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MICROBIAL BIOCONVERSION TO PRODUCE NUTRACEUTICAL AND PHARMACEUTICAL BIOACTIVE COMPOUNDS

Biokonversi Mikroorganisma untuk Memproduksi Senyawa-Senyawa Bioaktif Nutraseutikal dan Farmaseutikal

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ABSTRACT
The bioconversions of bioactive health supporting compounds using certain microorganisms have long been known, however it has not been widely applied in industrial scale to produce nutraceutical and products of high economic value. This review article will discuss the group of steroids, carotenoids and statins which were produced by microbial bioconversion using the various substrates and conditions. The information presented in this article were collected from scientific books and articles in national and international journals which can be validated and traced back to their sources through many sites such as google.com, google scholar, NCBI and Science Direct. From the information compiled in this article, it can be concluded that the bioconversion of bioactive compounds by microorganisms has shown positive results and potential to be further optimized and applied in an industrial scale to produce natural bioactive compounds as basic ingredients for nutraceutical and pharmaceutical products with high economic value.

Keywords: *bioconversion, carotenoids, lovastatin, nutraceutical, steroids*

ABSTRAK
Senyawa-senyawa bioaktif penunjang kesehatan hasil biokonversi oleh mikroorganisma telah lama dikenal namun dimanfaatkan secara maksimal pada industri nutraseutik dan farmaseutik untuk menghasilkan produk-produk bernilai ekonomi tinggi. Pada artikel ini akan ditinjau produk-produk nutraseutikal dan farmaseutikal dari golongan steroid, golongan karetinoid, dan golongan statin yang dihasilkan dari proses biokonversi oleh berbagai mikroorganime. Informasi-informasi yang disajikan didapatkan dengan metoda studi lieratur mengacu pada berbagai tulisan ilmiah nasional dan internasuonal yang dapat divalidasi dan dilacak kembali sumbernya melalui situs-situs google.com, google scholar, NCBI dan Science Direct. Dari artikel tinjauan ini dapat disimpulkan bahwa biokonversi senyawa bioaktif oleh mikroorganisma menunjukkan hasil-hasil positif dan berpotensi untuk dioptimasi dan diterapkan lebih lanjut pada skala industri untuk menghasilkan senyawa-senyawa sebagai bahan dasar produk nutraseutik dan farmasetik yang bernilai ekonomi tinggi.

Kata Kunci: biokonversi, karotenoid, lovastatin, nutraseutik, steroid

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INTRODUCTION

Bioconversion or biotransformation is the process of converting some substances into new or different products with the same chemical structure as the original compound utilizing biological catalysts such as bacteria and fungus. Biotransformation is described as a chemical's structural alteration aided by microbes or enzymes produced by microorganisms (Pervaiz et al. 2013). Microorganisms adapt to their surrounding conditions, which in this case are the substrates or growing media, and convert the substrate into specific metabolites through the bioconversion mechanism. In the food, pharmaceutical, agrochemical, and other industries, the process of microbial biotransformation in the form of fermentation technology has shown to be a key instrument in enhancing food quality and lowering the usage of synthetic chemicals for millennia.

The bioconversion process is used in pharmaceutical research and development to convert pharmacologically inactive chemicals into pharmacologically active ones to produce high-value bioactive compounds such as steroids, statins, carotenoids, and antibiotics. Various enzymes, such as hydrolytic enzymes, esterases, amidases, and phosphatases, are involved in this bioconversion process, while the conversion of *prodrugs* into appropriate active drugs can also occur non-enzymatically (De Carvalho and Da Fonsesca 2019).

Because microorganisms can naturally synthesize numerous types of enzymes in a short period as metabolites of their development process, the bioconversion process can reach excellence in the creation of bioactive chemicals. As an effective enzyme producer, microorganisms can be cultured and survive in extreme environments such as low or high temperatures, high acid or alkaline conditions. Because of these advantages, microbial bioconversion can catalyze chemical reactions that are difficult to achieve under ordinary synthetic conditions, shorten the synthesis step of the desired final compound, and enable reactions that would be required under conventional synthetic procedures (Kebamo et al. 2015, Smitha et al. 2017). Biotransformation is becoming more popular as a method for synthesizing medicinal molecules from natural sources. Aromatic chemicals, steroids, alkaloids, coumarins, flavonoids, and terpenoids have all been successfully produced on a commercial scale by biotransformation of microbes employing fermentation technology in modern bioreactors (Mishra et al. 2013, Hegazy et al. 2015). The bioconversion of bacterial and fungal microorganisms produces steroids, carotenoids, and statins, which are discussed in this article. The potential and bioconversion capacity of microbes to secondary metabolites can be enhanced and further employed on an industrial scale to manufacture basic components for nutraceutical and pharmaceutical products with high economic value using the information offered in this article.

STEROID PRODUCTION

Steroid hormones in the human body are naturally known to play a key role in controlling body metabolism, managing human fertility, menopause, osteoporosis, and regulating blood pressure (Boonyaratanakornkit and Pateetin 2015, Acconcia and Marino 2016). Within the pharmaceutical industry, steroids are among the most marketed medical products and represent the second largest category after antibiotics (Fernández-Cabezón et al. 2018). Steroid drugs are widely used as anti-estrogen, anticonvulsant, and antiallergic; for the prevention and treatment of various serious diseases such as hormone-dependent breast and prostate cancer, obesity, diabetes, rheumatoid arthritis, hypertension, asthma, eczema, inflammation, metabolic disorders, neurodegenerative diseases of the elderly (Shaikh et al. 2012, Raissy et al. 2013, Lossignol 2016).

In the pharmaceutical industry, steroid medicines are made through a series of lengthy chemical conversion processes with low yields. The synthesis of steroid molecules and their precursors becomes more successful with the help of microbial bioconversion as shown in much research (Bhatti and Khera 2012). The use of *deoxycholic acid* as a substrate for bioconversion of steroids by microorganisms has the potential to minimize the stages involved in the manufacture of some hormones such as cortisone hormone.

Cortisone manufacturing traditionally needs 31 stages of chemical processes with low yields (0.16 percent), however, biotransformation employing *Rhizopus arrhizus* ATCC 11145 and *Aspergillus niger* ATCC 9142 reduces the needed steps from 11 to 20 with lower production costs (Carballeira et al. 2009).

The use of phytosterols as a carbon source by *Mycobacterium sp.* and several species of the genus *Actinobacteria* for their growth and reproduction is the basis of the development of microbial bioconversion or biotransformation processes for the large-scale production of steroid compounds (Donova and Egorova 2012, Garcia et al. 2012, Galán et al. 2017b). *Mycobacetrium sp.* is currently widely used for sterol bioconversion into androstenedione (AD), which is the precursor for most other steroid class compounds such as testosterone, estradiol, ethinylestradiol, testosterone, progesterone, cortisone, cortisol, prednisone, prednisolone, and other steroids on an industrial scale (Wang et al. 2020, Zhou et al. 2020).

Mycobacetrium sp. is capable of selectively degrading the sterol side chain at C-17.1,2, triggering oxyfunctionalization of the steroid core, and triggering redox reactions at various positions on the steroid molecule to produce high concentration of androstenedione (AD) hormone which applied for a variety of industrial applications (Gerber et al. 2015, Josefsen et al. 2017). Other investigations have shown that *Mycobacterium sp.* can convert b-sitosterol substrate into AD and ADD with concentrations of 75.87% and 83.86%, respectively, proving the effectiveness of sterol bioconversion by *Mycobacterium sp.* Another advantage of the bioconversion of steroids with the help of *Actinobacteria* is to shorten the conversion stage as in the production of testosterone which requires only one step (Eisa et al. 2016, Liu et al. 2016), and the production of boldenone (VIII) from phytosterols which includes two steps, starting from the conversion of phytosterols into AD compounds and continued with the dehydrogenation of AD by *Fusarium* sp. to boldenone (Kutney et al. 2003).

For substrates other than sterol group compounds, the biosynthesis of progesterone and hydrocortisone from simple carbon sources such as galactose and even ethanol has been successfully produced by recombinant strains of *Saccharomyces cerevisiae* by engineering the endogenous sterol biosynthetic pathway to produce cholesterol-like molecules that function as heterologous-enzymatic multi-route precursors that mimic human steroid biosynthesis, or by detoxifying steroids as fungitoxic molecules with their structural modifications (Hull et al. 2017, Chen et al. 2019). The production rate of sterol bioconversion using *S. cerevisiae* is quite high, where under optimal conditions, the conversion of AD to Boldenone BD can reach 83% (Tang et al. 2019a).

Table 1 below shows several types of microorganisms that successfully produce an AD, ADD, and BD steroids through the bioconversion process.

CAROTENOID PRODUCTION

Carotenoids are terpenoid group compounds generally found in the form of natural pigments that are responsible for the red, orange, and yellow colors in plants and some animals, consisting of more than 600 compounds belonging to this pigment (Britton et al. 2004). Generally, carotenoids are hydrocarbons containing 40 carbon atoms and two terminal rings of conjugated C = C bonds so that carotenoid compounds can bind free radicals and act as effective antioxidants (Edge and Truscott 2018). Regarding its ability as antioxidants, carotenoid compounds play a role in increasing the function of the immune system (Dhinaut et al. 2017), as a “sun protector” to protect the skin from sunburn (Stahl and Sies 2012), inhibiting the development of certain types of cancer, and helping prevent heart disease by preventing the formation of harmful LDL cholesterol (Mozos et al. 2018).

The nutraceutical industry synthetically produces several types of major carotenoids on a large scale such as lycopene, β-carotene, canthaxanthin, zeaxanthin, and astaxanthin. These carotenoids are commonly used in a variety of food products, cosmetics, and health supplements. Synthetic carotenoid products which are mass-produced with low cost as additives, are generally of low quality and are less effective when used as health support so

Table 1. Steroid precursor compounds produced by microbial bioconverstion

Microorganism	Products	Production Strategies	References
Mycobacterium neoaurum	BD	Utilization of *phytosterol* as growth medium	Tang et al. 2019a
Arthrobacter simplex and *Pichia pastoris*	BD	Using DNA recombinant technology and inactivation of medium at specific stages	Tang et al. 2019b
Mycobacterium neoaurum	AD, ADD	Application of three stages fermentation	Shao et al. 2015
Pseudomonas aeruginosa	AD, ADD, BD	Utilization of selective medium containing containing 8-hydroxyquinoline	Eisa et al. 2016
Nocardioides simplex	AD, ADD	Utilization of selective medium containing Hydroxypropyl-β-cyclodextrin	Shtratnikova et al. 201
Mycobacterium smegmatis	AD, ADD	Utilization of selective medium containing Hydroxypropyl-β-cyclodextrin	Galán et al. 2017a
Saccharomyces cerevisiae	AD to BD	Utilization of natural medium containing rice bran oil.	Sallam et al. 2008
Deinococcus radiodurans	AD, ADD	Utilizationof natural medium containing rice bran oil	Sallam et al. 2008
Mycobacterium neoaurum JC-12	AD to ADD	Application of three stages fermentation	Shao et al. 2015
Arthrobacter simplex and Pichia pastoris recombinant	AD to BD	Optimization of transformation process	Tang et al. 2019b
Bacillus subtilis rekombinan	AD to ADD	Insertion of *Mycobacterium neoaurum* JC-12 DNA to *Bacillus subtilis*	Zhang et al. 2013
Mycobacterium smegmatis	Production of *synthons steroid*	Utilization of medium containing cholesterol	Galan et al. 2017
Arthrobacter simplex and *Streptomyces roseochromogenes*	Hydrocortisone to 16α-Hydroxyprednisolone	Optimization of temperature and pH	Restaino et al. 2020
Arthrobacter simplex	AD to ADD	Variation of medium concentration and elicitation using specifics ingredients	Luthra and Nishtha 201

synthetic products become less valuable. As an alternative source of carotenoids, microorganisms from various types of yeasts and bacteria show high potential because they can produce large biomass in a faster time, are not limited by seasons, are easy to cultivate, have wide color variations, non-toxic, more efficient extraction process and the final product would decompose faster naturally (Ram et al. 2020).

Compared to bacteria, yeast is known to produce β-carotene better in terms of quality and quantity. Beta carotene pigments are reported to be produced on an industrial scale using the fungus *Blakeslea trispora* and *Phycomyces blakesleeanus*. Cultivation of *B. trispora* in wastewater from Spanish olive processing. The fermentation process able to produce β-carotene of 61.2 to 64.1 mg L^{-1} cultivation media (Papadaki and Mantzouridou 2021). It is known that in the cultivation of the most productive strains of *P. blakesleeanu*s, the potential for carotenoid production reaches its maximum potential in the system or bioreactor without agitation. In addition to *P. blakesleeanus*, several types of yeast from the genus *Rhodotorula* such as *R. glutinis, R. minuta, R. mucilaginosa, R. acheniorum,* and *R. graminis* are known to have the ability to biosynthesize certain carotenoids such as β-carotene, torulen, and torularhodin, with different levels (Mannazzu et al. 2015, Zhao et al. 2019). The main carotenoids estimated to be produced by *R. glutinis* are β-carotene (about 25-43% of total carotenoids) and torulene (about 28-30% of

Table 2. Carotenoid products from microbial bioconvertion and optimization strategies

Products	Microorganism	Production Strategy	Reference
Astaxanthin 8.0 mg L^{-1}/day	*Haematococcus pluvialis*	Supplementation of 0.5 mmol g^{-1} nitrate to the medum and radiation on photobiorector (2500 mE/m^2 s) in open space.	García-Malea et al. 2009
Astaxanthin 119 mg L^{-1}	*Xanthophyllomyces dendrorhous*	Fermentation process using direct sunlight	De La Fuente et al. 2010
Canthaxanthin 13.1 mg L^{-1}	*Dietzia natronolimnaea* HS-1	Supplementation of *α-ketoglutarate* (9.69 mM), *oxaloacetate*, (8.68 mM) and *succinate* (8.51 mM) to fermentation medium.	Nasrabadi and Razavi 2010
Canthaxanthin 1.58 mg L^{-1}	*Dactylococcus dissociatus*	Application of stress salt (NaCl 7.5 g L^{-1}), nitrogen deficiency and oxidative stress using high light intensity (600 mE/m2 s).	Grama et al. 2014
Canthaxanthin, Echinenone, astaxanthin, Lutein/ zeaxanthin, 0,8% of total dry weight	*Chlorella protothecoides*	Application of stess salt (supplementation of 20 g L^{-1} NaCl to the medium).	Campenni et al. 2013
Lutein 5.18 mg g^{-1}	*Desmodesmus sp.* JSC3	Application of batch culture with supplementation of 1.0 g L^{-1} sodium nitrate as nitrogen source.	Yeh et al. 2017
Lycopene 775 mg L^{-1}	*Blakeslea trispora*	Supplementation of medium with 1000 ppm vitamin A acetate and 500 ppm *piperidine* inhibitor.	Choudhari et al. 2008
Total carotenoids 135.25 mg L^{-1}	*Rhodotorula glutinis*	Utilization of medium with 85 C/N ratio, addition of 9.5% glycerol and 0.15% tween 20 to optimize glycerol solubility.	Saenge et al. 2011
Zeaxanthin 1.47 mg g^{-1} of dry weight	*Muricauda sp. YUAB-SO-11*	Suplementation of medium with 0.1 M glutamamic acid	Prabhu et al. 2013
Zeaxanthin 0.62 mg L^{-1}	*Shingomonas (Blastomonas) natatoria* KODA19-6	Naturally produced from oleh coccus shaped gram negative bacterial isolated from sponge species in Thailand gulf.	Thawornwiriyanun et al. 2012
β-carotene 830 mg L^{-1}	*Blakeslea trispora*	Utilization of medium with 0.2% Span 20 surfactan and 1000 ppm vitamin A acetate	Choudhari et al. 2008

Source of table: Saini and Keum (2019)

total carotenoids). Torulene is more commonly produced by the yeasts *Sporobolomyces roseus, S. salmonicolor* and *S. patagonicus* identified as the carotenoids torulene, torularodine, and γ-carotene (Moliné et al. 2012).

Carotenoid production in the *Rhodotorula* genus varies between species due to differences in substrate composition and climatic circumstances (Elfeky et al. 2019). Davoli et al. (2004) categorised the amount of carotenoids generated by this genus as low (less than 100 µg g^{-1}), moderate (101 to 505 µg g^{-1}), and high (more than 500 µg g^{-1}) (Aksu and Eren 2007). After 4 days of fermentation in malt yeast broth, a novel strain of *Rhodotorula glutinis* isolated from pin cushion flowers was able to produce 266 µg g^{-1} of cellular carotenoids and 1.6 µg L^{-1} of volumetric carotenoids according to a recent study (El-Banna et al. 2012). The previous study of *R. glutinis* which is cultured in a variety of sources of substrate proved to produce

Table 3. Types of statin compounds and production sources

Types of statin	Sources
Mevastatin	*Penicillium citrinum*
Compactin	*P. citrinum, P. brevicompactum*
Pravastatin (derivate of compactin)	*Streptomyces carbophilus; Actinomadura sp. Bacillus megaterium; Nocordia autrophica*
Simvastatin (derivate of ilovastatin)	*Aspergillus tereus* (mutant strain)
Fluvastatin	Synthetic
Atorvastatin	Synthetic
Resorvastatin	Synthetic
Pitavastatin	Synthetic

Table source: Barrios-González and Miranda (2010)

relatively high outputs of carotene, for example when fermented using agricultural wastes such as onion peel waste extract, it could yield up to 204.29 mg L^{-1} of β-Carotene (Bagy et al. 2016).

Other types of yeast such as *Neurospora intermedia* N-1 isolated from fermented of traditional peanut dregs cake (traditionally known as *oncom*) are also known as carotenoid-producing yeasts and have been investigated as an alternative to food and beverage coloring. Several studies have recommended that soybean dregs from tofu production is a suitable substrate for *Neurospora crassa* fermentation which produces high spore volume and total carotenoid concentration, while *N. crassa* fermentation on tapioca pulp and tofu waste can produce high levels of β -carotene reaching 295.16 µg g^{-1} substrate (Nuraini et al. 2009, Priatni 2014).

One of the most commercially valuable carotenoid pigments is astaxanthin which is used as a potential neuroprotective agent to prevent cardiovascular disease, Parkinson's disease, *Alzheimer's disease* (AD), brain and nerve injury, neuropathic pain, depression, and autism (Fassett and Coombes 2011, Fakhri et al. 2019). Astaxanthin is mainly synthesized from the micro-alga *Haematococcus pluvialis*, with an optimized extraction method capable of producing up to 500 mg L^{-1} of compounds from raw materials (Bauer and Minceva 2019) and by the yeast *Xanthophyllomyces denrorhous/Phaffia rhodozyma*.

Carotenoids have also been discovered to be produced by a variety of bacteria, in addition to yeast. *Corynebacterium michiganense, Micrococcus roseus, Brevibacterium spp., Bradyrhizobium spp., Gordonia jacobaea*, and *Dietzia natronolimnaea* are among the most studied carotenoid-producing bacteria. *Flavobacterium sp.,* a marine bacteria, has been found to produce 3.8 g L^{-1} of zeaxanthin under optimal culture conditions (Chávez-Parga et al. 2012), whereas *H. pluvialis*, a microalgae, has a significant industrial potential for canthaxanthin synthesis (Panis and Rosales-Carreon 2016). Other bacteria such as *Agrobacterium aurantiacum* and modified *Escherichia coli, Mycobacterium brevicaie, M. lacticola, Rhodobacter sphaeroides, Rhodococcus maris, Streptomyces chrestomyceticus*, and *Erwinia uredovora* also can synthesize carotenoids. Table 2 shows several types of carotenoids produced through the microorganism bioconversion process and their optimization strategies.

STATIN PRODUCTION

Statins are cholesterol-lowering drugs that function by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), an enzyme that catalyzes the synthesis of cholesterol in the bloodstream. Statins are polyketide molecules produced spontaneously as secondary metabolites by numerous species of yeast, particularly under stressful situations. Acetyl Co-A serves as a precursor molecule for statins, bridging primary and secondary metabolism and resulting in the

formation of a variety of secondary metabolites such as terpenes and polyketides, including statins (Chiang et al. 2010, Shi and Tu 2015).

Natural statins, including lovastatin and mevastatin are commonly known as compactins. Chemically synthesized statins include atorvastatin, rosuvastatin, uvastatin, and pitavastatin. The statin compactin was discovered by Akira Endo in 1973 as a structural analogue for the substrate β-Hydroxy β-methylglutaryl-CoA (HMG-CoA), produced by *Penicillium citrinum*. Since his discovery, screening has been carried out for lovastatin, previously known as mevinolin K and mevinolin, produced from cultures of *Monascus ruber* and Aspergillus *terreus*, respectively, as the first statin to be approved by the FDA in 1987 (Endo 2010). Genomic studies of *Aspergillus spp* and *A. terreus* showed these molds have a unique gene cluster involved in the biosynthesis of lovastatin compounds (Keller 2015).

Lovastatin for commercial purposes is produced from the direct fermentation of the fungus *Aspergillus terreus* while the semi-synthetic statins simvastatin and pravastatin are synthesized by chemical treatment of esteroselective hydroxylation of lovastatin or monacolin (Belwal and Patel 2019). Table 3 shows the types of statins produced by bioconversion of microorganisms and synthetic statins that have been produced commercially.

The yeast strain utilized and the composition of the culture media, such as carbon and nitrogen sources, have an impact on lovastatin production. The highest lovastatin yield was obtained in fermentation with lactose as a carbon source derived from wheat bran substrate optimized to be the best solid substrate for SSF, according to research on the cultivation of two strains *of Aspergillus terreus, A. terreus 4* and *A. terreus 20*, using five different carbon sources. According to the study, the fermentation of *A. terreus* MIM A1 and A2 in soy flour and glycerol produced lovastatin, mevastatin, pravastatin, and monacolin, with 83% of lovastatin contained in the mycelium and 17% secreted in the culture filtrate (Jahromi et al. 2012, Kamath et al. 2015, Abdul-Rahim et al. al. 2017). *A. terreus* has also been reported to convert lovastatin directly and accumulate simvastatin as the end product of fermentation (Gulyamova et al. 2013), and up to 1.9% lovastatin can be produced compared to the weight of the substrate (Yulineri and Nurhidayat 2012). Recently, genetic engineering has been used to improve the efficacy of statins. For example, pravastatin manufacturing by single-stage fermentation can be generated by reprogramming the enzyme involved in the hydroxylation of compactin in cell of the antibiotics-producing fungus *Penicillium chrysogenum* (McLean et al. 2015).

In addition to lovastatin, other natural anticholesterol statin compounds generated from the fermentation method are monacolin compounds. Monacholine decreases cholesterol by blocking HMG-CoA (5-hydroxy-3-methylglutaryl-coenzyme A) reductase, which slows cholesterol synthesis in the liver (Kazi et al. 2017). Rice substrate fermented with *Monascus purpureus* yeast can be used to produce monacholine. The natural monacolin content of brown rice extract varies based on the *Monascus purpureus* strain and the type of rice used in fermentation, as well as other treatments like irradiation. According to antioxidation measures and histological examinations of rat liver, the lovastatin molecule obtained from fermentation of rice media by *Monascus purpureus* exhibits a high anticholesterol and glutathione peroxidase (GPx) activites with an optimal dose of 5 g/day of lovastatin (Kumar et al. 2011).

CONCLUSION

The finding from many researches and studies compilled of this article indicates the potential of microbial bioconvertion to produce pharmaceutical and nutraceutical compounds such as steroids, carotenoids, and lovastatin on an industrial scale. The amounts of active compounds produced by microorganisms on diverse substrates can be fairly high, such as the bioconvertion of steroid AD to Boldenone BD using *S. cerevisiae* can reach 83% of initial substrate of steroid. The carotenoids production by Rhodotorula genus reaching more than 500 μg g^{-1} media, and monacholine production by *Monascus purpureus* reaching 0.2% of the weight of substrate used.

The substrates used for microbial bioconversion to produce desired compunds are generally cheap and easily obtained. The

production of steroid by recombinant straint of *S. cerevisiae* worked successfully by using simple carbon source started from galactose to ethanol. The yield of carotenoid compounds could be increased by making use of various carbon sources including the carbon from food industry waste. For example, high concentration of β-caroten could be produce using *Neurospora crassa* mold with soybean and tapioca dregs as substrate. For the production of statin compounds, the substrate commonly used are quite available in the form of carbohydrates either the simple one such as lactose, or the complex one such as starch from corn or rice.

Referring to informations in this article, it is expected that there will be future research to optimize microbial bioconversion process by selection of proper and suitable subtrate and environmental factors such as pH, temperature, light and aeration. More sophisticated method using DNA recombinant also can be applied to design higher substrate conversion yields and product selectivity where the ultimate goal is the production of pharmaceutical and nutraceutical compounds on an industrial scale.

REFERENCES

Abd-Rahim MH, Harith HH, Montoya A, Abbas A (2017) Growth and lovastatin production by *Aspergillus terreus* under different carbohyrates as carbon sources. Biocatal Agric Biotechnol 10: 379-385. doi: 10.1016/j.bcab.2017.04.011

Acconcia F, Marino M (2016) Steroid hormones: synthesis, secretion, and transport. In: Belfiore A, LeRoith D (Eds). Principles of Endocrinology and Hormone Action. Endocrinology. Springer, Cham. doi: 10.1007/978-3-319-27318-1_4-1

Aksu Z, Eren AT (2007) Production of carotenoids by the isolated yeast of *Rhodotorula glutinis*. Biochem Eng J 35:107–113. doi: 10.1016/j.bej.2007.01.004

Bagy MMK, Abd-Alla MH, Nafady NA, Morsy FM, Mahmoud G (2016) Bioconversion of plant wastes to β-carotene by *Rhodotorula glutinis* KU550702. Eu J Biol Res 6:226-241. doi: 10.5281/zenodo.163649

Barrios-González J, Miranda RU (2010) Biotechnological production and applications of statins. Appl Microbiol Biotechnol 85:869–883. doi: 10.1007/s00253-009-2239-6

Bauer A, Minceva M (2019) Direct extraction of astaxanthin from the microalgae *Haematococcus pluvialis* using liquid–liquid chromatography. RSC Adv 9:22779–22789. doi: 10.1039/c9ra03263k

Belwal CK, Patel J (2019) Synthetic methods for simvastatin – An overview. J Pharm Appl Chem 5:23-43. doi: 10.18576/jpac//050103

Bhatti HN, Khera RA (2012) Biological transformations of steroidal compounds: A review. Steroids 77:1267-1290. doi: 10.1016/j.steroids.2012.07.018

Boonyaratanakornkit V, Pateetin P (2015) The role of ovarian sex steroids in metabolic homeostasis, obesity, and postmenopausal breast cancer: Molecular mechanisms and therapeutic implications. Biomed Res Int 2015:140196. doi: 10.1155/2015/140196

Britton S, Liaaen-Jensen S, Pfander H (2014) Carotenoids: Handbook. Birkhäuser, Boston, MA, USA

Campenni L, Nobre BP, Santos CA, Oliveira AC, Aires-Barros MR, Palavra AMF, Gouveia L (2013) Carotenoid and lipid production by the autotrophic microalga Chlorella protothecoides under nutritional, salinity, and luminosity stress conditions. Appl Microbiol Biotechnol 97:1383-1393. doi: 10.1007/s00253-012-4570-6

Carballeira JD, Quezada MA, Hoyos P, Simeó Y, Hernaiz MJ, Alcantara AR, Sinisterra JV (2009). Microbial cells as catalysts for stereoselective red-ox reactions. Biotechnol Adv 27:686-714. doi: 10.1016/j.biotechadv.2009.05.001

Chávez-Parga C, Munguía-Franco A, Aguilar-Torres M, Escamilla-Silva EM (2012) Optimization of zeaxanthin production by immobilized *Flavobacterium sp*. cells in fluidized

bed bioreactor. Adv Microbiol 2:598-604. doi: 10.4236/aim.2012.24078

Chen J, Tang JL, Xi YY, Dai ZB, Bi CH, Chen X, Fan FY, Zhang XL (2019) Production of 14 alpha-hydroxysteroids by a recombinant *Saccharomyces cerevisiae* biocatalyst expressing of a fungal steroid 14 alpha-hydroxylation system. Appl Microbiol Biotechnol 103:8363–8374. doi: 10.1007/s00253-019-10076-x

Chiang YM, Oakley BR, Keller NP, Wang CC (2010) Unraveling polyketide synthesis in members of the genus *Aspergillus*. Appl Microbiol Biotechnol 86:1719–1736. doi: 10.1007/ s00253-010-2525

Choudhari SM, Ananthanarayan L, Singhal RS (2008) Use of metabolic stimulators and inhibitors for enhanced production of β-carotene and lycopene by *Blakeslea trispora* NRRL 2895 and 2896. Bioresour Technol 99:3166-3173. doi: 10.1016/j.biortech.2007.05.051

Davoli P, Mierau V, Weber RWS (2004) Carotenoids and fatty acids in red yeasts *Sporobolomyces roseus* and *Rhodotorula glutinis*. Appl Biochem Microbiol 40:392–397. doi: 10.1023/B:ABIM.0000033917.57177.f2

De Carvalho CCCR, Da Fonseca MMR (2019) Biotransformations. pp 574-585. In: Moo-Young M (Ed). Comprehensive Biotechnology, 3rd edition. Pergamon, Oxford

De La Fuente JL, Rodríguez-Sáiz M, Schleissner C, Diez B, Peiro E, Barredo JL (2010) High-titer production of astaxanthin by the semi-industrial fermentation of *Xanthophyllomyces dendrorhous*. J Biotechnol 148:144-146. doi: 10.1016/j.jbiotec.2010.05.004

Dhinaut J, Balourdet A, Teixeira M, Chogne M, Moret Y (2017) A dietary carotenoid reduces immunopathology and enhances longevity through an immune depressive effect in an insect model. Sci Rep 7:12429. doi: 10.1038/s41598-017-12769-7

Donova MV, Egorova OV (2012) Microbial steroid transformations: Current state and prospects. Appl Microbiol Biotechnol 94:1423-1447. doi: 10.1007/s00253-012-4078-0

Edge R, Truscott TG (2018) Singlet oxygen and free radical reactions of retinoids and carotenoids - A review. Antioxidants (Basel) 7:5. doi: 10.3390/antiox7010005

Eisa M, El-Refai H, Amin M (2016) Single step biotransformation of corn oil phytosterols to boldenone by a newly isolated *Pseudomonas aeruginosa*. Biotechnol Rep 11:36-43. doi: 10.1016/j.btre.2016.05.002

El-Banna AAE, El-Razek AMA, El-Mahdy AR (2012). Isolation, identification and screening of carotenoid-producing strains of *Rhodotorula glutinis.* Food Nutr Sci 3:627-633. doi: 10.4236/fns.2012.35086

Elfeky N, Elmahmoudy M, Zhang Y, Guo J, Bao Y (2019) Lipid and carotenoid production by *Rhodotorula glutinis* with a combined cultivation mode of nitrogen, sulfur, and aluminium stress. Appl Sci 9:2444. doi: 10.3390/app9122444

Endo A (2010) A historical perspective on the discovery of statins. Proc Jpn Acad Ser B Phys Biol Sci 86:484-493. doi: 10.2183/pjab.86.484

Fakhri S, Aneva IY, Farzaei MH, Sobarzo-Sánchez E (2019) The neuroprotective effects of astaxanthin: Therapeutic targets and clinical perspective. Molecules 24:2640. doi: 10.3390/molecules24142640

Fassett RG, Coombes JS (2011) Astaxanthin: A potential therapeutic agent in cardiovascular disease. Mar Drugs 9:447-465. doi: 10.3390/md9030447

Fernández-Cabezón L, Galán B, García JL (2018) New insights on steroid biotechnology. Front Microbiol 9:958. doi: 10.3389/fmicb.2018.00958

Galán B, García-Fernández J, Felpeto-Santero C, Fernández-Cabezón L, García JL (2017a) Bacterial metabolism of steroids. In: Timmis KN (Ed). Handbook of Hydrocarbon and Lipid Microbiology. Springer, Switzerland. doi: 10.1007/978-3-319-39782-5_43-1

Galán B, Uhía I, García-Fernández E, Martínez I, Bahíllo E, de la Fuente J,

Barredo JL, Fernandez-Cabezon L, García JL (2017b) Mycobacterium smegmatis is a suitable cell factory for the production of steroidic synthons. Microb Biotechnol 10:138-150. doi: 10.1111/1751-7915.12429

García JL, Uhía I, Galán B (2012) Catabolism and biotechnological applications of cholesterol degrading bacteria. Microb Biotechnol 5:679-699. doi: 10.1111/j.1751-7915.2012.00331.x

García-Malea MC, Acién FG, Del Río E, Fernandez JM, Ceron MC, Guerrero MG, Molina-Grima E (2009) Production of astaxanthin by Haematococcus pluvialis: Taking the one-step system outdoors. Biotechnol Bioeng 102:651-657. doi: 10.1002/bit.22076

Gerber A, Kleser M, Biedendieck R, Bernhardt R, Hannemann F (2015) Functionalized PHB granules provide the basis for the efficient side-chain cleavage of cholesterol and analogs in recombinant *Bacillus megaterium*. Microb Cell Fact 14:107. doi: 10.1186/s12934-015-0300-y

Grama BS, Chader S, Khelifi D, Agathos SN, Jeffryes C (2014) Induction of canthaxanthin production in a *Dactylococcus microalga* isolated from the Algerian Sahara. Bioresour Technol 151:297-305. doi: 10.1016/j.biortech.2013.10.073

Gulyamova T, Ruzieva D, Nasmetova S, Sattarova R, Lobanova, K, Abdulmyanova L, Rasulova G (2013) Lovastatin production by *Aspergillus terreus* in solid state and submerged fermentations. Int J Eng Sci Technol 5:19-24. doi: 10.4314/ijest.v5i3.2

Hegazy MEF, Mohamed TA, El Shamy AI, Mohamed AEH, Mahalel UA, Reda EH, Shaheen AM, Tawfik WA, Shahat AA, Shams KA, Abdel-Azin NS, Hammouda FM (2015) Microbial biotransformation as a tool for drug development based on natural products from mevalonic acid pathway: A review. J Adv Res 6:17-33. doi: 10.1016/j.jare.2014.11.009

Hull CM, Warrilow AGS, Rolley NJ, Price CL, Donnison IS, Kelly DE, Kelly SL (2017) Co-production of 11α-hydroxyprogesterone and ethanol using recombinant yeast expressing fungal steroid hydroxylases. Biotechnol Biofuels 10:226. doi: 10.1186/s13068-017-0904-z

Jahromi MF, Liang JB, Ho YW, Mohamad R, Goh YM, Shokryazdan P (2012) Lovastatin production by *Aspergillus terreus* using agro-biomass as substrate in solid state fermentation. J Biomed Biotechnol 2012:196264. doi: 10.1155/2012/196264

Josefsen KD, Nordborg A, Sletta H (2017) Bioconversion of phytosterols into androstenedione by mycobacterium. Methods Mol Biol 1645:177-197. doi: 10.1007/978-1-4939-7183-1_13

Kamath PV, Dwarakanath BS, Chaudhary A, Janakiraman S (2015) Optimization of culture conditions for maximal lovastatin production by Aspergilus terreus (KM017963) under solid state fermentation. Hayati 22:174-180. doi: 10.1016/j.hjb.2015.11.001

Kazi DS, Penko JM, Bibbins-Domingo K (2017) Statins for primary prevention of cardiovascular disease: Review of evidence and recommendations for clinical practice. Med Clin North Am 101:689-699. doi: 10.1016/j.mcna.2017.03.001

Kebamo S, Tesema S, Geleta B (2015) The role of biotransformation in drug discovery and development. J Drug Metab Toxicol 6:196. doi: 10.4172/2157-7609.1000196

Keller NP (2015) Translating biosynthetic gene clusters into fungal armor and weaponry. Nat Chem Biol 11:671–677. doi: 10.1038/nchembio.1897

Kumar S, Srivastava N, Gomes J (2011) The effect of lovastatin on oxidative stress and antioxidant enzymes in hydrogen peroxide intoxicated rat. Food Chem Toxicol 49:898-902. doi: 10.1016/j.fct.2010.12.014

Kutney JP, Herrington EJ, Spassov G (2003) Process for fermentation of phytosterols to androstadiendione. WO 03/064674 A3. Patent Cooperation Treaty

Liu M, Zhu ZT, Tao XY, Wang FQ, Wei DZ (2016) RNA-Seq analysis uncovers non-coding small RNA system of *Mycobacterium neoaurum* in the

metabolism of sterols to accumulate steroid intermediates. Microb Cell Fact 15:64. doi: 10.1186/ s12934-016-0462-2

Lossignol D (2016) A little help from steroids in oncology. J Transl Int Med 4:52–54. doi: 10.1515/jtim-2016-0011

Luthra U, Singh N, Tripathi AR, Bhosle VR, Kain P, Mota K (2015) Optimization of various parameters for the bioconversion of AD to ADD by using *Arthrobacter simplex*. Int J Innov Res Sci Eng Technol 4:11783-11789. doi: 10.15680/IJIRSET.2015.0412017

Mannazzu I, Landolfo S, da Silva TL, Buzzini P (2015) Red yeasts and carotenoid production: Outlining a future for non-conventional yeasts of biotechnological interest. World J. Microbiol Biotechnol 31:1665–1673. doi: 10.1007/s11274-015-1927-x

McLean KJ, Hans M, Meijrink B, van Scheppingen WB, Vollebregt A, Tee KL, Van Der Laan JM, Leys D, Munro AW, van den Berg (2015) Pravastatin production in *Penicillium chrysogenum.* Proceedings of the National Academy of Sciences:2847-2852. doi: 10.1073/pnas.1419028112

Mishra S, Sachan A, Sachan SG (2013) Production of natural value-added compounds: An insight into the eugenol biotransformation pathway. J Ind Microbiol Biotechnol 40: 545-550. doi: 10.1007/s10295-013-1255-9

Moliné M, Libkind D, van Broock M (2012) Production of torularhodin, torulene, and β-carotene by Rhodotorula yeasts: Methods Mol Biol 898:275-283. doi: 10.1007/978-1-61779-918-1_19

Mozos I, Stoian D, Caraba A, Malainer C, Horbańczuk JO, Atanasov AG (2018) Lycopene and vascular health. Front Pharmacol 9:521. doi: 10.3389/fphar.2018.00521

Nasrabadi NMR, Razavi SH (2010) Use of response surface methodology in a fed-batch process for optimization of tricarboxylic acid cycle intermediates to achieve high levels of canthaxanthin from Dietzia natronolimnaea HS-1. J Biosci Bioeng 109:361-368. doi: 10.1016/j.jbiosc.2009.10.013

Nuraini N, Sabrina S, Latif SA (2009) Improving the quality of tapioca by product through fermentation by *Neurospora crassa* to produce β-carotene rich feed. Pak J Nutr 8:487-490. doi: 10.3923/pjn.2009.487.490

Panis G, Rosales-Carreon JR (2016) Commercial astaxanthin production derived by green alga *Haematococcus pluvialis*: A microalgae process model and a techno-economic assessment all through production line. Algal Res 18:175-190. doi: 10.1016/j.algal.2016.06.007

Papadaki E, Mantzouridou, FT (2021) Natural β-carotene production by *Blakeslea trispora* cultivated in spanish-style green olive processing wastewaters. Foods 10:327. doi: 10.3390/foods10020327

Pervaiz I, Ahmad S, Madni MA, Ahmad H, Khaliq FH (2013) Microbial biotransformation: A tool for drug designing (Review). Prikl Biokhim Mikrobiol 49:435-449. doi: 10.7868/s0555109913050097

Prabhu S, Rekha PD, Young CC, Hameed A, Lin SY, Arun AB (2013) Zeaxanthin production by novel marine isolates from coastal sand of India and its antioxidant properties. Appl Biochem Biotechnol 171:817-831. doi: 10.1007/s12010-013-0397-6

Priatni S (2014) Review: Potential production of carotenoids from Neurospora. Nusantara Biosci 6:63-68. doi: 10.13057/nusbiosci/n060111

Raissy HH, Kelly HW, Harkins M, Szeflr SJ (2013) Inhaled corticosteroids in lung diseases. Am J Respir Crit Care Med 187:798–803. doi: 10.1164/rccm.201210-1853PP

Ram S, Mitra SF, Tirkey SR, Mishra S (2020) Bacteria as an alternate biofactory for carotenoid production: A review of its applications, opportunities and challenges. J Funct Foods 67:103867. doi: 10.1016/j.jff.2020.103867

Restaino OF, Ferraiuolo SB, Perna A, Cammarota M, Borzacchiello MG, Fiorentino A, Schiraldi C (2020) Biotechnological transformation of hydrocortisone into 16α-Hydroxyprednisolone by Coupling *Arthrobacter simplex* and

Streptomyces roseochromogenes. Molecules 25:4912. doi: 10.3390/molecules25214912

Saenge C, Cheirsilp B, Suksaroge TT, Bourtoom T (2011) Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from biodiesel plant to lipids and carotenoids. Process Biochemistry 46:210-218. doi: 10.1016/j.procbio.2010.08.009

Saini RK, Keum YS (2019) Microbial platforms to produce commercially vital carotenoids at industrial scale: An updated review of critical issues, J Ind Microbiol Biotechnolo. 46 :657–674. doi: 10.1007/s10295-018-2104-7

Sallam LAR, Osman ME, Hamdy AA, Zaghlol GM (2008) Microbial transformation of phytosterols mixture from rice bran oil unsaponifiable mater by selected bacteria. World J Microbiol Biotechnol 24:1643-1656. doi: 10.1007/s11274-008-9659-9

Shaikh S, Verma H, Yadav M, Jauhari M, Bullangowda J (2012) Applications of steroid in clinical practice: A review. ISRN Anesthesiol 2012:985495. doi: 10.5402/2012/985495

Shao M, Zhang X, Rao Z, Xu M, Yang T, Li H, Xu Z (2015) Enhanced production of androst-1,4-diene-3,17-dione by *Mycobacterium neoaurum* JC-12 using three-stage fermentation strategy. PLoS One 10: e0137658. doi: 10.1371/journal.pone.0137658

Shi L, Tu BP (2015) Acetyl-CoA and the regulation of metabolism: Mechanisms and consequences. Curr Opin Cell Biol 33:125-131. doi: 10.1016/j.ceb.2015.02.003 9

Shtratnikova VY, Schelkunov MI, Fokina VV, Pekov YA, Ivashina T, Donova MV (2016) Genome-wide bioinformatics analysis of steroid metabolism-associated genes in Nocardioides simplex VKM Ac-2033D. Curr Genet 62:643-656. doi:10.1007/ s00294-016-0568-4

Smitha MS, Singh S, Singh R (2017) Microbial biotransformation: a process for chemical alterations. J Bacteriol Mycol 4:47-51. doi: 10.15406/jbmoa.2017.04.00085

Stahl W. Sies H (2012) β-Carotene and other carotenoids in protection from sunlight. Am J Clin Nutr 96:1179S-84S. doi: 10.3945/ajcn.112.034819

Tang R, Shen Y, Xia M, Tu L, Luo J, Geng Y, Gao T, Zhou H, Zhao Y, Wang M (2019a) A highly efficient step-wise biotransformation strategy for direct conversion of phytosterol to boldenone. Bioresour Technol 283:242-250. doi: 10.1016/j.biortech.2019.03.058

Tang R, Shen YB, Wang M, Zhou HJ, Zhao YQ (2019b) Highly efficient synthesis of boldenone from androst-4-ene-3,17-dione by *Arthrobacter simplex* and *Pichia pastoris* ordered biotransformation. Bioproc Biosyst Eng 42:933-940. doi: org/10.1007/s00449-019-02092-y

Thawornwiriyanun P, Tanasupawat S, Dechsakulwatana C, Techkarnjanaruk S, Suntornsuk (2012) Identification of newly zeaxanthin-producing bacteria isolated from sponges in the Gulf of Thailand and their zeaxanthin production. Appl Biochem Biotechnol 167:2357-2368. doi: 10.1007/s12010-012-9760-2

Wang H, Song S, Peng F, Yang F, Chen T, Li X, Cheng X, He Y, Huang Y, Su Z (2020) Whole-genome and enzymatic analyses of an androstenedione-producing Mycobacterium strain with residual phytosterol-degrading pathways. Microb Cell Fact 19:187. doi: 10.1186/s12934-020-01442-w

Yeh TJ, Tseng YF, Chen YC, Hsiao Y, Lee PC, Chen TJ, Chen CY, Kao CY, Chang JS, Chen JC, Lee TM (2017) Transcriptome and physiological analysis of a lutein-producing alga Desmodesmus sp. reveals the molecular mechanisms for high lutein productivity. Algal Res 21:103-119. doi: 10.1016/j.algal.2016.11.013

Yulineri T, Nurhidayat N. (2012) Analysis of lovastatin, pigments and citrinin in rice which fermented by *Monascus purpureus* mutant. Berita Biol 11:119-129. doi: 10.14203/beritabiologi.v11i1.1888

Zhang W, Shao M, Rao Z, Xu M, Zhang X, Yang T, Li H, Xu Z (2013) Bioconversion of 4-androstene-3,17-

dione to androst-1,4-diene-3,17-dione by recombinant *Bacillus subtilis* expressing ksdd gene encoding 3-ketosteroid-Δ1-dehydrogenase from *Mycobacterium neoaurum* JC-12. J Steroid Biochem Mol Biol 135:36-42. doi: 10.1016/j.jsbmb.2012.12.016

Zhao Y, Guo, L, Xia Y, Zhuang X, Chu W (2019) Isolation, identification of carotenoid-producing Rhodotorula sp. from marine environment and optimization for carotenoid production. Mar Drugs 17:161. doi: 10.3390/md17030161

Zhou X, Zhang Y, Shen Y, Zhang X, Zan Z, Xia M, Luo J, Wang M (2020) Efficient repeated batch production of androstenedione using untreated cane molasses by Mycobacterium neoaurum driven by ATP futile cycle. Bioresour Technol 309:123307. doi: 10.1016/j.biortech.2020.123307