [79].

Another feature of *Rhodococci* is their ability to 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 [28].

Some of *Rhodococcus* species have been shown to produce a number of commercially interesting and potentially useful products such as *R. rhodochrous, R. rhodochrous* J1 is used by the Nitto Chemistry Industry Company Ltd (Japan) to produce over 30 000 tons of acrylamide annually. This is said to be the first instance of successful industrial production of a commodity chemical using a microbe. The use of bacterial enzymes in the production of these chemicals is favored over synthetic processes because the products are more pure and can be made without the production of unwanted byproducts [29]. Another strains such as *R. erythropolis* are capable of carrying out several reactions such as dehydrogenation, hydrolysis, oxidation, desulfurization, hydroxylation, dehalogenation, and epoxidation [78].

6.1. Examples of enzymes produced by *Rhodococcus* genus

1) Alkene monooxygenase (AMO)

R. rhodochrous can grow using propene and certain other aliphatic alkenes as its sole source of carbon and energy. The first enzyme in the pathway for alkene metabolism is alkene monooxygenase (AMO). AMO catalysis the stereo selective epoxygenation of aliphatic alkenes, yielding primarily R enantiomers [80].

2) Limonene-1, 2-epoxide hydrolase

An epoxide hydrolase from *R. erythropolis* DCL14 catalyzes the hydrolysis of limonene-1, 2-epoxide to limonene-1, 2-diol. The enzyme is induced when *R. erythropolis* is grown on monoterpenes, reflecting its role in the limonene degradation pathway of this microorganism [81].

3) Naphthalene 1, 2-dioxygenase

Members of the genus *Rhodococcus* were found in many environmental niches and have a remarkable ability to metabolize a wide variety of xenobiotic compounds. Naphthalene is released into the environment as coal tar and coal tar products such as creosote, The catabolism of naphthalene by *Rhodococcus strain B4* to the metabolites salicylic acid and gentisic acid has been reported in Larkin *et al*. study [82].

4) 6-Oxocineole dehydrogenase

In 1989 Williams *et al*. isolated *Rhodococcus sp.* by elective culture with 1, 8-cineole as sole carbon source. 6-endo-Hydroxycineole and 6-oxocineole accumulated transiently during the latter part of the exponential growth phase and, together with 1, 8-cineole, were oxidized rapidly by the enzyme from *Rhodococcus C1* [83].

5) Alpha Mannanase

In 1988 Zacharova *et al*. described the production, purification, and characteristics of α-Mannanase from *R. erythropolis*. It uses to catalysis hydrolyses of terminal non-reducing alpha-D-mannosides and also hydrolyses alpha-D-lyxosides and heptopyranosides as mannose [84].

6) Endoglycoceramidases

In 1989 Ito *et al*. described purification and characterization of glycosphingolipid-specific endoglycosidases (endoglycoceramidases) from a culture fluid of the mutant strain of M-750 *Rhodococcus sp*., This enzyme catalyses hydrolyses of oligosaccharylceramide to produce an oligosaccharide and aceramide [85].

7) Phenylalanine dehydrogenase

Misono *et al*. 1989 purified to homogeneity NAD+-dependent phenylalanine dehydrogenase from a crude extract of *R. mans* K-18 isolated from soil. The enzyme catalyzed the oxidative deamination of L-phenylalanine and several other L-amino acids and the reductive amination of phenylpyruvate and p-hydroxyphenylpyruvate [86].

8) 3-Ketosteroid-Δ1-dehydrogenase

The gene encoding 3-ketosteroid-Δ1-dehydrogenase enzyme from *R. rhodochrous* was cloned, sequenced, ligated and introduced into *E. coli* cells by Morii, S. *et al*. in 1998. The transformed cells hyperexpressed the 3-ketosteroid-Δ1-dehydrogenase as an active and soluble protein at more than 30 times the level of *R. rhodochrous* cells. The purified recombinant dehydrogenase exhibited identical molecular and catalytic properties to the *R. rhodochrous* enzyme [87].

9) Novel Self-Sufficient Styrene Monooxygenase

In 2009 Tischler *et al*. identified a novel self-sufficient styrene monooxygenase from *R. opacus* 1CP. This discovery overcome the necessity for expensive cofactors which was a more striking drawback limited the industrial application of flavoprotein monooxygenases enzyme which used by microorganisms as a common initial step of the aerobic degradation of aromatic compounds [88].

10) N-Oxygenase enzyme

Recently, Indest *et al*. expressed and characterized N-oxygenase enzyme from *R. jostii* RHAI, this importance related to the fact of small number of nitro group-containing natural products founded in nature with fewer than 200 known, and also few examples of N-oxygenases, enzymes that incorporate atmospheric oxygen into primary and secondary amines in synthetically manufactured of alkaloids compound [89].

7. Conclusion and future perspectives

Actinomycetes are one of the most attractive sources of new natural products discovery. They have provided many important bioactive compounds of high therapeutics values, these bioactive compounds related to various classes of natural products. Although, *Rhodococcus* is actinobacteria meaningful for its environmental and industrial biotechnology applications, interest of *Rhodococcus* in natural product discovery field has increased due to the discovery of a large number of genes for secondary metabolism. However, only few secondary metabolites have been characterized from the *Rhodococci*, it's may be due to the production of antimicrobial compounds by organisms is very dependent on the environmental conditions in which the organism was grown, so, the establish of new protocols to facilitate the recovery, cultivation and isolation of novel *Rhodococcus* species their metabolites must be a big challenge and occupies a great place in future researches.

8. References

1. Yoshida A *et al*. Actinomycetal community structures in seawater and freshwater examined by DGGE analysis of 16S rRNA gene fragments. Mar Biotechnol (NY), 2008; 10(5):554-63.
2. Ventura M *et al*. Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev, 2007; 71(3):495-548.
3. Choi H *et al*. Microbial community composition in the marine sediments of Jeju Island: next-generation sequencing surveys. J Microbiol Biotechnol, 2016.
4. Rodrigues RR *et al*. Plant invasions associated with change in root-zone microbial community structure and diversity. PLoS One, 2015; 10(10):e0141424.
5. Pawlowski K, Demchenko KN. The diversity of actinorhizal symbiosis. Protoplasma, 2012; 249(4):967-79.
6. Berlanga M. Functional symbiosis and communication in microbial ecosystems. The case of wood-eating termites and cockroaches. Int Microbiol, 2015; 18(3):159-69.
7. Kurtboke DI *et al*. Eco-taxonomic insights into actinomycete symbionts of termites for discovery of novel bioactive compounds. Adv Biochem Eng Biotechnol, 2015; 147:111-35.
8. Abdelmohsen UR, Bayer K, Hentschel U. Diversity,

abundance and natural products of marine sponge-associated actinomycetes. Nat Prod Rep. 2014; 31(3):381-99.
9. Mahmoud HM, Kalendar AA. Coral-associated actinobacteria: diversity, abundance, and biotechnological potentials. Front Microbiol, 2016; 7:204.
10. Van Goethem MW *et al*. Characterization of bacterial communities in lithobionts and soil niches from Victoria Valley, Antarctica. FEMS Microbiol Ecol, 2016; 92(4).
11. Crits-Christoph A *et al*. Phylogenetic and functional substrate specificity for endolithic microbial communities in hyper-arid environments. Front Microbiol, 2016; 7:301.
12. Eltamany EE *et al*. New antibacterial xanthone from the marine sponge-derived Micrococcus sp. EG45. Bioorg Med Chem Lett, 2014.
13. Hentschel U *et al*. Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges Aplysina aerophoba and Aplysina cavernicola. FEMS Microbiol Ecol, 2001; 35(3):305-312.
14. Zheng Z *et al*. Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. FEMS Microbiol Lett, 2000; 188(1):87-91.
15. Mann J. Natural products as immunosuppressive agents. Nat Prod Rep, 2001; 18(4):417-30.
16. Umezawa H. Low-Molecular-Weight Enzyme-Inhibitors of Microbial Origin. Annual Review of Microbiology, 1982; 36:75-99.
17. Boonlarppradab C *et al*. Marineosins A and B, cytotoxic spiroaminals from a marine-derived actinomycete. Organic letters, 2008; 10(24):5505-5508.
18. Oh D-C *et al*. Salinipyrones and pacificanones, mixed-precursor polyketides from the marine actinomycete Salinispora pacifica. Journal of natural products. 2008; 71(4):570-575.
19. Asolkar RN *et al*. Arenimycin, an antibiotic effective against rifampin-and methicillin-resistant Staphylococcus aureus from the marine actinomycete Salinispora arenicola. The Journal of antibiotics. 2010; 63(1):37.
20. Kitagawa W, Tamura T. A quinoline antibiotic from Rhodococcus erythropolis JCM 6824. J Antibiot (Tokyo), 2008; 61(11):680-2.
21. McLeod MP *et al*. The complete genome of Rhodococcus sp. RHA1 provides insights into a catabolic powerhouse. Proc Natl Acad Sci U S A, 2006; 103(42):15582-7.
22. Van der Geize R, Dijkhuizen L. Harnessing the catabolic diversity of rhodococci for environmental and biotechnological applications. Curr Opin Microbiol, 2004; 7(3):255-61.
23. Lechevalier. In Bergey's Manual of Systematic Bacteriology. 1989; 4:2348-2350.
24. Goethals K *et al*. Leafy gall formation by Rhodococcus fascians. Annu Rev Phytopathol, 2001; 39:27-52.
25. Muscatello G *et al*. Rhodococcus equi infection in foals: the science of 'rattles'. Equine Vet J. 2007; 39(5):470-8.
26. Giles C *et al*. An adenoviral vector based vaccine for Rhodococcus equi. PLoS One, 2016; 11(3):e0152149.
27. Goodfellow M, Alderson G. The actinomycete-genus Rhodococcus: a home for the rhodochrous complex. J Gen Microbiol. 1977; 100(1):99-122.
28. Bell KS *et al*. The genus Rhodococcus. J Appl Microbiol. 1998; 85(2):195-210.
29. Borisova RB. Isolation of a Rhodococcus Soil Bacterium that Produces a Strong Antibacterial Compound. 2011.
30. Yassin AF, Schaal KP. Reclassification of Nocardia corynebacterioides Serrano *et al*. 1972 (Approved Lists 1980) as Rhodococcus corynebacterioides comb. nov. Int J Syst Evol Microbiol. 2005; 55(3):1345-8.
31. Goodfellow M. Reclassification of Corynebacterium-Fascians (Tilford) Dowson in the Genus Rhodococcus, as Rhodococcus-Fascians Comb Nov. Systematic and Applied Microbiology. 1984; 5(2):225-229.
32. Jones AL *et al*. Rhodococcus gordoniae sp. nov., an actinomycete isolated from clinical material and phenol-contaminated soil. Int J Syst Evol Microbiol, 2004; 54(2):407-11.
33. Takeuchi M *et al*. Rhodococcus jostii sp. nov., isolated from a medieval grave. Int J Syst Evol Microbiol, 2002; 52(2):409-13.
34. Helmke E, Weyland H. Rhodococcus-Marinonascens Sp-Nov, an Actinomycete from the Sea. International Journal of Systematic Bacteriology. 1984; 34(2):127-138.
35. Klatte S, Kroppenstedt RM, Rainey FA. Rhodococcus Opacus Sp-Nov, an Unusual Nutritionally Versatile Rhodococcus-Species. Systematic and Applied Microbiology, 1994; 17(3):355-360.
36. Tsukamura M. A further numerical taxonomic study of the rhodochrous group. Jpn J Microbiol. 1974; 18(1):37-44.
37. Yassin AF. Rhodococcus triatomae sp. nov., isolated from a blood-sucking bug. Int J Syst Evol Microbiol. 2005; 55(4):1575-9.
38. Matsuyama H *et al*. Rhodococcus tukisamuensis sp. nov., isolated from soil. Int J Syst Evol Microbiol. 2003; 53(5):1333-7.
39. Goodfellow M *et al*. Transfer of Tsukamurella wratislaviensis Goodfellow et a. 1995 to the genus Rhodococcus as Rhodococcus wratislaviensis comb. nov. Int J Syst Evol Microbiol, 2002; 52(3):749-55.
40. Zhang YQ *et al*. Rhodococcus yunnanensis sp. nov., a mesophilic actinobacterium isolated from forest soil. Int J Syst Evol Microbiol. 2005; 55(3):1133-7.
41. Stoecker MA, Herwig RP, Staley JT. Rhodococcus zopfii sp. nov., a toxicant-degrading bacterium. Int J Syst Bacteriol. 1994; 44(1):106-10.
42. Okami YHK. Search and discovery of new antibiotics. In:Goodfellow M, Williams ST, Mordarski M (eds) Actinomycetes in biotechnology. 1988, 33-67.
43. Chiba H *et al*. Rhodopeptins (Mer-N1033), novel cyclic tetrapeptides with antifungal activity from Rhodococcus sp. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. J Antibiot (Tokyo). 1999; 52(8):695-9.
44. Iwatsuki M *et al*. Lariatins, novel anti-mycobacterial peptides with a lasso structure, produced by Rhodococcus jostii K01-B0171. J Antibiot (Tokyo). 2007; 60(6):357-63.
45. Kitagawa W, Tamura T. Three Types of Antibiotics Produced from Rhodococcus erythropolis Strains. Microbes Environ. 2008; 23(2):167-71.
46. Nachtigall J *et al*. Two new aurachins from Rhodococcus sp. Acta 2259. J Antibiot (Tokyo), 2010; 63(9):567-9.
47. Kurosawa K *et al*. Rhodostreptomycins, antibiotics biosynthesized following horizontal gene transfer from Streptomyces padanus to Rhodococcus fascians. J Am Chem Soc. 2008; 130(4):1126-7.
48. Li L *et al*. Characterization of the saframycin A gene cluster from Streptomyces lavendulae NRRL 11002

revealing a nonribosomal peptide synthetase system for assembling the unusual tetrapeptidyl skeleton in an iterative manner. J Bacteriol. 2008; 190(1):251-63.
49. Arai T *et al*. Some chemotherapeutic properties of two new antitumor antibiotics, saframycins A and C. Gan, 1980; 71(6):790-6.
50. Mikami Y *et al*. Saframycin S, a new saframycin group antibiotic. J Pharmacobiodyn. 1981; 4(4):282-6.
51. Yazawa K *et al*. Bioconversions of saframycin A specific to some genera of actinomycetes. J Antibiot (Tokyo), 1982; 35(7):915-7.
52. Gillor O, Etzion A, Riley MA. The dual role of bacteriocins as anti- and probiotics. Appl Microbiol Biotechnol. 2008; 81(4):591-606.
53. Lee JH *et al*. Indole-3-acetaldehyde from Rhodococcus sp. BFI 332 inhibits Escherichia coli O157:H7 biofilm formation. Appl Microbiol Biotechnol, 2012; 96(4):1071-8.
54. Undabarrena A *et al*. Exploring the Diversity and Antimicrobial Potential of Marine Actinobacteria from the Comau Fjord in Northern Patagonia, Chile. Front Microbiol, 2016; 7:1135.
55. Cheng C *et al*. Biodiversity, Anti-Trypanosomal Activity Screening, and Metabolomic Profiling of Actinomycetes Isolated from Mediterranean Sponges. Plos One, 2015; 10(9).
56. Blakie B. Production of omega-3 fatty acids in Rhodococcus opacus PD630 Borom Blakie. University of Otago, Dunedin, New Zealand, 2015.
57. Armstrong GA, Hearst JE. Carotenoids 2: Genetics and molecular biology of carotenoid pigment biosynthesis. FASEB J. 1996; 10(2):228-37.
58. Ichiyama S, Shimokata K, Tsukamura M. Carotenoid pigments of genus Rhodococcus. Microbiol Immunol, 1989. 33(6): p. 503-8.
59. Osawa, A., *et al*., Isolation of a novel carotenoid, OH-chlorobactene glucoside hexadecanoate, and related rare carotenoids from Rhodococcus sp. CIP and their antioxidative activities. Biosci Biotechnol Biochem, 2011. 75(11): p. 2142-7.
60. Neilands Siderophores J. structure and function of microbial iron transport compounds. Journal of Biological Chemistry. 1995; 270(45):26723-26726.
61. Bosello M *et al*. Biosynthesis of the siderophore rhodochelin requires the coordinated expression of three independent gene clusters in Rhodococcus jostii RHA1. J Am Chem Soc. 2011; 133(12):4587-95.
62. Carran CJ *et al*. Heterobactins: A new class of siderophores from Rhodococcus erythropolis IGTS8 containing both hydroxamate and catecholate donor groups. Biometals, 2001; 14(2):119-25.
63. Dhungana S *et al*. Purification and characterization of rhodobactin: a mixed ligand siderophore from Rhodococcus rhodochrous strain OFS. Biometals, 2007; 20(6):853-67.
64. Miranda-Caso Luengo R *et al*. The intracellular pathogen Rhodococcus equi produces a catecholate siderophore required for saprophytic growth. Journal of Bacteriology. 2008; 190(5):1631-1637.
65. Wei G, Yu B. Isoflavone glycosides: Synthesis and evaluation as alpha-glucosidase inhibitors. European Journal of Organic Chemistry. 2008; (18):3156-3163.
66. Rawat M, Av-Gay Y. Mycothiol-dependent proteins in actinomycetes. FEMS Microbiol Rev, 2007; 31(3):278-92.
67. Abbas ANM, Hasan A, Hadda TB. Synthesis and cytotoxicity studies of 4-alkoxychalcones as new antitumor agents. J Mater Environ Sci. 2014; 5:281-292.
68. Rozmer Z, Perjesi P. Naturally occurring chalcones and their biological activities. Phytochemistry Reviews. 2016; 15(1):87-120.
69. Ivkovic BM *et al*. Phenylpropiophenone derivatives as potential anticancer agents: synthesis, biological evaluation and quantitative structure-activity relationship study. Eur J Med Chem. 2013; 63:239-55.
70. Stompor M, Kaluzny M, Zarowska B. Biotechnological methods for chalcone reduction using whole cells of Lactobacillus, Rhodococcus and Rhodotorula strains as a way to produce new derivatives. Appl Microbiol Biotechnol, 2016; 100(19):8371-84.
71. Lee SH *et al*. Production of 3-Hydroxypropionic Acid from Acrylic Acid by Newly Isolated Rhodococcus erythropolis LG12. Journal of Microbiology and Biotechnology. 2009; 19(5):474-481.
72. Brown SF. Bioplastic fantastic. Fortune, 2003; 148(2):92-4.
73. Wang BX *et al*. Cultivation of lipid-producing bacteria with lignocellulosic biomass: Effects of inhibitory compounds of lignocellulosic hydrolysates. Bioresource Technology. 2014; 161:162-170.
74. Na KS *et al*. Isolation and characterization of benzene-tolerant Rhodococcus opacus strains. J Biosci Bioeng. 2005; 99(4):378-82.
75. Kosa M, Ragauskas AJ. Bioconversion of lignin model compounds with oleaginous Rhodococci. Appl Microbiol Biotechnol. 2012; 93(2):891-900.
76. Zaitsev GM *et al*. Utilization of Halogenated Benzenes, Phenols, and Benzoates by Rhodococcus opacus GM-14. Appl Environ Microbiol, 1995; 61(12):4191-201.
77. Yoneda A *et al*. Comparative transcriptomics elucidates adaptive phenol tolerance and utilization in lipid-accumulating Rhodococcus opacus PD630. Nucleic Acids Res, 2016; 44(5):2240-54.
78. De Carvalho CCCR, da Fonseca MMR. The remarkable Rhodococcus erythropolis. Applied Microbiology and Biotechnology, 2005; 67(6):715-726.
79. Finnerty WR. The biology and genetics of the genus Rhodococcus. Annu Rev Microbiol, 1992; 46:193-218.
80. Smith TJ *et al*. Heterologous expression of alkene monooxygenase from Rhodococcus rhodochrous B-276. Eur J Biochem. 1999; 260(2):446-52.
81. Van der Werf MJ, Overkamp KM, de Bont JAM. Limonene-1, 2-epoxide hydrolase from Rhodococcus erythropolis DCL14 belongs to a novel class of epoxide hydrolases. Journal of Bacteriology. 1998; 180(19):5052-5057.
82. Larkin MJ *et al*. Purification and characterization of a novel naphthalene dioxygenase from Rhodococcus sp. strain NCIMB12038. J Bacteriol, 1999; 181(19):6200-4.
83. Williams DR, Trudgill PW, Taylor DG. Metabolism of 1, 8-Cineole by a Rhodococcus Species - Ring Cleavage Reactions. Journal of General Microbiology. 1989; 135:1957-1967.
84. Zacharova IY, Tamm VY, Pavlova IN. Alpha-Mannanase from Rhodococcus-Erythropolis. Methods in Enzymology. 1988; 160:620-626.
85. Ito M, Yamagata T. Purification and characterization of glycosphingolipid-specific endoglycosidases (endoglycoceramidases) from a mutant strain of Rhodococcus sp. Evidence for three molecular species of

endoglycoceramidase with different specificities. J Biol Chem. 1989; 264(16):9510-9.
86. Misono H *et al*. Purification and characterization of a dimeric phenylalanine dehydrogenase from Rhodococcus maris K-18. J Bacteriol, 1989; 171(1):30-6.
87. Morii S *et al*. 3-Ketosteroid-delta1-dehydrogenase of Rhodococcus rhodochrous: sequencing of the genomic DNA and hyperexpression, purification, and characterization of the recombinant enzyme. J Biochem, 1998; 124(5):1026-32.
88. Tischler D *et al*. Identification of a novel self-sufficient styrene monooxygenase from Rhodococcus opacus 1CP. J Bacteriol, 2009; 191(15):4996-5009.
89. Indest KJ, Eberly JO, Hancock DE. Expression and characterization of an N-oxygenase from Rhodococcus jostii RHAI. J Gen Appl Microbiol, 2015; 61(6):217-23.