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REVIEW ARTICLE

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Biologically active metabolites of *Alcaligenes faecalis:* diversity, statistical optimization, and future perspectives

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ABSTRACT

Alcaligenes faecalis is a Gram-negative, rod-shaped bacterium that is common in the environment and has been reported to have various bioactive metabolites of industrial potential applications, including antifungal, antibacterial, antimycobacterial, antiparasitic, anticancer, antioxidant activities. In this review, we highlighted and discussed the respective metabolites, pointing out their chemical diversities, purification, current challenges, and future directions. *A. faecalis* has an industrial role in biodegradation, biosurfactants, and different enzyme production. In this review, the up-to-date various Response Surface Methodology methods (RSM) that can be employed for statistical optimization of the bioactive secondary metabolites have been discussed and highlighted, pointing out the optimal use of each method, current challenges, and future directions.

LAY ABSTRACT

This study aimed to discuss and highlight all the biologically active metabolites produced by various members of *Alcaligenes faecalis*. In the literature, it has been reported that this species produces a wide variety of biologically active metabolites for potential industrial applications, including those having antibacterial, antifungal, antimycobacterial, antiparasitic, anticancer, and antioxidant activities. In this review, we point out the chemical diversity and future perspectives of the respective metabolites and different methods that can be employed to optimize their production and make these metabolites of potential industrial use by the producer strains.

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Alcaligenes faecalis; biocontrol activity; antifungal; antibacterial; eco-friendly; industrial; response surface methodology; purification; optimization; secondary metabolites

1. Introduction

Natural product (NP) chemistry has evolved over the last four decades. Natural products (NPs) have played an essential role in pharmaceutical research due to their chemical variety and diverse bioactivities against medical conditions. Furthermore, because of their low cost and availability, crude natural products have been a major source of medication, particularly in developing countries around the world [1]. In addition, secondary bacterial metabolites are stable because they are end products and are not altered by the microorganisms that produce them [2]. These natural biofactories reduce the possibility of contamination of water and the environment, as well as the risk to animal and human health [3]. Of these metabolites are the secondary metabolites of the genus *Alcaligenes*.

Several metabolites extracted from *Alcaligenes* species have previously been investigated and have shown different biological activities [4,5]. There are many different sources from which one can isolate *Alcaligenes* species e.g., marine [6], soil [7], wastewater [8], other natural environmental sources [8], and humans. *A. faecalis*

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and *A. xylosoxidans* inhabit the intestines and respiratory tracts of vertebrates [9]. The genus *Alcaligenes* is usually non-pathogenic, but it can cause wound infections in hospitalized patients, and opportunistic infections in humans with compromised immune systems [10]. First identified in feces, the genus *Alcaligenes*, and more specifically *A. faecalis*, was frequently found in soil, water, and other environmental samples [11]. This bacterium is being used extensively in pharmaceutical and sewage treatment in the environmental industry [10]. Some *A. faecalis* strains produce enzymes that break down organic pollutants. Several *A. faecalis* strains can also manufacture R-(-)-mandelic acid, a crucial precursor to many drugs [12].

Members of the genus *Alcaligenes* have exhibited a wide bioremediation capacity for a variety of pollutants, like phenols [13], the common pollutant phenanthrene [14], polyaromatic hydrocarbon [15,16], pesticides [17,18], and azo dye degradation [19]. *A. faecalis* has also been found to convert arsenite, the most poisonous type of arsenic, to arsenate, a less harmful form. Heavy metal tolerance has also been recorded [20,21]. Nanoparticle production [22], nematicidal [23], and biocontrol activity [23] have been reported in addition to the ability to produce chemicals [24], detergents [25], gums [26], and bioplastics [27]. *Alcaligenes* species represent high applicability in important research areas, as well as potential benefits in the industrial, agricultural, and environmental domains.

Therefore, in this review, we highlighted and discussed the various bioactive metabolites, pointing out their chemical diversity produced by *A. faecalis*. The up-to-date industrial role of *A. faecalis* in biodegradation, biosurfactants, and different enzyme production has also been investigated and discussed. In this review, a comprehensive electronic searches of published studies of prominent databases including, PubMed, Scopus, Web of Science, EMBL, EMBASE, ChemSpider and NCBI GenBank nucleotide/protein database for the biosynthetic gene clusters of the bioactive metabolites produced by different strains of *A. faecalis* were conducted. We also highlighted the various methods that can be employed for statistical optimization of the previously mentioned bioactive metabolites, pointing out the optimal use of each method, current challenges, and future paths. The information provided in this review will act as a platform for researchers to get, develop, identify the nature and chemical identity of the various bioactive metabolites produced by various *A. faecalis* strains. Moreover, we discussed the different methods that can be employed for statistical optimization of the respective bioactive metabolites and the rationale behind the selection of certain optimizing method(s) based on the physiological/environmental, nature of metabolites (primary or secondary), and cultural conditions affecting their production by the selected *A. faecalis* strains.

2. The genus *Alcaligenes*

The genus *Alcaligenes* is well-known among bacteria for having antibacterial activity against a wide range of microorganisms [28–30]. The 9th edition of Bergey's Manual of Determinative Bacteriology assigned seven species including, *A. faecalis*, *A. xylosoxidans* subsp. *xylosoxidans*, *A. eutrophus*, *A. paradoxus*, *A. piechaudii*, *A. xylosoxidans* subsp. *Denitrificans* and *A. latus*. *Alcaligenes* now has many different species and subspecies on the list of prokaryotic names [31]. Although some strains are cultured under anaerobic conditions, metabolism is purely respiratory. *Alcaligenes* sp. is a chemoorganotrophic bacterium that can grow in a wide range of carbon sources [32]. A short list of the antimicrobial activities of some species of the genus *Alcaligenes* is tabulated in Table S1 (supplementary file).

A. faecalis is a Gram-negative rod, an obligate aerobic, positive for catalase and oxidase, mobile, peritrichous, and non-fermenting bacterium [33]. This bacterium is commonly isolated and found in water, soil, and healthcare settings like respirators, intravenous devices, and hemodialysis systems [34]. These bacteria have different applications in various fields, such as the production of organic acids [35,36], supporting and promoting the growth of plants [37,38], removal of water wastes, e.g., NH_4C- N [39], bioremediation of phenol contamination [40], and biological activity [41]. Kakar et al. [42] reported that *A. faecalis* strains P1 and Bk1 can produce many compounds that help in plant growth promotion, like indole acetic acid, ammonia, and siderophores. Both isolates have phosphate solubilizing activity and can increase the mineral nutrient content in seedlings, resulting in better plant growth. Figure 1 highlights the significant importance of *A. faecalis* and its different bioactivities thanks to the many different metabolites produced by it that aid in combating many infections caused by different species of different microorganisms vis-à-vis the industrial and environmental importance of some of its other metabolites.

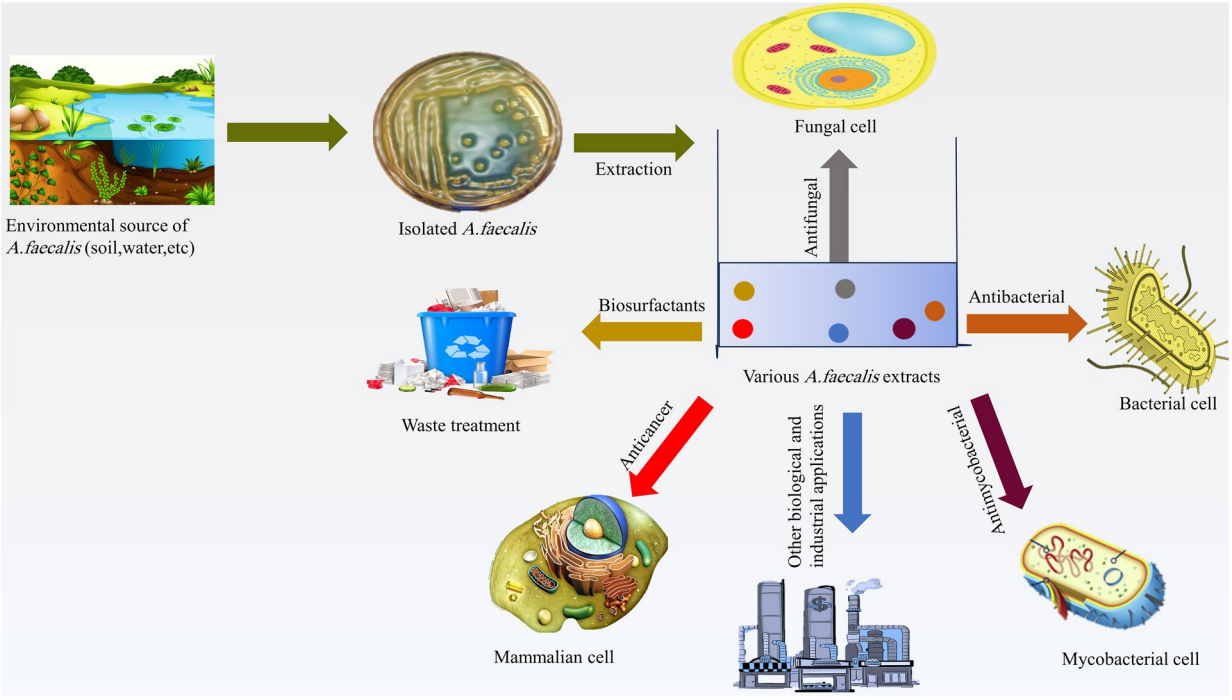

Figure 1. Different activities of various extracts of different strains of *A. faecalis*.

Table 1. Summary of some antifungal metabolites produced by the members of *A. faecalis* group and its activity against various fungal species.

Antifungal compound	Producing *A. faecalis* isolate	Test fungi	Reference(s)
Volatile compounds	*A. faecalis* N1-4 recovered from tea rhizosphere soil	*Aspergillus flavus*	[49]
Indole acetic acid, ammonia, and siderophores	*A. faecalis* strains P1 and Bk1	*Rhizoctonia solani*	[42]
1-Butanamine, benzaldehyde and methenamine	*A. faecalis* MHS033 and MHS013	*Pae. lilacinus* and *Po. chlamydosporia*	[50]
Siderophores	*A. faecalis* BCCM 2374	*Fusarium oxysporum* NCIM 1008, *Aspergillus niger* NCIM 1025, *Alternaria alternata* IARI 715 and *A. flavus* NCIM 650	[54]
Hydroxylamine	*A. faecalis* sp. No. 4	*F. oxysporum* f. sp. *lycopersici race* J1 SUF119	[51,52]
Hydroxamate and catecholate	- *A. faecalis* sp. - *A. faecalis* BCCM ID 2374	Phytopathogenic fungi	[46]
Hydroxylamine	*A. faecalis* AD15	*Colletotrichum gloeosporioides*	[53]
Glucanase, chitinase, and hydrogen cyanide	*Alcaligenes* sp. AE1.16	*Phytophthora cinnamomi*, *P. nicotianae* and *Rhizoctonia solani*	[55]
Biomolecule with antifungal activity	*A. faecalis* (MRbS12)	*C. albicans* CCMB286	[56]
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate	*A. faecalis* MT332429	Clinical isolate of *Aspergillus niger* and *C. albicans* ATCC 10231	[43]

3. Antifungal metabolites

Since fungal infections have many serious consequences, especially in immunosuppressed populations and patients with malignant health problems [43], and because phytopathogenic fungi can destroy many different types of crops, leading to commercial loss [44], researchers employ many ways to discover new natural antifungal drugs with fewer side effects and less resistance patterns. Different studies have reported the antifungal activities of several secondary metabolites from *A. faecalis* that were isolated from different sources (Table 1). According to Sayyed et al., an *A. faecalis* strain recovered from the local rhizospheric soil of a banana plant at North Maharashtra University's research farm has shown the ability to produce two forms of siderophores on modified succinic acid medium (SM): hydroxamate and catecholate. Siderophores are low molecular weight metal chelating compounds secreted by microorganisms growing under low iron stress [45]. Siderophores and their derivatives have a wide range of applications in agriculture, including soil fertility enhancement and

biocontrol of fungal diseases. They also have a crucial function in weathering soil minerals, promoting plant growth, and chelating agents that serve as, biocontrols, biosensors, and bioremediation agents [46]. These siderophores were also produced by *A. faecalis* BCCM ID 2374 [47] (Figure 2(a)). Indole-3-acetic acid, ammonia, and siderophores that are produced by *A. faecalis* strains and Bk1 prevent the spread of sheath blight illnesses caused by *Rhizoctonia solani* fungus by more than 70% [42]. The antifungal activity of Indole-3-acetic acid (IAA) based biopolymeric hydrogel has been studied against three fungi, namely *Aspergillus fumigates*, *Candida albicans* and *Rhizopusoryzae* [48]. Indole-3 acetic acid is the most important metabolite of tryptophan in animals, being shaped in body tissues by intestinal bacteria [48]. The chemical structure of indole-3-acetic acid is illustrated in Figure 2(b).

Many antifungal volatiles were formed by the bacteria *A. faecalis* N1-4 isolated from tea rhizosphere soil. In un-contacted dual culture testing, these volatile chemicals suppressed the growth of *Aspergillus flavus* [49]. Infectivity bioassays against plant pathogenic fungi (PPF) and entomopathogenic fungi (EPF) were also performed using another symbiotic, *A. faecalis* isolated from the soil. Both EPF and PPF were negatively affected by symbiotic bacterial volatile and non-volatile exudations. This deterrent impact on a variety of fungal strains shows that *A. faecalis* bacterial exudates have a common mechanism of action that could affect the microbial communities in soil [41]. A fungistatic activity was exerted by volatile organic compounds (VOCs) such as 1-butanamine, benzaldehyde, and methanamine (Figure 2(c)) that were produced by *A. faecalis* MHS033 and MHS013 [50]. Volatiles of microbial origins are potentially very important and were found to be able to inhibit spore germination and mycelial growth of two fungi, *Paecilomyces lilacinus* and *Pochonia chlamydosporia* [50].

Another fungistatic activity was noticed by *A. faecalis* sp. No. 4 which was reported as a fungistatic bacterium, due to its ability to produce hydroxylamine [51,52]. Also, *A. faecalis* AD15 strain was found to be able to produce hydroxylamine to exert antifungal activity against *Colletotrichum gloeosporioides* [53]. Hydroxylamine was fungistatic, but not fungicidal because its biocontrol effect was gradually diminished after a long period of cultivation, even when hydroxylamine was supplied above the MIC [53]. In contrast to *A. faecalis* AD15, *A.faecalis* BCCM 2374 exerted a fungicidal action by excretion of the fungicidal siderophore bavistin (Figure 3(d)) that had a role in the biocontrol of pathogenic fungi [54]. Moreover, glucanase, chitinase, and hydrogen cyanide were reported by Kavroulakis et al. [55] to be produced by *Alcaligenes* sp. AE1.16 as biocontrol agents in contrast to *A. faecalis* AD15 strain from which these secondary metabolites were not detected [53]. Partial sequencing of the 16S rRNA of a bacterial isolate recovered from *Salacia crassifolia* rhizosphere revealed that it was *A. faecalis* (MRbS12) demonstrated an inhibitory effect on *C. albicans* CCMB286 which proved the ability of *A. faecalis* (MRbS12) to produce bioactive molecule with biological activity against fungi and can be regarded as promising bioprospecting sources for the pharmaceutical industry [56].

A soil bacterial isolate was found to have strong inhibitory activity against *Aspergillus niger* clinical isolate and *C. albicans* ATCC 10231 in our recent investigation. This isolate was identified as *A. faecalis* MT332429 using phenotypic and molecular methods. The antifungal biomolecule was identified as octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoate using 1D and 2D NMR (HMBC, HSQC, and COSY), which is the first

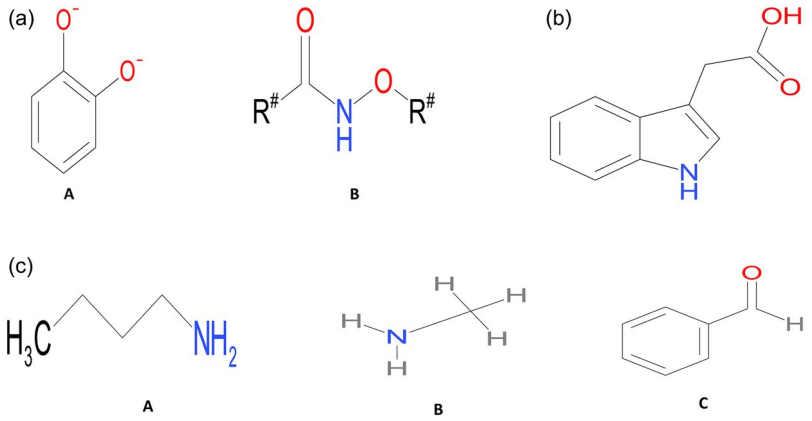

Figure 2. Chemical structures of (a) (A) catecholate and (B) hydroxamate produced be *A. faecalis* BCCM ID 2374. (b) Indole-3-acetic acid produced by *A. faecalis* strains P1 and Bk1. (c) (A) 1-butanamine, (B) Methanamine and (C) Benzaldehyde produced by *A. faecalis* MHS033 and MHS013. (d) Bavistin (structures were created using the ChemSpider | Search and share chemistry) (https://www.chemspider.com/FullSearch.aspx; accessed on 16 December 2023).

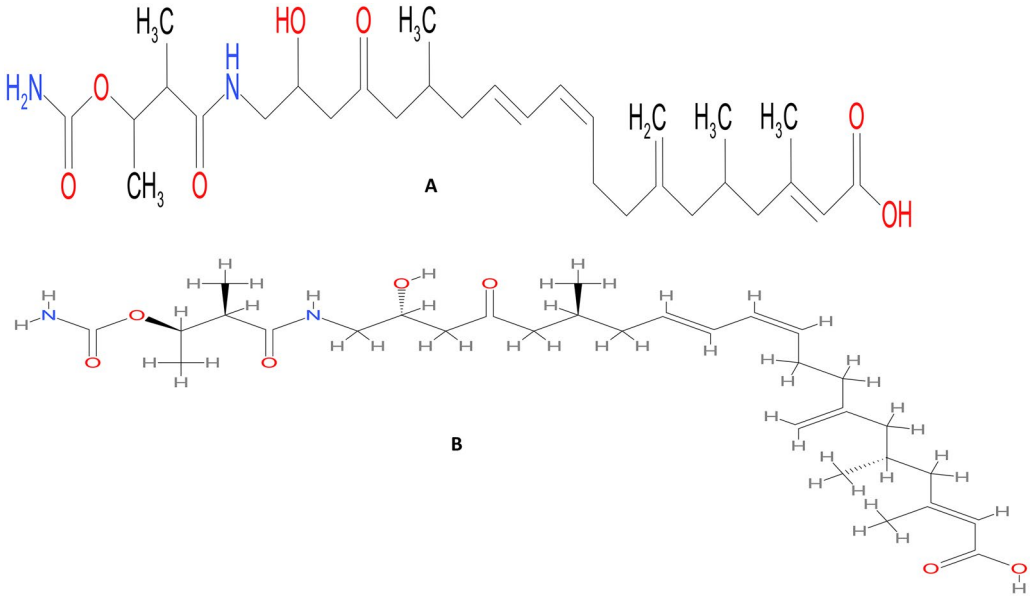

Figure 3. Chemical structures of (A) Kalimantacin and (B) Batumin. (structures were created using the ChemSpider | Search and share chemistry) (https://www.chemspider.com/FullSearch.aspx (accessed on 16 December 2023).

Table 2. Summary of some antibacterial metabolites produced by the members of *A. faecalis* group and their activity against various bacterial species.

Antibacterial compound	Producing *A. faecalis* isolate	Test bacteria	References
Polyketides kalimantacin/batumin	*Alcaligenes* sp. YL-02632S	*S. aureus*	[57]
Hydroxylamine	*A. faecalis* AD15	*Pantoea agglomerans*	[53]
Unknown antimicrobial agent	*A. faecalis*, isolated from a wetland substrate.	Broad-spectrum antibacterial activity	[62]
Burkholderic acid, bacillibactin, ectoine and quinolobactin ectoine, bacteriocin and terpene.	*A. faecalis* isolate MZ921504	MDR Gram-positive (*S. epidermidis* and vancomycin-resistant *S. aureus* VRSA) and Gram-negative (*K. pneumoniae* and *E. coli* ATCC 25922) .	[63]
Crude chloroform extract containing compound of unknown chemical nature	Marine *A. faecalis* grown in marine broth extract	A sulfate-reducing bacterium, *Desulfovibrio* sp.	[64]
Glycolipid biosurfactant	*A. faecalis* isolated from soil contaminated with crude oil in Upper Assam, India.	*B. circulans*, *B. subtilis*, *E. coli*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*	[15]
Unknown antimicrobial agent	*A. faecalis*	*S. aureus*	[65]
Synthesis of silver nanoparticles containing compound of unknown chemical nature	*A. faecalis* isolate GH3	Gram-positive bacteria (*S. aureus, B. subtilis, S. typhimurium*, and *B. cereus*) and Gram-negative (*P. aeruginosa, K. pneumoniae*, and *E. coli*)	[66]

time for this secondary metabolite to be extracted from the culture broth of *A. faecalis* MT332429 [43]. Moreover, the thermophilic bacterium *A. faecalis* was found to have the potential ability to produce a glycolipid biosurfactant that has shown antifungal activity against fungal species, e.g., *C. albicans* (MTCC 227) and *Fusarium oxysporium* (MTCC 284) [15]. A list of some antifungal metabolites produced by the members of the *A. faecalis* group and its activity against various fungal species is illustrated in Table 1.

4. Antibacterial metabolites

Different multisource *A. faecalis* species have been mentioned and studied in different reports for their potential ability to produce many antibacterial metabolites (Table 2), e.g., *Alcaligenes* sp. YL-02632S [57,58] produces bactericidal compounds (polyketides kalimantacin/batumin; Figure 3) that have a killing effect on *S. aureus*. In addition to its fungistatic properties against *Colletotrichum gloeosporioides*, Polyketides and nonribosomal peptides are two primary types of antibiotics produced on multimodular enzymatic assembly lines. The flexibility of scaffold biosynthesis, along with a variety of postassembly alterations by tailoring enzymes, opens up the

possibility of developing new antibiotics with improved action, manufacturing, and pharmacokinetic features. The antibiotic batumin, with the same molecular makeup as kalimantacin A, was isolated from a culture medium of *Pseudomonas batumici* and had similar antibacterial activity [59]. There have been no reports of batumin-resistant clinical *staphylococcus* isolates. Batumin has previously been demonstrated to have only moderate activity against enterobacteria (*Salmonella, Bordetella, Escherichia, and Klebsiella* species), with no activity against micrococci and streptococci. Chemical and UV-induced mutagenesis enabled the creation of strains with 3.5-fold higher batumin production [60]. During these trials, histidine auxotrophy was associated with a decrease in batumin production, indicating a relationship between batumin biosynthesis and the histidine operon [59]. Batumin's limited commercial availability and low purity (<80%) limit its potential for further investigation. Because the absolute configurations of the stereogenic carbon centers of all the aforementioned compounds are unknown, it is still unclear whether they are the same. *A. faecalis* AD15 also showed a bacteriostatic activity against *Pantoea agglomerans* due to hydroxylamine production in synthetic medium [53,61]. Bacic and Yoch found that filtrate from *A. faecalis* strains isolated from a wetland substrate had antagonistic activity against clinically relevant pathogens [62]. The presence of burkholderic acid, bacillibactin, ectoine, and quinolobactin was confirmed by advanced spectroscopic analysis and metagenomics sequence analysis of the soil samples which have confirmed the existence of the biosynthetic gene clusters of burkholderic acid, bacillibactin, ectoine, and quinolobactin ectoine, bacteriocin, and terpene. These metabolites granted this isolate a potential broad-spectrum antibacterial activity on MDR gram-positive bacteria (*S. epidermidis* and vancomycin-resistant *S. aureus* VRSA) and gram-negative (*K. pneumoniae* and *E. coli* ATCC 25922) pathogens [63].

Moreover, crude chloroform extract of the marine *A. faecalis* grown in marine broth extract can inhibit sulfate-reducing bacterium, *Desulfovibrio* sp., growth and hinders biofilm formation and H2S production which leads to biocorrosion of carbon steel coupons surface [64]. Moreover, *P. aeruginosa* (MTCC 7815), *B. circulans* (MTCC 61), *E. coli* (ATCC 9637), *S. aureus* (ATCC 11632), *B. subtilis* (ATCC 11774), *K. pneumoniae* (ATCC 10031) were all inhibited in the presence of a partially purified biosurfactant produced by the thermophilic bacterium *A. faecalis* recovered from soil contaminated with crude oil in Upper Assam, India [15]. According to a new study, *A. faecalis* isolate has been proven to inhibit *S. aureus* and other bacterial species. *S. aureus* planktonic growth was reduced by 94.5%, while biofilm formation was reduced by 76.6 percent, by *A. faecalis*. When combined with bacitracin, *A. faecalis* had a synergistic impact that reduced biofilm growth by 99.7% and planktonic growth by 99.9% [65].

A potent uricase-producing bacteria, *A. faecalis* isolate GH3, was recovered from poultry soil samples, which are often high in uric acid. *A. faecalis* GH3 cell extract was combined with an aqueous solution of silver nitrate to produce silver nanoparticles. Silver nanoparticles made from uricase-producing *Alcaligenes* cell extract have strong antibacterial action against *B. cereus, S. Typhimurium, B. subtilis, S. aureus, E. coli, P. aeruginosa,* and *K. pneumoniae* making them useful in antibacterial development approaches [66].

5. Antimycobacterial metabolites

Recently, Fernandes et al. investigated the whole genome sequence of *A. faecalis* HZ01 which has shown growth inhibitory activity against *Mycobacterium chubuense* ATCC 27278 culture [67]. In addition, *A. faecalis* BW1 (accession number HG737341) isolated from tannery waste in Morocco was proven to have broad-spectrum antibacterial activity, including *Mycobacterium smegmatis*. The produced metabolites were also shown to have a synergistic impact with rifampicin in the treatment of *M. smegmatis*. As a result, after purification and identification, it might be given orally as an antitubercular drug [68].

6. Miscellaneous bioactivities

6.1. Anticancer potential

The antioxidant potential of sulfated extracellular polysaccharides (EPS) from *A. faecalis*, which has previously been isolated from Mauritius saltwater has been previously reported [6]. The EPS were then screened for cytotoxicity C33A HepG2, CaSki, A549, and HeLa cell lines using the MTT assay [6]. These EPS from *A. faecalis* have been considered as potential antioxidant and cytotoxic compounds [6]. Furthermore, purified L-Glutaminase

generated by *A. faecalis* KLU102, which was isolated from the marine realm (Bay of Bengal), had cytotoxic action against HeLa cells, as measured by the MTT experiment, with an IC_{50} value of 12.5 µg/mL [69].

6.2. Eco-friendly activities of A. faecalis

Some strains of *A. faecalis* are utilized in the environmental industries to break down numerous xenobiotics to obtain the highest production of bioflocculant. The culture conditions of a novel *A. faecalis* HCB2 strain, which was isolated from a sediment sample from Sodwana Bay in South Africa, were optimized [70]. This bioflocculant has been utilized successfully to remove colloidal water contaminants, flocculate inorganic solid suspensions, microalgae, and heavy metals [71]. Due to their eco-friendliness and safety for humans, bioflocculants are also considered alternatives to chemical and synthetic flocculants [72]. The employment of such methods could have significant industrial implications, particularly in developing countries where about 90% of effluent is released into water bodies without being treated [73]. The use of *A. faecalis* strain No. 4 showed high potential for environmentally friendly bioremediation of dairy effluent [74].

A. faecalis strain ZWS11 was identified as a potential nicosulfuron-degrading strain by removing nicosulfuron residues from soil and water surfaces [75]. *A. faecalis* JBW4 was isolated from activated sludge as an endosulfan-degrading bacterial strain [18] as the endosulfan is particularly harmful to microorganisms [76]. *A. faecalis* PMS-1 isolated from soil samples showed enzymatic degradation of textile dye Reactive Orange 13 (RO 13) which is discharged in textile industrial effluent [19] and resists degradation because of its structure complexity [77]. Some *A. faecalis* strains are producers of biosurfactants [15], act as denitrifying bacteria [78], can oxidize arsenic [79], and act as biocontrol of nematodes and insects [80]. That is why some *A. faecalis* strains are important biofertilizers, bioremediation agents, and biocontrol agents.

A previous work describing *A. faecalis* strain Mc250 has excellent potential as a plant growth-promoting bacterium [81]. These preliminary findings, together with the biotechnological potential, have led to the whole genome sequence and compare it to other published genomes of this species [82]. *A. faecalis* thermophilic strain was recovered from oil-contaminated soil in India and tested for the ability to produce a new class of biosurfactant glycolipid [15]. This could increase the solubility of hydrophobic substrates, improving bioavailability and leading to increased oil degradation [15]. Based on biochemical characterization such as FTIR and mass spectroscopy investigations, the biosurfactant was identified as a glycolipid. After 96 h of growth on hydrocarbon, tensiometric studies demonstrated that the biosurfactant was produced by *A. faecalis*. The strain belonged to a new class of biosurfactant producers and acts as a contender for producing glycolipid biosurfactant, which is highly valuable in industrial and biotechnological processes [15].

Certain *Alcaligenes* sp. have also been employed in the degradation of phenolic substances and melanoidin when combined with *B. thuringiensis* [83]. Moreover, *A. faecalis* strain SAG5 isolated from distillery effluent and soil samples has been utilized for biodegradation and detoxification of melanoidin [84]. A new *A. faecalis* XF1 strain isolated from soil samples containing plant material waste and sawdust collected from a variety of green localities was discovered to produce a laccase enzyme, which catalyzes the oxidation of a diverse range of aromatic compounds, organic pollutants, and inorganic substrates as well as the reduction of oxygen to water [85].

A. faecalis LNDR-1 produces extracellular enzymes having the ability to break down the polyethylene surface, including lipase, xylanase, carboxymethyl cellulase, and protease. The existence of multiple compounds in the bags treated with bacterial biofilm, as compared to the control setup, was revealed by FTIR data. This suggests various sorts of metabolic pathways. In this experiment, it was also found that the *A. faecalis* LNDR-1 strain can use the used polyethylene bag as a carbon source without any prior treatment. The literature also indicated that the strain was capable of biodegrading plastic at a significant rate. This suggests a workable method for the biodegradation of plastic through the creation of biofilms and extracellular enzymes [86].

Additionally, the effectiveness of an isolated mixed bacterial culture containing *K. oxytoca* KF303807 and *A. faecalis* JF339228 was investigated for the treatment of wastewater. Both strains have demonstrated a promising effect on the bioremediation and removal of cyanide and phenol from coke oven wastewater [87]. Hospital wastewater was made safer for irrigation by being treated with *A. faecalis* isolate P1, which was promoted and retrieved from wastewater samples taken from several locations in Lahore, Pakistan [88]. The combination of TiO2 nanoparticles and *A. faecalis* HP8 (isolated from crude oil-contaminated soil) decreases anthracene concentration in liquid cultures by up to 21.3% after 7 days and 37.9% after 30 days in soil

treatments [89]. The *A. faecalis* JQ191 gene cluster quiA1A2A3A4 was found to be responsible for the initial catabolism step of quinolinic acid which is a nitrogen-containing aromatic heterocyclic molecule that is vital in organisms and serves as a key intermediary in the chemical industry. It possesses high neurotoxicity and cytotoxicity [90]. In a newly published study, *A. faecalis* RB-10, *P. guariconensis* RB-12, and *P. monteilii* RB-29 were isolated from a petroleum-contaminated location to form a consortium for indole biodegradation [91].

6.3. Other biological activities

A nitrilase that changes racemic mandelonitrile to R-(-)-mandelic acid (an optical resolving reagent and the source of semisynthetic cephalosporins and an excellent starting material for the synthesis of antibiotics, anti-obesity medicines, and anticancer agents) was purified from a broth culture of *A. faecalis* ATCC 8750, allowing R-(-)-mandelic acid to be produced naturally rather than through chemical pathways [35]. *A. faecalis* AU02, a chitinase-producing bacteria isolated from seafood effluent, was employed for the manufacture of chitinase antioxidants in marine waste, followed by the extraction of antioxidants from the same marine waste [92]. Silver nanoparticles generated by the extract of *A. faecalis* GH3, an uricase-producing bacteria, exhibited excellent antioxidant properties plus their high antimicrobial action [66].

An *A. faecalis* strain from abattoir wastewater effluent was found to be a potent producer of hemolysin, which is significantly toxic and used as a molecular marker for pathogenicity [8]. Curdlan, being an important carbohydrate polymer that holds significant importance for the food and pharmaceutical industries, was biosynthesized through an engineered approach by *A. faecalis* from orange peels [93]. However, curdlan was produced as a secondary metabolite directly by *A. faecalis* ATCC 31749 [94]. *A. faecalis* was capable of producing a small amount of various l-amino acids in a recent study [95]. To overcome feedback inhibition utilizing the l-methionine analog l-ethionine, strain improvement via chemical and physical mutations were utilized to establish l-methionine overproducers. In further submerged fermentation studies, the l-ethionine-resistant mutant *A. faecalis* ATCXT3624 could yield 20.1 ± 0.32 mg l-methionine/mL [95]. The phenol elimination by biochar-immobilized *A. faecalis* JH1 was studied and was found to be a dual action of adsorption and degradation [96].

6.4. Statistical optimization of bioactive metabolites production

Bioactive metabolite production primarily depends on physical and chemical parameters such as, temperature, pH, incubation time, and carbon and nitrogen sources. These parameters have very powerful influences on microbial metabolite yield [97]. Process and media optimization is a crucial step in permitting the commercialization of microbial manufacturing products, but poses numerous challenges too [98]. Formerly, optimization was done using the classical one-factor-at-a-time technique (OFAT) [99]. Several previous reports studied the optimization of secondary metabolite production from different bacterial and fungal strains using OFAT [97,100]. The foremost advantage of this technique is its simplicity, whereby a range of runs can be completed, and findings can be studied with the help of simple graphs without sophisticated statistical software. However, its main downsides are the complexity of estimating "interactions" from the experiments, the time spent, and the cost involved, since it demands a huge sum of runs to reach the target level. Additionally, the optimal point may be skipped using this technique. Nonetheless, the OFAT method can be the most suitable when nothing about the media is recognized due to its convenience [99].

Recently, OFAT was used to optimize the growth conditions of Alcaligenes sp. with the capability to degrade used engine oil [101]. The microbial processes, being biological, include vast natural variations and many factors affect the different parts of the complex networks associated. Optimization of microbial products has become more brilliant, efficient, frugal, and robust with the introduction of advanced mathematical/statistical procedures. Design of experiments (DOE) is a sequence of runs that are cleverly designed and implemented to acquire a greater sum of data about the influence of more than one factor simultaneously on the product yield, in a reduced count of experiments. Using this technique, numerous variables are evaluated and ranked based on the results. Then, statistical presentation parameters are created from the ensuing evaluation [99,102].

Response surface methodology (RSM) is a powerful, resilient, and competent mathematical methodology that involves statistical experimental designs and multiple regression analysis often applied in the optimization of fermentation processes. RSM considers interaction effects and can fit polynomial calculations to the

laboratory data describing the performance of the data sets. The main aim of RSM designs is to enhance many factors simultaneously to reach optimal system achievements. One of the valuable contributions of RSM is the representation of results as 2D contour or 3D surface plots [99]. The RSM model has proved to be simple, efficient, and capable of optimizing microbial metabolite production in both submerged liquid fermentation [103] and solid-state fermentation [104,105]. It investigates how a certain response is influenced by variations in the factor levels. With RSM, responses can be predicted, even in factor combinations not run in the lab [99]. RSM has become a familiar tactic to optimize and analyze various microbial products due to accessibility to various computer software which has made its application easier [106].

Many studies declare considerable advances in over-optimization obtained using the OFAT method by using RSM methods. For instance, Abdel-Rahman et al. studied OFAT and RSM for increased lactic acid (LA) production by *Enterococcus hirae* ds10 and exhibited a maximum LA concentration through a statistical design that was almost double the amount obtained by classical OFAT [107]. The RSM-based preparations were also supervised to improve the lysine–methionine biosynthesis of *Pediococcus pentosaceus* RF-1 by as much as 100% against that of the OFAT method in another study [108]. RSM should be carried out after a cautious assortment of factors that have a significant impact on the responses. This can be done using first-order designs such as Plakett Burman and Taguchi designs.

6.5. Plackett Burman design (PBD)

PBD is a two-level screening design, and very suitable for efficiently finding the main contributing factors and eliminating the noncontributing ones. Even though PBD successfully identifies the significant parameters, some disadvantages to its efficiency exist. This design should be applied only when the variables have additive effects and are without interactions on the response, since it neglects the effect of one factor on another. PBD is usually used as a starting point in RSM [99]. For example, Plakett Burman's design was used to screen 11 variables for optimized production of L-Glutaminase by A. faecalis KLU102, with potential anticancer activity in a recent study [69].

6.6. Taguchi design

This is a method based on an orthogonal array which was developed to defeat the issues seen with the PBD method. This method allows us to test how different factors affect the product in a few runs as an alternative to testing all the probable combinations. It analyzes the principal effect and two-factor interactions, unlike PBD. However, higher-order interactions are presumed to be insignificant. The Taguchi approach is a fully developed design that has the benefits of saving money, time, and quality [99]. Earlier, studies reported the optimization of polyhydroxybutyrate (PHB) production by *Alcaligenes* sp. NCIM 5085 using Taguchi (DOE) methodology [109]. The Taguchi design was also used to optimize a biosurfactant produced by *Alcaligenes* sp. MS-103 [110]. Second-order designs like Central Composite and Box-Behnken designs assess the interaction of the variables and are in the form of quadratic equations.

6.7. Central composite design (CCD)

The CCD is the most popular design in the RSM experiment [99]. It includes up to 5 levels for each factor and is considered less sensitive than other designs to missing data [111]. In our previous study, CCD was used to optimize the antifungal metabolite, octadecyl 3-(3, 5-di-tert-butyl-4-hydroxyphenyl) propanoate from *A. faecalis* MT332429 [43]. In addition, CCD has been used in another study to optimize the process variables for increased PHB production in batch cultivation by *Alcaligenes* sp. [27]. The optimum conditions for biosurfactant production from *Alcaligenes* sp. S-XJ-1 was also identified using the CCD of RSM [112].

6.8. Box Behnken design (BBD)

BBD is an alternative to CCD, where the factor combinations are at the mid-points of the edges of the process space and the center. This design never includes runs where all factors are at their extreme setting. BBDs are

rotatable and require 3 levels of each factor [99]. An advantage of BBD is that it requires fewer experimental runs than CCD, and is accordingly less expensive [113]. Lately, RSM based on BBD was used to optimize the Congo Red decolorization process by *A. faecalis* H77 [114]. Moreover, RSM using BBD demonstrates the optimal conditions for *Alcaligenes* sp. R3 as a tetracycline removal strain [115].

6.9. Methods for purification of bacterial bioactive compounds

Purification and isolation of valuable secondary metabolites from nature is a challenging process, requiring multiple steps. First, these compounds need to be extracted from the source material. However, these initial extracts are a complex mixture with very low concentrations of the desired compounds. To be useful for research or development, these extracts must be purified to obtain highly concentrated, pure forms of the bioactive compounds. This purification step is crucial for further work, such as identifying the exact structures of the molecules, understanding their properties, and exploring potential applications. In some cases, especially for complex mixtures, researchers may need to combine several purification techniques to achieve the desired level of purity [116]. The following section will discuss some common methods used to purify secondary metabolites from bacteria.

6.10. Thin-layer chromatography

Thin-layer chromatography (TLC) is a method for analyzing a mixture of solids and liquids by adsorption. This approach is beneficial as it is rapid and requires little material. As part of TLC, a mixture is distributed between a stationary and mobile phase. On a glass plate, the adsorbent (stationary phase) is applied in a thin, uniform layer. As a result of capillary action, the organic solvent (mobile phase) moves upward and affects separation. A thin layer of adsorbent material is applied to the surface of the TLC plate, which is typically made of glass, plastic, or aluminum. The most common materials used are aluminum oxide, cellulose, and silica gel [117]. As soon as the solvent reaches the top of the plate, it is removed from the chamber. A TLC plate shows spots for each component, and each component is rated based on its retention factor (Rf). According to how much they adsorb in the stationary phase and how much they dissolve in the mobile phase; the components of the samples will separate.

6.11. Column chromatography

Column chromatography is the most popular method for purifying biomolecules due to its versatility and simplicity. Compounds are separated by their polarity and differential adsorption to an adsorbent. Depending on their polarity, molecules move through the column at varying rates, essentially separating them. The organic solvent (mobile phase) flows through a thin tube that encloses the stationary phase, either under pressure or gravity. To purify a mixture, the mobile phase is eluted from the column and collected in test tubes. Column chromatography is a versatile and simple-to-use method for purifying substances [118].

6.12. Low pressure liquid chromatography

This approach forces the mobile phase to move through the column under low pressure. The performance of low-pressure liquid column chromatography (LPLC) depends on particle size and column pressure. Sample components split and arrive at different rates at the detector as they pass through the column. Liquid-phase liquid chromatography is commonly used for separating compounds from crude samples. LPLC, unlike HPLC, is a non-destructive and preparative method [116].

6.13. High performance liquid chromatography

Separation research has embraced HPLC in recent years. This chromatography approach allows for quick and accurate structural and functional investigation, as well as purification of many compounds. This chromatography method uses high pressure to separate analytes, making it a more advanced version of column

chromatography. This technique involves pushing mobile phases through a stationary phase column at high pressures (10–400 atm) and directing them to a detector at a steady flow rate [117]. The separation performed under high pressure yields improved resolution. Compounds in a column are detected and quantified via a detector. Since the 1990s, preparative high-performance liquid chromatography (HPLC) has gained popularity. Prep-HPLC isolates and purifies target substances, whereas HPLC detects mixtures. Prep-HPLC requires huge columns, high pressures, and high flow rates.

6.14. High-speed countercurrent chromatography

High-speed countercurrent chromatography (HSCCC) is a recent type of countercurrent chromatography invented by Ito and his colleagues in the early 1980s. This approach is commonly used for natural product separation and purification [116,119]. In this chromatographic process, the stationary phase is liquid rather than solid. Separation is performed by partitioning the solute into two immiscible solvents. An HSCCC typically includes a movable phase tank, injection valve, pump, detectors, a column, fraction collector, and data processor. HSCCC offers several advantages over traditional solid-liquid separation procedures, including complete sample recovery, low risk of denaturation, no irreversible adsorption, low solvent consumption, and no need for expensive columns. Once the initial investment is made, only common solvents are used [120].

6.15. Supercritical fluid chromatography

In supercritical fluid chromatography (SFC), a supercritical fluid serves as the mobile phase. A supercritical fluid is a liquid or gas that, when heated or compressed over its critical temperature and pressure, exhibits both gas and liquid qualities. Compared to organic solvents used in other chromatography, supercritical fluids have various desired physical qualities such as low viscosity and high diffusivity, resulting in significantly faster mass movement [116]. Essentially, this approach combines gas chromatography (GC) and HPLC. SFC can be employed with nearly any stationary phase in HPLC and GC. An SFC system consists of a CO2 pump, injector, oven, column, detector, and recorder. Carbon dioxide is commonly utilized as a supercritical fluid in this approach, however nitrous oxide and xenon can also be used to isolate and purify secondary metabolites [121].

6.16. Crystallization

Crystallization is a process for separating solids and liquids. It is the physical transformation of a liquid, solution, or gas into a solid, with atoms or molecules arranged in a three-dimensional crystal structure. To remove contaminants from a mixture, the crystallization process must meet certain structural specifications. Saturation and solubility are two critical elements in crystallization. When a solid material is mixed with a liquid, it dissolves in the liquid. Furthermore, as an additional solid is introduced into the liquid, it loses its ability to dissolve. The stage is referred to as a saturation point, and the fluid as a saturated solution [121].

7. Conclusion

The genus *Alcaligenes* specifically *A. faecalis* bacterium, has the potential to produce many valuable bioactive metabolites that possess antagonistic activities against different bacterial, fungal strains, *Mycobacterium*, and parasites. Some others have valuable industrial properties by producing biomolecules that act as bio-surfactants and biodegrading enzymes. Moreover, they could have potential anticancer and antioxidant activities by producing compounds possessing these activities. These sundry activities of *A. faecalis* provide it with a great industrial significance and put a spotlight on this group of bacteria to be well investigated, screened, and inspected for more medical and industrial applications. Statistical optimization using RSM using various multifactorial designs has proved to be a useful tool for increasing the yield of the respective valuable active metabolites. However, large-scale production of these different beneficial metabolites should be applied to obtain the highest yield and marketed to achieve the best benefit to humanity, the community, the environment, and health care providers.

8. Future perspectives

A. faecalis is a versatile bacterium with diverse applications in various fields. Its ability to produce a wide range of bioactive metabolites makes it a promising candidate for future research and development. One of the main obstacles is the low production level of the respective biologically active metabolites. However, statistical optimization techniques can be employed to solve this problem via studying various physiological parameters influencing the production process and optimizing the yield of the respective metabolites and rendering the production process economically applicable. In addition, a full genome sequence of the promising strains should be undertaken to determine and define the biosynthetic gene clusters of these valuable metabolites and to become able to define the key genes/enzymes involved in the biosynthetic pathways. By this way, researchers can manipulate the genetic pathways to not only optimize production of the respective metabolites but also to produce new biologically active metabolites that never existed in nature.

Lately, with the widespread knowledge of the biosynthetic pathways of most of the respective biologically active metabolites and profound use of gene manipulations, biocombinatorial formation of new metabolites or pathway engineering can be done using genetically modified antibiotic-producing strains. Therefore, protein pathway engineering of the active metabolites can be considered a promising strategy to get newly discovered antimicrobial metabolites to combat the clinically relevant MDR pathogens and overcome the newly emerged pathogens. This possible genetic manipulation, can include, site-directed mutagenesis (through knock-out certain repressor genes that are involved in the catabolite repression of the biosynthetic gene(s)/operon or via knock-in strategy (insertion of additional copy of a key biosynthetic gene to enhance biosynthesis of the respective metabolites), protoplast fusion (between different bacterial species in order to decreases time required for the production or secretion of the respective metabolites extracellularly), or co-expression of certain gene(s) previously known to be involved in unique biosynthetic steps (gene duplication by including a copy of the desired gene(s) on cloning/expression vectors in the producer strains and by this way, the producing strains will have two copies of the desired gene one on the chromosomal and another one on the cloning vectors). This way, new biological entities with new biological activities could be isolated and used in the treatment of infectious diseases, conferring resistance to current treatment.

9. Article highlights

9.1. Alcaligenes as a source of antimicrobial activity

- The genus *Alcaligenes* is well-known for its broad-spectrum antimicrobial activity against various microorganisms.
- This makes it a promising candidate for research and development in areas like antibiotic discovery and control of pathogenic bacteria.

9.2. Taxonomy and nomenclature of the genus Alcaligenes

- The 9th edition of Bergey's Manual listed seven species within the genus, but the current list of prokaryotic names is much more extensive.
- Several new species and subspecies have been identified and classified, including *A. denitrificans, A. faecalis* subsp. *parafaecalis*, etc.
- This highlights the ongoing research and diversification within the genus.

9.3. Metabolic characteristics of the genus Alcaligenes

- Despite some strains being able to be cultured under anaerobic conditions, *Alcaligenes* species are primarily respiratory in nature.
- They are chemoorganotrophic, meaning they obtain energy from the oxidation of organic compounds.
- This characteristic makes them adaptable and capable of utilizing a wide range of carbon sources for growth.

9.3.1. A. faecalis

- *faecalis* is a readily available source of beneficial biomolecules.
- These biomolecules have multiple potential uses in healthcare and industry.
- There are challenges and opportunities for further development, scaling up and applications.

9.4. Applications of A. faecalis

- Industrial: Production of organic acids, bioremediation, waste removal.
- Agricultural: Plant growth promotion, disease suppression.
- Environmental: Biocontrol of fungal and bacterial pathogens.
- Medical: Production of antifungal and antibacterial, antimycobacterial and cytotoxic metabolites.

9.5. Statistical optimization

- Optimization of microbial products has become more brilliant, efficient, frugal, and robust with the introduction of advanced mathematical/statistical procedures.
- RSM is a powerful, resilient, and competent mathematical methodology that involves statistical experimental designs and multiple regression analysis.
- The main aim of RSM designs is to enhance many factors simultaneously to reach optimal system achievements.
- RSM should be carried out after a cautious assortment of factors significantly impacting the responses. This can be done using either first-order designs such as Plackett Burman and Taguchi designs or second-order designs like Central Composite and Box-Behnken designs.

9.6. Purification of bioactive compounds

- Different methods of purification include thin layer chromatography, column chromatography, low pressure liquid chromatography, HPLC, Supercritical fluid chromatography and crystallization

9.7. Future directions

- Statistical optimization techniques can be employed to optimize yield of the respective metabolites and render the production process economically applicable.
- Define the biosynthetic gene cluster of the produced active metabolites and determine the key genes/enzymes.
- Protein pathway engineering is highly recommended to allow not only increase in production but also establish new routes for the biosynthetic pathways.

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