MICROBIAL TRANSFORMATIONS OF NATURAL PRODUCTS

S. R. Gopishetty
Center for Biocatalysis and Bioprocessing, The University of Iowa, Iowa, U. S. A

M. T. Louie
Department of Chemical and Biochemical Engineering The University of Iowa, Iowa, U. S. A

M. V. Subramanian
*Center for Biocatalysis and Bioprocessing, * Department of Chemical and Biochemical Engineering The University of Iowa, Iowa, U. S. A*

Keywords: biotransformations, natural products, terpenes, steroids, alkaloids, caffeine, alkyl xanthines, theophylline, theobromine, demethylation, decaffeination.

Contents

Summary

Natural products are organic compounds that are made by living systems. Naturally occurring compounds may be divided into two board categories namely primary and secondary metabolites. Secondary metabolites have attracted attention because of their diverse biological effects on other organisms. Microorganisms which are one of the primitive forms of life show a great ability to adapt to a wide variety of natural products. They also have remarkable ability to make and carryout diverse reactions on natural products. Microorganisms have been widely applied for steroid biotransformations to prepare specific derivatives, the production of which is difficult

by traditional synthetic methods. As a major example of microbial metabolism/transformations a review on caffeine alkaloids is presented (this sentence is not clear; seems misplaced). Most of the population of the world is exposed to caffeine (1,3,7-trimethyl xanthine) since it occurs in a number of plants. Caffeine is also widely consumed as drinks, and has found limited therapeutic use. Caffeine is the major constituent of coffee and tea. Other methyl xanthines like theobromine (3, 7-dimethyl xanthine), theophylline (1,3-dimethyl xanthine) and mono-methylxanthines are also present in minor quantities in these beverages. Caffeine degrading microorganisms have great potential in developing decaffeination process, which will replace the use of toxic organic solvents. Other applications of microbial caffeine degradation include decontamination of these compounds in the environment and production of high value alkyl xanthines.

1. Introduction

Natural products are molecules derived from plants, marine organisms and microorganisms. They can be classified majorly into i. Polyketides and fatty acids, ii. Terpenoids, iii. Steroids, iv. Alkaloids and v. Phenolic compounds (see Figure 1). This review focuses on the microbial transformations of steroids, terpenoids, alkaloids, and flavonoids (phenolic compounds). Existence of microbes was recognized only in the 17th century by Dutch microscopist Anton Van Leeuwenhoek. By 1857, sufficient understanding had been developed to provide the necessary background for the work of Louis Pasteur on the fermentation of sugar to lactic acid and ethanol. Pasteur discovered that all fermentative processes are the result of microbial activity and that individual microbial species are responsible for discrete chemical alteration of selected substrates.

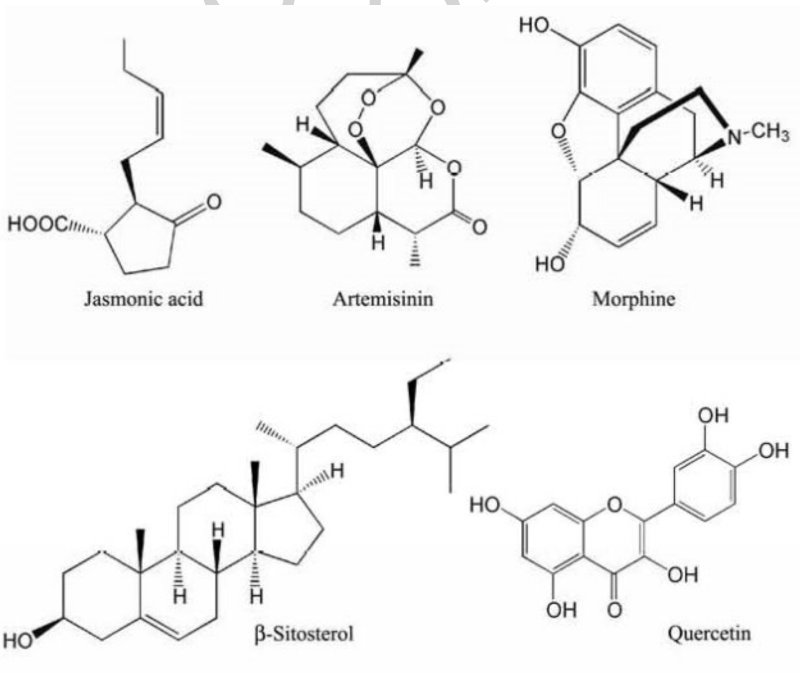

Figure 1. Representative Structures Natural Products

Targeted application of microbial transformations emerged only after the 19th century. The fusion of two sciences namely, organic chemistry and microbiology was the driver of tremendous growth of this field. The technology of microbial transformation deals with harnessing the enzymes in microorganisms to catalyze useful reactions on organic compounds. Great advances have been made in terms of exploiting microorganisms for biotransformations.

Many industrial processes for the production of amino acids and polysaccharides have been developed. The use of microorganisms for the synthesis of antibiotics and steroidal hormones evolved into large-scale industrial processes. Nowadays, a whole array of methods have been developed for using microorganisms in novel processes of transformations of both natural and synthetic bioactive compounds. Microbial transformations make use of enzyme catalyzed reactions with living cells, typically exploiting single chemical reactions like oxidation, reduction, hydrolysis, and degradation, formation of C-C or C-hetero atom bonds.

Some of the advantages in selecting microbial reactions as alternative or supplement to chemical synthesis are, i. microbial reactions can be used to functionalize specific positions in the molecules which are not normally possible by chemical methods, ii. Oxygen function or other substituent's can be introduced stereospecifically or regiospecifically. iii. Several individual reactions can be combined in one microbial step, iv. The conditions under which microbial reactions take place are mild; hence compounds that are sensitive to heat, acid and base become amenable to microbial transformations, v. In some cases, it is cheaper to use a microorganism for the preparation of organic compounds than to synthesize it chemically. Thus it is not surprising to note that a large number of antibiotics and several of the medicinally important steroids hormones are currently produced on a large scale by microbial processes.

Microbial transformations are of considerable economic importance in the manufacture of alkaloids, antibiotics, vitamins, amino acids, fermented beverages and fermented foods. They also catalyze simple and chemically well-defined reactions like conversion of acrylonitrile to acrylamide. This has matured to an industrial process where the production is carried out at 10,000 tons per year. In addition, microorganisms are employed in many studies of synthetic, structural, stereochemical and kinetic problems in organic chemistry to functionalize non-activated carbon atoms including (i) to introduce centers of chirality into optically inactive substrates, and (ii) to carry out optical resolutions of racemic mixtures.

Another feature of microbial transformations is its ability to imitate mammalian metabolism of drugs. Thus key intermediates or metabolites of drugs can be produced in adequate amounts rapidly. This enables structure determination of drug metabolites for use in preclinical trials, toxicity studies and regulatory process. Microorganisms do not always form the same metabolites as mammals; nevertheless, they are good models of drug metabolism. This approach has found wide success, that led Smith and Rosazza to coin the term "Microbial Models of Mammalian Metabolism", to describe the use of microbial transformation systems as tool to facilitate mammalian metabolic studies.

-
-
-

Bibliography

Bicas JL, Fontanille P, Pastore GM and Larroche C (2008). Characterization of monoterpene biotransformation in two pseudomonads, *J. Appl. Microbiol*. 105:1991-2001 [This article reports study of metabolic profile of *Pseudomonas rhodesiae* and *Pseudomonas fluorescens* in water–organic solvent systems using terpene substrates for both growth and biotransformation processes].

Boonstra B, Rathbone DA and Bruce NC (200)1. Engineering novel biocatalytic routes for production of semisynthetic opiate drugs, Biomol. Engineering. 18:41-47. [This article illustrates the biotransformation of morphine and codeine to the potent analgesic hydromorphone and the mild analgesic/antitussive hydrocodone, respectively, by recombinant *Escherichia coli*].

Cécile J. B van der Vlugt-Bergmans and Mariët J. van der Werf (2001). Genetic and biochemical characterization of a novel monoterpene ε-lactone hydrolase from *Rhodococcus erythropolis* DCL14, *Appl. Environ. Microbiol*.67:733-741.[This article reports biochemically and genetically characterization of a monoterpene α- lactone hydrolase from *R. erythropolis* DCL14 is a novel enzyme involved in the degradation of monoterpenes].

Das S and Rosazza J.P.N (2006), Microbial and enzymatic transformations of flavonoids. *J. Nat.Prod.* 69:499-508. [This article reviews flavonoids in microbial systems].

Dash, SS and Gummadi SN (2006). Catabolic pathways and biotechnological applications of microbial caffeine degradation, *Biotechnol Lett.* 28:1993-2002.[This article reviews caffeine degradation in microbial system and its biotechnological applications].

Dhavalikar RS and Bhattacharyya PK (1966). Microbiological transformations of terpenes. 8. Fermentation of limonene by a soil pseudomonad. *Indian J Biochem*. 3:144–157. [This article reports the transformation of limonene by a pseudomonad].

Duetz WA, Bouwmeester H, van Beilen JB and Witholt B (2003).Biotransformation of limonene by bacteria, fungi, yeasts, and plants, *Appl. Microbiol. Biotechnol.* 61:269-277. [This article is a mini review in the field of limonene biotransformation, especially with regard to the regiospecificity of microbial biocatalysts and utility of other systems on biotransformation of limonene].

Eaton RW (1997**) .**p-Cymene catabolic pathway in Pseudomonas putida F1: cloning and characterization of DNA encoding conversion of p-cymene to p-cumate *J. Bacteriol.* 179**:** 3171 **–** 3180. [This article describes the cloning and characterization of enzymes in the conversion of *p*-cymene].

Fernandes P, Cruz A, Angelova B, Pinheiro HM, and Cabral JMS (2003). Microbial conversion of steroid compounds: recent developments. *Enzyme and Microbial Technology*. 32: 688-705. [This article presents brief review on microbial transformations of steroids].

Griffiths ET, Harries PC, Jeffcoat R, and Trudgil PW (1987). Purification and properties of α-pinene oxide lyase from Nocardia sp. strain P18.3. *J. Bacteriol.* 169: 4980- 4983. [This article describes the purification and biochemical properties of α-pinene oxide lyase for *Norcardia*].

Hylemon PB, and Harder J, (1998). Biotransformation of monoterpenes, bile acids, and other isoprenoids in anaerobic ecosystems, *FEMS Microbiol. Rev.* 22:475-488. [This review describes the anaerobic metabolism of isoprenoids, mainly by denitrifying and fermentative bacteria].

Jan B. van Beilen, René H, Daniel L, Ulrich B, Bernard W, and Wouter A. D (2005). Biocatalytic production of perillyl alcohol from limonene by using a novel *Mycobacterium sp*. cytochrome P450 alkane hydroxylase expressed in *Pseudomonas putida*, *Appl. Environ. Microbiol.* 71: 1737-1744. [This article reports expression of cytochrome P450, a ferredoxin, and a ferredoxin reductase. in *Pseudomonas putida* conversion of limonene to perillyl alcohol with the recombinant *P. putida*].

Julsing MK, Koulman A, Woerdenbag, HJ, Quax WJ and Kayser O (2006). Combinatorial biosynthesis of medicinal plant secondary metabolites, Biomol. Engineering. 23:265-279. [This article reviews combinatorial biosynthetic approach for important classes of natural products, including alkaloids, terpenoids and flavonoids. The role and importance of today's used host organisms is critically described].

Kaiser JP, Feng Y and Bollag JM (1996). Microbial metabolism of pyridine, quinoline, acridine and their derivatives under aerobic and anaerobic conditions, *Microbiol. Rev.* 60:483-498. [This article illustrates the microbial metabolism of pyridine heterocyclic compounds under aerobic and anaerobic conditions].

Khan MTH and Ather A **(**2007). Microbial transformation of nitrogenous compounds, *Top Heterocycl Chem*. 10:99-122. [A comprehensive review on the microbial transformation of alkaloids].

Laurent-Philippe B, Agnès C, and Annie R (2007)**.** In vitro degradation of 10 mono- and sesquiterpenes of plant origin by caprine rumen micro-organisms, *J. Sci. Food Agri* 87:1653-1658. [This article reports exposure of rumen micro-organisms to a specific blend of essential oils compounds, containing mainly thymol, guaiacol and limonene and their degradation].

Leak DJ, Aikens PJ, and Seyed-Mahmoudian M (1992). The microbial production of epoxides, *Trends in Biotechnol.*10:256-261. [This article illustrates use of biocatalysts for production of epoxides as chiral intermediates].

Mariët J. van der Werf, Henk J.S, and Jan A. M. de Bont (1999). *Rhodococcus erythropolis* DCL14 contains a Novel Degradation Pathway for Limonene, *Appl. Environ. Microbiol*. 65: 2092-2102. [This article details the isolation of *R. erythropolis* DCL14 and the degradation of limonene and monoterpenes].

Mariët J. Van der werf (2000). Purification and characterization of a Baeyer–Villiger mono-oxygenase from *Rhodococcus erythropolis* DCL14 involved in three different monocyclic monoterpene degradation pathways, *Biochem. J* 347:693-701. [This article reports the homogeneous purification and characterization of Baeyer-Villiger mono-oxygenase].

Mariët J. van der Werf, Jan A. M. de Bont and David J. L, (1997). Opportunities in microbial biotransformation of monoterpenes, *Adv. Biochem. Eng/Biotechnol* 55:147-177. [An in depth review article on microbial transformations of monoterpenes].

Mars AE, Gorissen JPL, van den Beld I and Eggink G, (2001). Bioconversion of limonene to increased concentrations of perillic acid by *Pseudomonas putida* GS1 in a fed-batch reactor, *Appl. Microbiol. Biotechnol.* 56:101-107. [This article explains the ability of *P.putida* transformation of limonene to perillic acid in a fed-batch reactor].

Rathbone DA, Lister DL and Bruce NC (2002). Biotransformation of alkaloids, *The Alkaloids* 58:1-82. [This article is an exhaustive review on microbial transformations of alkaloids].

Rathbone DA and Bruce NC (2002). Microbial transformation of alkaloids, *Curr. Opin. Microbiol.* 5:274-281. [A brief review on biotransformation of alkaloids].

Rosazza JPN and Duffel MW (1986). Metabolic transformations of alkaloids, *The Alkaloids* 27:323-405.[This article is comprehensive review on alkaloids transformations in microbial system].

Schafer H and Wink M (2009). Medicinally important secondary metabolites in recombinant microorganisms or plants: Progress in alkaloid biosynthesis, *Biotechnol. J*. 4:1684-1703. [This article is up to date review on recent developments in medicinally important alkaloids biosynthesis].

Sedlaczek, L and Smith LL**.** (1988). Biotransformations of steroids, *Critical Reviews in Biotechnology*. 7: 187-236. [A critical review on the biotransformations of steroids].

Speelmans G, Bijlsma A and Eggink G (1998). Limonene bioconversion to high concentrations of a single and stable product, perillic acid, by a solvent-resistant *Pseudomonas putida* strain, *Appl. Microbiol. Biotechnol.* 50:538-544. [This article describes production of perillic acid by solvent-resistant *P. putida*].

Tong WY and Dong X (2009). Microbial biotransformations: recent developments on steroid drugs. *Recent Patents on Biotechnology* 3:141-153. [An up to date review on microbial transformations of steroidal drugs].

Trudgill PW (1990). Microbial metabolism of monoterpenes recent developments, *Biodegradation* 1:93-105. [A review on the metabolism of monoterpenes].

Wackett L.P and Hershberger C.D (2001). Biocatalysis and Biodegradation: Microbial transformation of organic compounds. [ASM Press, This book is designed for use as a textbook in courses in biodegradation, this volume details the fundamental concepts of the microbial transformations of organic compounds]

Yu CL, Kale Y, Gopishetty S, Louie TM and Subramanian, M (2008). A novel caffeine dehydrogenase in *Pseudomonas* sp. strain CBB1 oxidizes caffeine to trimethyluric acid, *J. Bacteriol.* 190:772-776. [This articles reports a novel caffeine dehydrogenase from *Pseudomonas* sp grows on caffeine as sole source of carbon and nitrogen].

Yu CL, Louie TM, Summers R, Kale Y, Gopishetty S and Subramanian M (2009). Two distinct pathways for metabolism of theophyllilne and caffeine are coexpressed in *Pseudomonas putida* CBB5, *J. Bacteriol*. 191:4624-4632. [This article illustrates the presence of the two different pathways in the metabolism of theophylline and caffeine structurally related alkylxanthines].

Biographical Sketches

Sridhar Gopishetty was born in India and obtained his M.Sc. (in 1992) and Ph.D. (in 2000) in bioorganic chemistry from Indian Institute of Science, India. After four years of postdoctoral research in carcinogenesis at the Department of Pharmacology at University of Pennsylvania, he joined Center for Biocatalysis and Bioprocessing, University of Iowa as postdoctoral researcher; in 2006 he was appointed as Assistant Research Scientist, currently he is working as Project Manager. His major interests lie in the biocatalysis, fermentation and protein purification.

Michael Tai-Man Louie was born in Hong Kong, and obtained his B.Sc. (in 1992) and Ph.D. (in 1998) in microbiology at the University of British Columbia, Canada. After four years of postdoctoral research in microbial biochemistry and enzymology at the School of Molecular Biosciences at Washington State University, he joined Kemin Industries at Des Moines, Iowa as an Associate Scientist. In 2006, he was appointed to the position of Senior Scientist at Kemin Industries and led a project in engineering metabolic pathways in *E. coli* to produce novel carotenoids. In 2008, he joined the Chemical and Biochemical Engineering Department, and the Center for Biocatalysis and Bioprocessing at the University of Iowa as a Research Scientist. His major interests lie in the discovery of novel enzymes and the application of these enzymes in biocatalytic processes.

Mani V. Subramanian was born in India and obtained his M.Sc (1974) and Ph.D (1978) in Biochemistry from the Indian Institute of Science, India. After 3 years of postdoctoral work in microbial metabolism of hydrocarbons at the University of Texas at Austin, Texas, USA. He joined The Dow Chemical Company as Sr. Research Chemist in 1981. After 25 years in various biotechnology companies, including Sandoz, Maxygen and Dow Chemical, he joined The University of Iowa in 2005 as Professor, Chemical & Biochemical Engineering, and as Department Executive Officer, Center for Biocatalysis & Bioprocessing. His major interests are biocatalysis applications, metabolic engineering, metabolism of xenobiotics, enzyme discovery, protein expression, fermentation and downstream processing.