Chapter 5
Modern Analytical Techniques for Extraction, Purification, and Structural Characterization of Microbial Bioactive Compounds

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Abstract Analytical techniques play a vital role in extraction, purification, and molecular characterization of bioactive molecules. The selection of appropriate analytical method depends mainly on the specific properties of the bioactive compound being isolated. Since these bioactive compounds derived from microorganisms found in different environmental conditions, ranging from moderate to extreme, therefore it is impossible to apply a single analytical method universally. Moreover, conventional analytical methods often fall short, especially when multiple nonessential compounds co-elute during the initial solvent extraction and chromatographic purification processes. Nevertheless, significant improvements and advancements are being made in existing analytical methods to enhance the speed and accuracy of the isolation process. Several advanced techniques, such as solid phase extraction (SPE), supercritical fluid extraction (SFE), liquid chromatography-mass spectrometry (LC–MS), single-crystal X-ray diffraction (SCXRD), and two-dimensional nuclear magnetic resonance (2D-NMR), are uncovering the way for future advancements in the characterization of bioactive compounds.

Keywords Bioactive molecules · Microorganisms · Solvent extraction · Chromatographic purification · Structural characterization

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R. Soni et al. (eds.), *Microbial Bioactive Compounds*,
https://doi.org/10.1007/978-3-031-40082-7_5

5.1 Introduction

Microbial habitats often present challenging conditions, including high temperature, high pressure, high salinity, and high pressure. In order to thrive in these harsh environments, microorganisms have evolved various adaptation mechanisms, one of which involves the synthesis of specific bioactive molecules [1, 2]. Bacteria, actinomycetes, fungi, and microalgae isolated from diverse environments are a rich source of various valuable bioactive compounds, such as antibiotics, food enzymes, industry-used enzymes, vitamins, biopesticides, biodegradable plastics, antifungal compounds, anticancer compounds, antioxidants, and immunomodulators [3]. Nonetheless, researchers face substantial challenges when it comes to isolating and characterizing these bioactive molecules from complex biological sources. These compounds often exist in minuscule quantities, buried within a sea of complex mixtures, necessitating sophisticated analytical techniques to unravel their secrets. Consequently, a diverse range of powerful tools and methodologies has been developed to facilitate their discovery, isolation, and characterization.

Initially for the extraction of bioactive molecules, microbial isolates are cultured in the laboratory using appropriate growth media and conditions. Generally, the culturing process starts on a small scale, and once optimized, it is scaled up in fermenters or bioreactors and photobioreactors. Following mass culturing, the focus shifts toward the separation and purification of bioactive molecules, which usually starts with different conventional and advanced extraction methods. Various extraction methods are frequently employed to separate bioactive molecules from microorganisms, including solid-phase extraction, liquid–liquid extraction, supercritical fluid extraction, microwave-assisted extraction, and enzymatic extraction, among others [4]. Each technique has its own advantages, limitations, and compatibility with microorganism sources. Moreover, the influence of extraction parameters, including solvent selection, extraction time, temperature, and methods for microbial cell disruption, should not be disregarded, as they significantly affect the efficiency and selectivity of extracting bioactive compounds.

However, the crude extract, which contains various nonessential components, cannot undergo further characterization until it is subjected to purification. To achieve purification of the bioactive compound, a range of chromatographic procedures are employed, including gas chromatography (GC), high-performance liquid chromatography (HPLC), and mass spectrometry (MS) interfaced chromatographic techniques, such as LC–MS, and GC–MS [5]. Subsequently, after chromatographic purification, molecular-level characterization can be carried out using advanced spectroscopic methods, such as tandem mass spectrometry (MS/MS), X-ray crystallography, nuclear magnetic resonance (NMR), and Fourier-transform infrared spectroscopy (FT-IR) [6].

However, the crude extract, which contains different nonessential components, cannot be further characterized until it is purified. To purify the bioactive compound, various chromatographic procedures are used, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry

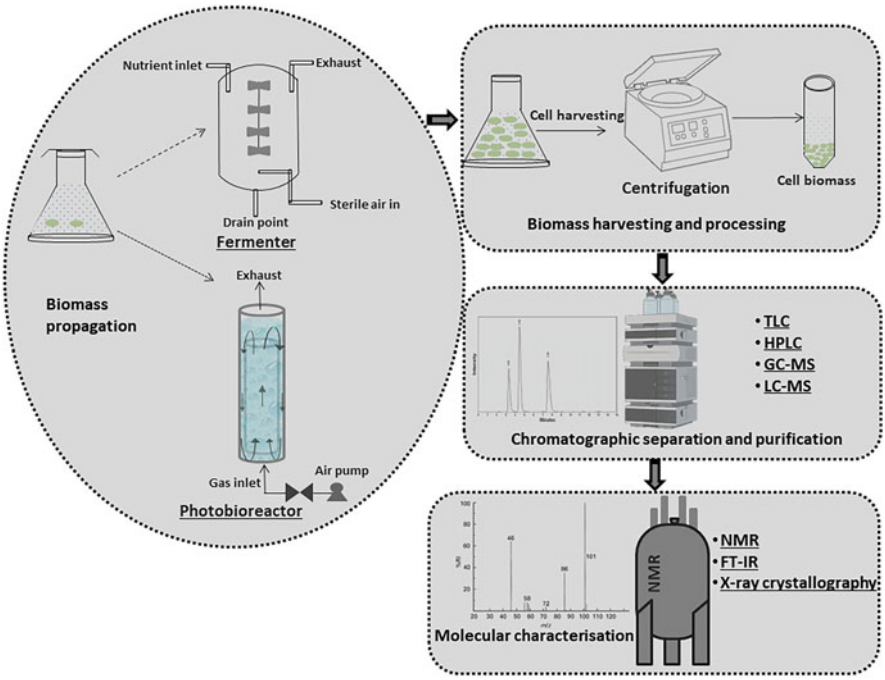

Fig. 5.1 General schematic approaches for extraction, purification, and characterization of bioactive molecules

(MS) interfaced chromatographic techniques, such as GC–MS and LC–MS. After chromatographic purification, molecular-level characterization can be performed using highly sophisticated spectroscopic methods, such as nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FT-IR), tandem mass spectrometry (MS/MS), and X-ray crystallography.

In all these processes, the extraction of bioactive compounds from microorganisms is a critical step for isolating and studying their potential therapeutic properties. The choice of an appropriate extraction method greatly influences the yield, purity, and bioactivity of the isolated compounds. Therefore, it is essential to evaluate various extraction techniques to optimize the extraction process and obtain maximum recovery of bioactive compounds. The second most important step for the characterization of bioactive compounds is chromatographic purification, the credibility of which depends on the purity of the bioactive component for further characterization at the molecular level using different spectroscopic techniques. The choice of extraction, purification, and characterization methods depends on different factors, such as compound stability, target bioactivity, ease of scale-up, and downstream applications, which should be considered during method selection. General schematic bioprocess methods are indicated in Fig. 5.1. By optimizing the extraction process, researchers can enhance the discovery and development of novel

bioactive compounds from microorganisms, leading to potential breakthroughs in pharmaceutical and biotechnological applications.

5.2 Biomass Processing; Extraction, Purification, and Characterization of Bioactive Molecules

5.2.1 Biomass Propagation

Microorganisms isolated from the environment are grown in the laboratory and optimized for cell biomass propagation. However, the main fundamental challenge lies in maintaining the growth characteristics of microbial isolates under laboratory conditions to ensure long-term sustainability and facilitate their later use in the scale-up process [7]. Different types of fermenters and bioreactors including photobioreactors are used to scale up the biomass yield [8–11]. Batch, fed-batch, pulsed fed-batch, continuous (chemostate and perfusion culture system), solid-state fermenters [12], and photobioreactors are commonly used for the cell biomass propagation for different types of microorganisms including microalgae [13]. Nonetheless, the biomass production differs considerably across various types of fermenters and relies on multiple factors including design, size, sensor-based control, regulation of nutrient supply, gas exchange, and mixing [14]. Yet, lot of improvements are required in bioreactor design to optimize the cost of cell biomass propagation and growth media compositions to grow viable but nonculturable cells (VBNC) [15].

5.2.2 Solvent Extraction

The extraction of bioactive compounds from microorganisms is a critical step in the process of isolating and studying their potential therapeutic properties. The choice of an appropriate extraction method greatly influences the yield, purity, and bioactivity of the isolated compounds. Therefore, it is essential to choose right extraction method to optimize the maximum bioactive compound recovery. A number of extraction methods from conventional [16–18] (soxhlet, maceration, and hydrodistillation) to emerging methods such as supercritical fluid [19, 20], subcritical fluid [21], microwave assisted [22], ultrasonic assisted [23, 24], and enzyme assisted [25] have been used for the extraction of bioactive molecules from microorganisms. However, most of the solvent extraction methods are more popularized for the extraction of plant-based bioactive compounds, and they have been less commonly utilized for isolating microbial bioactive compounds. The emerging advanced extraction methods including green extraction methods could be suitable

methods over conventional extraction methods for the isolation of bioactive compounds.

5.2.3 *Chromatographic Purification*

Different microbial extracts (solvent extracts and fractional parts) can be further purified through various types of column chromatography (e.g., liquid chromatography and gas chromatography). The selection of appropriate solvent systems and stationary phases, tailored to the polarity of the bioactive fraction, allows for effective purification. Liquid chromatographic methods commonly used for the isolation of antibiotics and bioactive compounds [26] include normal phase [27] [28], reversed phase [19], ion exchange [29], size exclusion [30], and affinity chromatography [31]. The selection of the appropriate mode depends on the properties of the compounds of interest and the desired separation objectives. Among all the liquid chromatographic procedures, reverse phase is the most commonly employed procedure [26] because many bioactive compounds often possess varying degrees of hydrophobicity, making reversed phase chromatography an excellent choice for their isolation. The nonpolar stationary phase, such as C_{18}, interacts with the hydrophobic regions of the compounds, allowing for efficient separation [32]. Table 5.1 indicates the some selected chromatographic procedures employed for the isolation of bioactive compounds from microorganisms.

In the near future, the requirements of chromatographic procedures employed for the isolation of bioactive compounds from microorganisms are expected to evolve in response to advancements in technology and the growing demand for novel therapeutic agents. One key requirement will be the development of high-throughput and automated chromatographic systems that can efficiently handle large sample volumes and minimize manual intervention. Additionally, there will be a growing need for improved resolution and selectivity in separating complex mixtures of bioactive compounds. This will drive the development of advanced stationary phases, such as novel sorbents and hybrid materials, which can provide enhanced separation capabilities. Another important aspect will be the integration of chromatographic techniques with complementary analytical methods, such as mass spectrometry and nuclear magnetic resonance spectroscopy, to enable rapid compound identification and structural elucidation. Furthermore, there will be an increased emphasis on sustainability, pushing for the use of greener solvents, reduced energy consumption, and recycling of chromatographic materials. Overall, the future requirements of chromatographic procedures for isolating bioactive compounds from microorganisms will revolve around efficiency, selectivity, integration, and sustainability to meet the ever-expanding needs of drug discovery and natural product research.

Table 5.1 Selected chromatographic procedures used for the isolation and purification of bioactive compounds from microorganisms

S. No.	Chromatographic procedure	Microorganism	Bioactive compound isolated	References
1.	TLC	*Bacillus* sp.	Bacitracin	[33]
2.	SPE	*Bacillus lichenform*	Bacitracin	[34]
3.	Cation exchange, SPE	*Micrococcus luteus*	Bacitracin	[35]
4.	C_{18}-HPLC	*Bacillus* sp.	Bacitracin	[36]
5.	HPLC and supercritical fluid extraction (SFE)	*Penicillium expansum*, *Aspergillus fumigatus*, and *Streptomyces* sp.	Chaetogiobosin A, mycolutein, and luteoreticulin, 7,8-dihydro-7,8-epoxy-1-hydroxy-3-hydroxymethylxanthone-8-carboxylic acid methyl ester, and sydowinin B	[19]
6.	HPLC	*Nocardiopsis* sp., SCA21	4-bromophenol, and Bis (2-ethylhexyl) phthalate	[37]
7.	HPLC, LC–MS/MS	*Fusarium proliferatum* CECT 20569	Beauvericin (BEA)	[38]
8.	TLC, HPLC, and LC–MS/MS	*Streptomyces cavourensis* TN638	Cyclo-(Leu-Pro), Cyclo-(Val-Pro), Cyclo-(Phe-Pro), nonactin, monactin, dinactin, and trinactin	[39]
9.	GC–MS	*Streptomyces albidoflavus* 321.2	Dibutyl phthalate	[40]
10.	TLC and GC	*Streptomyces* sp., TN256 strain	N-[2-(1H-indol-3-yl)-2 oxo-ethyl] acetamide 'alkaloid' derivative; di-(2-ethylhexyl) phthalate, a phthalate derivative; 1-Nonadecene and Cyclo (L-Pro-L-Tyr) a diketopiperazine 'DKP' derivative	[41]
11.	HPLC	*Cladosporium* sp., F14	3-phenyl-2-propenoic acid, cyclo-(Phe-Pro), cyclo-(Val-Pro) 3-phenyl-2-propenoic acid, and bis (2-ethylhexyl)phthalate	[42]
12.	TLC, HPLC	*Aspergillus ostianus*	Circumdatins A and B and benzodiazepine alkaloids	[43]
13.	HPLC	*Chondrostereum* sp.	Hirsutane sesquiterpenoid	[44]
14.	HPLC	*Aspergillus* sp.	Aspergilone A & B	[45]

(continued)

Table 5.1 (continued)

S. No.	Chromatographic procedure	Microorganism	Bioactive compound isolated	References
15.	TLC	*S. chibaensis* AUBN1/7	Resistoflavine	[46]
16	TLC, HPLC	*Nocardiopsis alba* MSA10	Lipopeptide biosurfactant	[47]
17.	TLC, HPLC, and LC–MS	*Nocardiopsis* sp., GRG 2 (KT 235641)	1,4-diaza-2, 5-dioxo-3-isobutyl bicyclo[4.3.0] nonane (DDIBN)	
18.	TLC, HPLC, GC–MS, and LC–MS	*Streptomyces akiyoshiensis* GRG 6 (KY457710)	pyrrolo[1,2-a]pyrazine-1,4-dione, and hexahydro-3	[48]
19.	Sephadex G-25 gel column chromatography, and, IRC-50 ion-exchange resin, and TLC	*Streptomyces ahygroscopicus*	ε-poly-l-lysine (ε-PL)	[49]
20.	Ion exchange chromatography through DEAE Sepharose CL-6B column	*Streptomyces fradiae* NEAE-82	L-asparaginase	[50]
21.	Thin-layer chromatography (TLC)	*Nocardiopsis dassonvillei*	Tetrodotoxin	[51]
22.	Anion-exchange chromatography	*Pseudonocardia thermophila*	Thermoactive amidase	[52]
23.	Supercritical fluid extraction (SFE)	*Myxococcus xanthus* DK1622	Chloroxanthic acid A	[53]

5.2.4 Structural Characterization of Bioactive Molecules

The structural characterization of bioactive compounds isolated from microorganisms plays a vital role in understanding their therapeutic potential and mechanisms of action. Various types of advanced analytical techniques are used to determine the chemical structure, stereochemistry, and conformational properties [54]. Spectroscopic methods such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry are commonly used to identify the composition and structure of compounds [55].

NMR spectroscopy provides valuable information about the arrangement of atoms in the molecule and helps in the determination of the compound's stereochemistry. Mass spectrometry, on the other hand, enables the measurement of the compound's molecular weight and fragmentation patterns, facilitating compound identification and providing insights into its structural features. Additionally, other techniques like X-ray crystallography and HR-TEM may be employed to visualize the three-dimensional structure of the bioactive compound, allowing for a more comprehensive understanding of its shape and spatial arrangement. Advanced NMR

techniques are extensively employed in the structural characterization of bioactive compounds isolated from microorganisms. One such technique is multidimensional NMR spectroscopy, which involves the acquisition of multiple NMR spectra with different pulse sequences to correlate nuclear spins and establish connectivity between atoms [56]. Through techniques like COSY (correlation spectroscopy) [57], HMQC (heteronuclear multiple-quantum coherence) [58], and HMBC (heteronuclear multiple-bond correlation) [58], the interatomic relationships, and bond connectivity within the compound can be determined. Additionally, advanced NMR techniques such as NOESY (nuclear overhauser effect spectroscopy) provide valuable information about the spatial arrangement of atoms in the molecule, allowing for the determination of molecular conformation and stereochemistry [59]. The use of selective NMR experiments, such as selective TOCSY (total correlation spectroscopy) and selective HSQC (heteronuclear single-quantum coherence) [60], enables the identification and assignment of specific functional groups within the compound. Overall, advanced NMR techniques play a critical role in elucidating the structural features of bioactive compounds from microorganisms, helping in their characterization and understanding of their biological activities.

Another most advanced technique used to characterize bioactive molecules isolated from microorganism is X-ray crystallography. Similar to 2D-NMR techniques, it also allows researchers to determine the three-dimensional structure of these compounds at an atomic level, providing crucial insights into their chemical composition and spatial arrangement. By growing single crystals of the bioactive compound and subjecting them to X-ray diffraction, scientists can measure the angles and intensities of diffracted X-rays, which are then used to calculate the electron density distribution within the crystal [61]. This information enables the generation of an accurate molecular model, revealing the positions of individual atoms and their connectivity within the compound. X-ray crystallography helps in understanding the stereochemistry, molecular interactions, and overall conformation of the bioactive compound, aiding in the design of more effective drugs and therapeutic interventions. Furthermore, this technique contributes to the elucidation of structure–activity relationships [62], facilitating the optimization and development of novel pharmaceuticals derived from microorganisms (Table 5.2).

To determine the molecular mass of isolated bioactive compound, mass spectroscopy is used. Mass spectrometry utilizes various ionization processes, including electrospray ionization (ESI) [73], matrix-assisted laser desorption/ionization (MALDI) [82], and atmospheric pressure chemical ionization (APCI) [83], depending on the structural complexity and size of the bioactive molecule. Similarly, based on the desired resolution, accuracy, mass range, and other factors to achieve optimal results in their experiments, various types of mass analyzers are used such as quadrupole [84], time-of-flight (TOF) [85], Ion Trap, Orbitrap, Fourier Transform Ion Cyclotron Resonance (FT-ICR), and magnetic sector. Even, among these, quadrupole and TOF are most commonly used methods. However, further interfacing of chromatographic techniques to mass spectroscopic methods, such as LC–MS, and GC–MS, has facilitated the characterization process fast and more accurate.

Table 5.2 Different spectroscopic methods; NMR, MS, and X-ray used for characterization of bioactive molecules

S. No.	Spectroscopic method	Microorganism	Bioactive compound isolated	References
1.	NMR, mass, single-crystal X-ray diffraction (SCXRD)	*Nocardia* sp. ALAA 2000	Chrysophanol 8-methyl ether, asphodelin; 4,7′--bichrysophanol, and justicidin B, in addition to a novel bioactive compound ayamycin; 1,1-dichloro-4-ethyl-5-(4-nitro-phenyl)-hexan-2-one	[32]
2.	X-ray diffraction	*Penicillium vinaceum* (strain no. X17)	Quinazoline alkaloid ((-)-(1R,4R)-1,4-(2,3)-indolmethane-1-methyl-2,4-dihydro-1H-pyrazino-[2,1-b]-quinazoline-3,6-dione)	[63]
3.	Single crystal X-ray diffraction (SCXRD)	*Periconia* sp.	piperine (5-(3, 4-methylenedioxyphenyl)-1-piperidinopent-2, 4-dien-1-one)	[64]
4.	1D, 2D NMR, ESI HR-Mass, and X-ray crystallography	*Aspergillus* sp., ASCLA	Isoshamixanthone, epiisoshamixanthone, sterigmatocystin, arugosin C, norlichexanthone, diorcinol, ergosterol, and methyllinoleate	[65]
5.	NMR and X-ray diffraction analyses	*Aspergillus glaucus*	Aspergiolide A	[66]
6.	1D- and 2D NMR, HRESIMS, MS/MS, and electronic circular dichroism calculation and single-crystal X-ray diffraction	*Penicillium* sp., ZZ380	Penicipyrrodiether A and phenol A derivative	[67]
7.	1D NMR, HRESIMS, and X-ray crystallography	*Diaporthe* sp., GZU-1021	Diaporthichalasins A–C, and biatriosporin N	[68]
8.	1D NMR, 2D (COSY, HMQC, HMBC, NOESY) NMR, X-ray	*Nocardiopsis* sp.	Terretonin N	[69]
9.	NMR, HRESIMS, electronic circular dichroism (ECD) calculation, and X-ray diffraction	*Streptomyces* sp., ZZ446	Streptopyrazinones A − D	[70]
10.	MS, NMR, and X-ray crystallography	*Streptomyces* sp. SN194	Diterpenoids (chloroxaloterpin A and B)	[71]

(continued)

Table 5.2 (continued)

S. No.	Spectroscopic method	Microorganism	Bioactive compound isolated	References
11.	HR-ESI-MS, NMR, and single-crystal X-ray diffraction (SCXRD)	*Streptomyces anandii* H41-59	Anandins A and B	[72]
12.	ESIMS, 1D and 2D NMR data, and X-ray crystallography	*Aspergillus carbonarius*	Carbonarones A, and B	[73]
13.	HR-ESI-MS, X-ray diffraction, and NMR	*Chaetomium globosum*	Azaphilones	[74]
14.	NMR, HRESIMS, ECD, single-crystal X-ray diffraction (SCXRD)	*Streptomyces* sp. ZZ1956	Hygrocins K–U and Streptophenylpropanamide A	[75]
15.	ESIMS, 1D and 2D NMR data, and X-ray crystallography	*Micromonospora echinospora* SCSIO 04089	Angucyclinone derivatives and anthracene	[76]
16.	X-ray analysis	*Alternaria alternata*	Alternariol methyl ether (AME)	[77]
17.	HRESIMS, NMR and single-crystal X-ray diffraction (SCXRD)	*Penicillium* sp. SY2107	Mixed 16 metabolites	[78]
18.	Single-crystal X-ray diffraction (SCXRD)	*Emericella dentata* Nq45	Meleagrin, haenamindole, isorugulosuvine, secalonic acid D, ergosterol, and cerebroside A	[79]
19.	NMR, HRESIMS, electronic circular dichroism (ECD), ^{13}C NMR, and X-ray single-crystal diffraction (SCXRD)	*Penicillium* sp. ZZ380	Penicipyrroether A and Pyrrospirone J	[80]
20.	1D, 2D NMR and ECD	*Talaromyces scorteus* AS-242	Talascortenes A–G and 5α,9β dihydroxyisocupressic acid	[81]

LC–MS is a hybrid technique that combines liquid chromatography (LC) and mass spectrometry (MS) to separate and detect individual components within a complex mixture. In this method, a liquid mobile phase carries the sample through a stationary phase, separating the components based on their physicochemical properties. The eluted compounds are then introduced into the mass spectrometer, where they are ionized and analyzed based on their mass-to-charge ratio (m/z). LC–MS provides high sensitivity, selectivity, and the ability to handle complex mixtures. Liquid chromatography-mass spectrometry (LC–MS) and tandem mass spectrometry (MS/MS) are powerful analytical techniques used in the structure determination of bioactive compounds. MS/MS, also known as tandem mass spectrometry or MS2,

is a technique that involves performing a second round of mass spectrometry on selected precursor ions obtained from the LC–MS analysis. In this process, the selected precursor ion is fragmented into smaller product ions using collision-induced dissociation (CID) or other fragmentation techniques. The resulting fragmentation patterns provide valuable structural information about the compound, including the arrangement of atoms and the presence of specific functional groups. Moreover, LC–MS and MS/MS can be combined with other techniques such as nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry to further enhance the structural determination of bioactive compounds. The combination of multiple analytical techniques increases confidence in the structural elucidation and can help researchers understand the chemical diversity and biological activities of natural bioactive compounds derived from microorganisms. In spite of significant contribution of these techniques in structural characterization of bioactive compounds, many challenges exist depending on purity, and structural complexity of biomolecules.

5.2.5 *Challenges and Future Scope*

A number of challenges exist from culturing of microorganisms to isolation, purification, and finally spectroscopic structural characterization of bioactive molecules. Once culture conditions are optimized, further extraction and purification remain major tasks. Chromatography techniques, such as high-performance liquid chromatography (HPLC), are commonly used for compound separation and purification. However, challenges can arise in the determination of the compound's structure:

(a) *Co-elution*: Sometimes, compounds with similar physicochemical properties can co-elute, making it difficult to differentiate and assign structures. In such cases, additional separation methods, such as preparative chromatography or orthogonal chromatographic techniques, may be employed to isolate individual compounds for further analysis.
(b) *Impurities and matrix effects*: Presence of impurities or complex matrices can interfere with the detection and identification of the target compound. Extensive sample preparation techniques, such as solid-phase extraction or sample derivatization, can be used to reduce interference and enhance the compound's detectability.

Mass spectrometry (MS) also faces challenges in mass determination, mainly due to different ionization efficiencies and fragmentation capabilities of bioactive compounds.

(a) *Ionization efficiency*: Different compounds exhibit different ionization efficiencies, which can affect the accuracy of mass spectral data. Careful optimization of ionization techniques, such as electrospray ionization (ESI) or matrix-assisted

laser desorption/ionization (MALDI), is necessary to ensure efficient ionization and accurate mass determination.

(b) *Fragmentation pattern analysis*: Interpreting the fragmentation patterns obtained from MS analysis can be complex, particularly for large and structurally diverse compounds. The use of tandem mass spectrometry (MS/MS) or high-resolution MS can provide more detailed fragmentation data, aiding in structural elucidation.

NMR is a highly sophisticated tool used to elucidate atomic arrangement inside the molecules, but it also depends on:

(a) *Compound solubility*: Poor solubility of the compound in NMR solvents can impede data acquisition and spectral analysis. Optimization of solvents or the use of advanced NMR techniques, such as microscale NMR or diffusion-ordered spectroscopy (DOSY) [86], can overcome solubility issues.

(b) *Complex spectra*: In the case of structurally complex compounds, overlapping peaks and multiplicity can make spectral interpretation difficult. Advanced NMR techniques like 2D-NMR spectroscopy (e.g., COSY, HSQC, and HMBC) can be employed to resolve overlapping signals and provide additional structural information.

Again similar to NMR, single-crystal X-ray diffraction or crystallography is a powerful method for determining the 3D structure of bioactive compounds. However, it has its own challenges:

(a) *Obtaining suitable crystals:* Obtaining high-quality single crystals can be a significant challenge, especially for compounds with low crystallinity or limited availability. Techniques such as recrystallization, co-crystallization, or cryocrystallography can be employed to improve crystal quality or increase the chances of obtaining suitable crystals.

(b) *Radiation damage*: Exposure to X-rays during crystallographic data collection can lead to radiation damage to the crystal, resulting in poor data quality or structural changes. To mitigate this, low-temperature data collection, limited exposure time, and advanced data collection strategies like multicrystal or serial crystallography are employed.

5.3 Conclusion

Microorganisms are rich source of many value-added bioactive compounds, and their isolation, purification, and structural characterization always remain a challenge. However, various types of analytical techniques such as solvent extraction, chromatographic purification, and spectroscopic methods are used to characterize the bioactive molecules. By uncovering the chemical diversity present in microorganisms, these techniques open up avenues for bioprospecting and drug discovery, offering potential solutions to unmet medical needs and challenges in various

industries. In summary, the isolation, purification, and characterization of bioactive compounds from microorganisms using analytical techniques enable researchers to harness the vast potential of these microorganisms as a source of valuable molecules. These techniques provide critical insights into the structural features and functional properties of bioactive compounds, paving the way for their further exploration and application in diverse fields.

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