Chapter

Microorganisms as Alternative Sources of New Natural Products

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Abstract

Microbial natural products have become important over the last decades due to the ability of bacteria and fungi to subsist in different habitats such as marine and extreme environments. Microorganisms are able to synthesize new compounds with diverse therapeutic activity equal to or better than the activity of compounds already known, thus being promising for the treatment of different diseases such as cancer or the solution to health problems such as antibiotic resistance. The production of microbial natural compounds can be improved by modifying culture media, growing conditions, amplifying gene expression or by co-cultivation techniques, which are the major challenges in the industrial production of such compounds.

Keywords: microorganisms, antioxidants, antibiotics, antitumor, polymers

1. Introduction

The lack of effectiveness in current therapeutics using already known compounds has made necessary the rediscovery of natural products, either for obtaining new compounds or modifying their structure to improve their activity, where plants are the most popular sources.

However, due to seasonal and environmental conditions that influence their production, alternative sources have been searched for. Microorganisms have been considered as good alternative sources due to the self-sustainability and controllable growth conditions such as carbon source, nitrogen source, pH and temperature [1, 2], thus leading to the possibility of discovering new compounds.

In this chapter, we will focus on the uses of microbial secondary metabolites as antioxidants, antibiotics, antitumor and polymers from mainly *Streptomyces* genus, which have been important in soil bioremediation and biocatalysis for the obtention of enantiopure compounds [3–5].

2. Microorganisms as sources of natural products

Since the discovery of penicillin and streptomycin in 1928 and 1943 respectively [6, 7], microorganisms have become fascinating alternative sources because of the diversity of natural products with new structures to be elucidated and studied for biological activity.

Microorganisms can be found in very extreme environments (soil/marine, high/low temperature, acid/alkaline) [8], with the isolation of these microorganisms being a major challenge to date because there are uncultivable microbes, complicating

natural product discovery. To overcome this problem, different techniques have been applied such as co-cultivation, as well as exploration of isolation techniques on natural habitats [9]. Co-cultivation has attracted attention because it can induce the biosynthesis of new compounds [10] such as libertellenone A, B, C and D from co-cultivating α-proteobacterium and *Libertella* sp. [11] and stearidonic acid from *Rhizobium* strain 10II and *Ankistrodesmus* sp. [12].

Terrestrial fungus and actinobacteria are the most important sources of antimicrobials, cytotoxic compounds and antioxidants, among others [13]. However, in the last few years, marine environment has attracted attention due to the diversity and effectivity of natural products [14], such as apratoxins from cyanobacteria from the *Lyngbya* genus used as cytotoxic agents to induce apoptosis [15], as well as salinisporamides isolated from *Salinispora tropica* with activity against human colon carcinoma [16].

3. Antibiotics

The inadequate use of current antibiotics has led to antibiotic resistance, which is a global threat because of the adaptation rate of microorganisms [17]. Natural product discovery as a potential solution to antibiotic resistance has been important if we recall the discovery of penicillin and streptomycin. Nevertheless, actinobacteria isolated from soil have already been widely exploited, limiting the search of new antibiotics [18].

Due to the latter, the need to search new microorganisms associated with higher life forms or from unknown environments such as marine and extreme ecosystems [19, 20], as well as co-cultivation techniques between antagonists strains have been useful [21, 22], as the case of the co-cultivation of a *Micromonospora* sp. with a *Rhodococcus* strain to enhance the production of keyicin [23], as well as the co-cultivation of a marine *Pestalotia* sp. with an unidentified bacteria to obtain pestalone which resulted in high activity against *Staphylococcus aureus* and *Enterococcus faecium* [24].

Among the examples of marine microbial sources is a *Streptomyces* strain isolated from a marine sediment in India that produced ala-geninthiocin along with val-geninthiocin, geninthiocin and staurosporine; all compounds were found to be effective against *Staphylococcus aureus* and *Candida albicans* [25]. Another example is tetrahydroanthra-γ-pyrone from marine *Streptomyces* sp. (isolated from Binzhou shell island), which presented activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Enterococcus faecalis* with a minimum inhibitory concentration (MIC) from 3 to 46 μg/mL [26].

The presence of metals has been explored to increase the production of antibiotics, such as the presence of nickel chloride in the cultivation of *Streptomyces pratensis* (isolated from the east coast of China), which enhanced the production of angucycline-type antibiotics, with moderate antimicrobial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus* with a MIC of 16 μg/mL [27]. Other minerals that have been tested to increase antimicrobial production on other *Streptomyces* strains are magnesium, calcium, manganese, cobalt, copper and iron salts, where copper sulfate and iron chloride resulted in the best induction of antimicrobial biosynthesis [28].

The *Micromonospora* is *a* genus of actinobacteria known to produce other antibiotics such as aminorifamycins and sporalactams with a good antimicrobial activity against *Mycobacterium tuberculosis* [29], as well as phocoenamicins with a potent activity against *Staphylococcus aureus* and *Mycobacterium tuberculosis* (MIC 32–64 μg/mL); the differences in their activity are attributed to different functional groups in the macrocyclic core [30].

Marine fungi have been considered as antibiotic sources such as *Penicillum* sp. (isolated from the coast of China), which produced four new compounds (neocitreoviridin, 10z-isocitreoviridinol, penicillstresseol and isopencillstressol) in the presence of cobalt. Penicillstresseol and isopencillstressol presented a MIC of 0.5 μg/mL against *Staphylococcus aureus*, followed by 10z-isocitroviridinol with a MIC value of 1–4 μg/mL, while neocitreoviridin exhibited a strong activity against *Pseudomonas aeruginosa* with a MIC around 4 μg/mL [31].

Marine *Engyodontium album* (isolated from a sponge) produced six new polyketides, where engyodontochone A and engyodontochone B were the ones that exhibited the best antimicrobial activity against *Staphylococcus aureus*, which was better than that of chloramphenicol [32].

Emerimicin IV extracted from *Emericellopsis minima* (isolated from a bay in Chile) exhibited a strong antimicrobial activity against *Enterococcus faecalis* and moderate to low activity against *Staphylococcus aureus* with a MIC value of 12.5 and 100 μg/mL respectively [33].

Extremophiles have also been useful in the discovery of new antibiotics due to the extreme growth conditions such as salinity (>1.0 M NaCl), pH (<5.0, >8.0), temperature (1–15°C and >45°C) and pressure (380 atm and >500–1200 atm); such conditions can be found on oceans, hypersaline lakes, hot springs and hydrothermal vents, among other places [34]. Actinobacteria are known to survive a range of the conditions previously reviewed such as the ones isolated from Kazakhstan where screening for antagonistic strains against *Escherichia coli* and *Aspergillus niger* [35].

Co-cultivation techniques have also been used for antibiotic synthesis such as *Penicillium fuscum* with *Penicillium camemberti/clavigerum*, whose co-culture allowed the extraction and purification of new macrolides named berkeleylactones. Berkeleylactone A was the one that exhibited the best activity against *Staphylococcus aureus, Bacillus anthracis, Streptococcus pyogenes, Candida albicans* and *Candida glabrata* [36].

4. Antioxidants

Antioxidants are molecules capable of counteracting at low concentrations the damage of mainly reactive oxygen and nitrogen species (ROS and RNS), which are generated from metabolic pathways such as mitochondrial respiratory chain and lipid β-oxidation among others [37, 38]; depending on the ROS/RNS, they can attack different targets [39, 40] whether biomolecules such as proteins, lipids and nucleic acids or cell organelles [22, 41]. Usually ROS and RNS at moderate concentration are useful for defense, signaling mechanisms and cellular maturation [42–45]; however when ROS and RNS concentration are in excess, different pathologies can be caused due to oxidative stress by causing tissue damage [41, 43, 45, 46].

In this regard actinobacteria have played their role as potential sources of antioxidants where [47] isolated *Streptomyces* strains in the Oman sea presented an inhibitory concentration 50 (IC_{50}) that ranges from 356.8 to 566.4 μg/mL against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition.

Growth media is important for the production of antioxidants such as the case reported by [48] on *Streptomyces variabilis* (isolated from the Gulf of Khambhat) using six different media: starch casein agar, yeast malt extract agar (ISP2), glycerol asparagine agar (ISP5), inorganic salt agar (ISP4), tyrosine agar (ISP7) and gause' synthetic agar (GSA), and incubated at 30°C for 7–9 days. GSA medium was selected because there was a larger quantity of cell mass compared to other media;

its metabolites were extracted with ethyl acetate and antioxidant activity was tested against DPPH, metal and hydrogen peroxide (H_2O_2) radical in a concentration range from 0.5 to 2.0 mg/mL. The best radical scavenging activity was against H_2O_2 radical (64% of antioxidant activity) at a concentration of 0.5 mg/mL.

Specific radical scavengers can be obtained depending on the microorganism such as the strain of *Streptomyces antioxidans* (isolated in the forest of Tanjung Lumpur), in a research reported by [49], which exhibited 79.84% of antioxidant activity against superoxide radical at an extract concentration of 1.5 mg/mL; most compounds present in the extract were pyrazines, fatty acids and a phenolic compound. Similar compounds have been found by [50] in a strain of *Streptomyces monachensis* isolated from a mangrove in Malaysia with an antioxidant activity against superoxide radical as well as metal chelating activity of 83.80 and 75.50% respectively.

Among other antioxidants found on microorganisms extracted due to their possible coloring properties are carotenoid pigments mainly used as vitamins in the case of carotenes and xanthophylls, which can be found on bacteria (*Gordonia rubropertincta*), yeast (*Blakeslea trispora)* and microalgae (*Haematococcus pluvialis*) [51].

In this regard, 50 carbon atom carotenoids identified as bacterioruberin derivatives have been detected as main pigments of *Haloterrigena turkmenica* grown in halobacterium medium, which were tested with DPPH and ferric reducing antioxidant power (FRAP) assays [52].

As mentioned earlier, growth media can influence in the production of antioxidants. Three yeasts isolated from Brazil were tested in different media. The highest carotenoid producer was *Rhodotorula mucilaginosa* in malt and yeast extract medium (MYM) followed by glycerol and corn steep liquor (GCSLM) with a biomass production of 13.5 and 7.9 g/L and a carotenoid content of 1068.5 and 224.8 μg/L respectively.

The authors noticed changes in the carotenoid profile with a higher content of β-carotene followed by astaxanthin and lutein in MYM (91.8, 6.9 and 1.3% respectively). With GCSLM, astaxanthin and lutein content increased (23.3 and 71.2% respectively) and β-carotene content decreased (71.2%).

This change in the carotenoid profile influenced greatly in the antioxidant activity where the pigments presented antioxidant activity against DPPH, 2.7 and 14.7% for MYM and GCSLM respectively. A similar, yet higher behavior was observed with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and FRAP [53]. The increase in antioxidant activity could be due to the increase of xanthophyll content since the presence of oxygenated moieties in the carotenoid structures increases the antioxidant activity [54].

Similar experiments have been carried out by adding bivalent ions such as ferrous, calcium, copper and zinc among others as initiators of the Fenton reaction or as cofactors for carotenoid biosynthesis [55–57]. However, in a research reported by [58], such behavior was not observed on carotenoid pigments from marine strains of *Rhodococcus* and *Gordonia* genera (isolated from the Gulf of Mexico). However, a change in the carotenoid profile was observed on *Rhodococcus* sp., which may improve the antioxidant activity for two reasons:

1. The increase on the selective carotenoids that may present the best antioxidant activity [58].

2. The possible formation of carotenoid-metal complexes, mainly in the oxygenated groups [59].

These carotenoids were identified as glycosidic carotenoids; such carotenoid extracts demonstrated a better antioxidant activity against DPPH radical (IC_{50} of 1.07 and 0.09 μg/mL for *Rhodococcus* sp. and *Gordonia* sp. respectively) than β-carotene (IC_{50} of 19.59 μg/mL) [60]. Furthermore, these extracts were compared against those reported by [61] where the authors calculated an IC_{50} of 11.6 and 9.1 μg/mL for carotenoid extracts from two varieties of *Bactris gasipae*, presenting a better antioxidant activity than the bacterial extracts.

Some of these microbial carotenoid pigments are already commercially available for their use as supplement like Lycogen™, which is a carotenoid pigment from a mutant strain of *Rhodobacter sphaeroides* [62], which contains spheroidenone, bixin (a carotenoid found on *Bixa orellana* L.) and hydroxyspheroidenone [63, 64]. Another pigment that is already available is astaxanthin from microalgae *Haematococcus pluvialis*, whose production cost is estimated at $552/Kg, being competitive with synthetic carotenoids ($1000/Kg) [65].

5. Antitumor

Tumoral cells are submitted to high levels of ROS and RNS, manifesting uncontrolled proliferation, death evasion, angiogenesis, invasiveness and metastasis, causing loss of cellular function due to changes in the DNA [66].

The action of ROS and RNS may trigger different factors that stimulate angiogenic processes such as the vascular endothelial growth factor inducing proliferation, migration and tubule formation [67], as well as the induction of epithelial-mesenchymal transition by upregulation of transforming growth factor β [68].

In this regard, antioxidants serve as chemopreventive agents on healthy tissue while increasing the damage on cancerous cells; this phenomenon has been studied on secondary metabolites of plant origin such as soy isoflavone, and polyphenols such as resveratrol and hydroxychalcones [69].

Among microbial compounds that presented a correlation between antitumor and antioxidant activity were in extracts of *Streptomyces malaysiense* with compounds identified as pyrrolizidines and deferoxamine, exhibiting antioxidant as well as antitumor activities. Deferoxamine, which is listed in the World Health Organization's List of Essential Medicines, presents antioxidant activity by chelating iron and antitumoral activities [70].

Another interesting example of antitumoral compounds is an already known compound that is widely used for breast cancer stage III and IV treatment, which is doxorubicin [71], isolated from a mutant strain of *Streptomyces peuceticus*. Doxorubicin works as a DNA intercalating agent by inhibiting the activity of topoisomerase II in DNA replication [72].

Since the 1950s there has been an increase in the interest of studying marine microbial sources for drug discovery in the area of anticancer drugs such as tetracenoquinocin and 5-iminoarianciamicina, extracted from *Streptomyces* sp. in 2010, which were effective against human cervical carcinoma HeLa cells and myelogenous leukemia LH-60 [73].

A similar case is the research reported by [74], where they isolated 32 strains from lagoon sediment in Lagos. The strains were identified mostly as *Streptomyces* and *Micromonospora.* Nine isolates from *Streptomyces* genus presented cytotoxic activity against human acute myelocytic and promyelocytic leukemia, cervical carcinoma, human gastric, breast adenocarcinoma cell lines varying their effectiveness at a concentration below 1 mg/mL. The compounds present in the extracts were identified as kigamicin and staurosporine analogues.

Other kind of compounds found in *Streptomyces* strains are pyrrolopyrazines (found on *Streptomyces colonosanans*), which presented anticancer activity against human colon cancer cell lines [75].

Diketopiperazines from *Streptomyces nigra* (isolated from a mangrove soil) were effective against several human cancer-derived cell lines, while with normal cell lines they were inactive at a concentration range of 50–100 μg/mL. Other compound found on *Streptomyces nigra* was β-carboline, which is a compound widely found in plants with anticancer activity against a variety of cancer cell lines that act inhibitis DNA topoisomerase as well as intercalates in the DNA strands, changing the DNA structure; and tamoxifen [76] which is commonly used to control breast cancer after chemo and radiotherapy have been applied to the patient.

Another actinobacteria with discovered antitumor activity is *Rhodococcus*, where [77] a *Rhodococcus* strain was isolated from a contaminated soil. The extract exhibited cytotoxic activity against HepG2 and Hela cell line with an IC_{50} of 33 and 73 μg/mL respectively.

Another class of compounds that exhibit antitumor properties are polysaccharides, which inhibit cell growth and induce apoptosis as well as exert a synergistic effect with other chemotherapeutical agents such as doxorubicin [78], such as that reported on resveratrol [79, 80]. Examples of these kind of compounds are exopolysaccharides (EPs) produced by *Bacillus mycoides* composed of a sugar mixture containing galactose, mannose, glucose and glucuronic acid; such EPs exhibited antitumor activity by observing morphological abnormalities in HepG2 and Caco-2 cancer cell lines with an IC_{50} of 138 and 159 μg/mL respectively, while on normal cells the IC_{50} was 245 μg/mL [81]. A similar activity was observed with *Bacillus licheniformis* EP constituted by glucose, galactose, fructose, mannose and galacturonic acid on MCF cancer cell lines with an IC_{50} value of 840 μg/mL [82].

It can be observed from both *Bacillus* species that changes in the polysaccharide composition may influence the antitumor activity; as observed by [83] in three EPs of *Streptococcus thermophilus*, two of them were mainly composed by mannose, while the other contained mainly glucose with a protein moiety. The latter exhibited a higher antitumor activity on HepG2 cells with an IC_{50} of 313.75 μg/mL, while for the other two compounds the antitumor activity was below 50%.

Some *Trichoderma* species are also able to synthesize EP constituted by mannose, glucose, galacturonic acid and glucuronic acid with a mannan core, where the antitumor activity was more effective on HeLa cells than on MCF-7 cells by arresting G2/M phase and inducing apoptosis [84].

Fungal endophytes are another kind of microorganisms that could be used as alternative sources of bioactive compounds found in plants. Such as taxol (a chemotherapeutic), pestalactams and penicestorids. Taxol was discovered initially on *Taxus brevifolia*, and it presents a similar activity as doxorubicin [85]. Another example, camphotecin, found commonly on *Campotheca acuminata*, was also found on *Fusarium solani*. Camphotecin from *Fusarium solani* was proved to induce apoptosis on Vero cells at a concentration of 30 μg/mL for 24 h with a maximum apoptosis of 15% [86].

Endophytic fungi are also able to produce EP with antitumor activity. An example is *Bionectria ochroleuca* whose activity was proved to be effective against liver, gastric and colon cancer cell lines in a concentration range from 100 to 450 μg/mL without exhibiting toxicity in healthy cells [87].

Fungal co-cultivation techniques have also been used in the obtention of antitumor compounds. For example, *Isaria felina* with *Aspergillus sulphureus* was used for obtaining oxirapentyn L, which exhibited antitumor activity at IC_{50} greater than 100 μg/mL [88].

6. Polymers

Biopolymers such as lipopolysaccharides (LPSs), EP and extracellular polymeric substances (EPSs) are high-molecular weight substances secreted by microorganisms [89]. In the case of EP, their antitumor properties have been observed in some bacteria as well as in endophytic fungi. EPSs are exopolymers, constituted by polysaccharides, lipids, proteins and nucleic acids; the composition provides these biopolymers unique properties that can be manipulated for a variety of technological applications [90].

LPSs from Gram negative bacteria possess a lipid moiety and a glucosamine fraction with phosphate groups to improve membrane stability [91, 92]. Some of these LPSs have been studied as flocculating and emulsifying agents; for example, the one produced by *Trichosporon mycotoxinivorans* at a concentration of 8.6 mg/mL was able to flocculate kaolin and charcoal with 80 and 78% of efficiency respectively, while the emulsifying activity by mixing water and kerosene presented an emulsification efficiency of 81% [93].

Another application of LPSs is to enhance the immune response by accelerating the maturation of dendritic cells using immobilized LPS nanostructures; compared to LPS solutions and LPS monolayers, such structures could be useful in HIV patients [94]. In a similar manner, inactivated LPSs from non-sulfur photosynthetic bacteria have been used to stimulate immune response [95].

EPs have become important in material science, being useful as storage molecules, protective capsular layers and as matrix components of biofilms due to their water-binding capacity because of hydroxyl and carboxyl groups. EPs can be used in drug delivery, enzyme immobilization, tissue engineering, among other uses [96], their production depends on composition and growth conditions applied on the culture media [97].

EPs from lactic acid bacteria have been used as emulsifiers and viscosifiers because of their pseudoplastic rheological behavior; the sugar identified have been dextran, reuteran, levan and insulin, pullulan (homopolysaccharides), kefiran and hyaluronic acid (heteropolysaccharides) among others depending on the strain used to produce EP [98, 99].

An example of this kind of EP is levan produced by *Bacillus licheniformis* reported by [100] where the authors studied its physicochemical properties and concluded its utility in stabilizing topical formulations. Other uses that have been studied of levan but from *Halomonas smyrnensis* were on tissue engineering and prosthetics [101].

Hyaluronic acid from *Streptococcus equi* was compared against kefiran isolated from kefir grains (also produced on lactic acid bacteria) demonstrating antioxidant and immunostimulatory activities [102].

Marine EPs are mainly heteropolysaccharides composed of pentoses, hexoses, aminosugars or uronic acids [103]. The EPs of *Pantoea* sp. [97] presented wound healing activity by facilitating cell migration on fibroblasts. The EPs of *Bacterium polaribacter* increased 1.42-fold the wound closure at an EP concentration of 1 mg/mL.

EPS in microbial cells aids in the fixation to marine surfaces, thus forming biofilm communities though a three-dimensional arrangement in which the cells can localize extracellular activities and conduct agonist/antagonist interactions. In marine bacteria, EPSs generally contain higher levels of glucuronic and galacturonic acids. Among the sugars found on EPSs are glucose, galactose, mannose, fructose, rhamnose, uronic acids, N-acetyl-glucosamine and N-acetyl-galactosamine; the protein moiety can occur as peptides, aminosugars, glycoproteins, proteoglycans and amyloid proteins. Proteins can occur as peptides, aminosugars, glycoproteins, proteoglycans and amyloid proteins. Extracellular DNA and extracellular nucleases can be found, thus influencing on the physical consistency [90].

EPS production depend on the presence of divalent cations [90], as it is in the case of *Bacillus vallismortis;* which EPS was better in composition in the presence of zinc enhancing the adsorption capacity [104]; while in the presence of the ferric ion the EPS production is limited [90].

An application of EPS is in microencapsulation of vitamins to formulate functional foods as demonstrated on *Cyanoteche* sp. The authors extracted its EPSs and made encapsulation tests of vitamin B12 either alone or in the presence of arabic gum by spray-drying technique. EPS alone presented a particle diameter of 8 μm and when combined with arabic gum the particle diameter was smaller than that of EPS alone; both microcapsules presented different release kinetics due to the different swelling mechanisms of the EPS [105]. EPS from another *Cyanoteche* strain was found to be useful for controlled delivery of small molecules such as procainamide as well as proteins. The authors found out that adding bivalent cations such as Ca^{2+}, as well as considering the protein charge, the release kinetics could improve [106].

Other encapsulation studies were performed on the EPSs of *Bacillus subtilis* in the preservation of *Lactobacillus plantarum* as probiotic, facilitating its survival in gastric conditions during co-cultivation of both strains [107].

EPSs have been widely used in sludge treatment for pharmaceutically active ingredients removal such as ciprofloxacin as well as sulfonamides. EPS from *Klebsiella* sp. was tested against sulfonamides; the high protein content of EPS (mainly tryptophan and tyrosine) is a critical factor in the adsorption of sulfonamides for hydrophobic interactions with sulfonamides [108]. The same thing happens with ciprofloxacin being important to reach the isoelectric point of the protein moiety as well as the use of iron salts to enhance the adsorption of ciprofloxacin [109–111].

The latter ability of EPSs to adsorb antibiotics needs further studies in order to model and improve the kinetics of controlled release dosage forms giving us a natural and possible biocompatible alternative material for design of molecular pharmaceutical forms.

7. Challenges and trends in the discovery and development of microbial natural products

Even though the plethora of natural products of microbial origin mainly isolated from marine and extreme environments is a large field of research, developments in technological aspects such as the increase in natural product production for industrial scale-up or overcoming the difficulty in isolating microorganisms are needed [112].

Genome mining focused on the activation of silent genes, to search gene clusters serving as molecular markers, with complementary informatic tools has been a solution [113, 114]. This technique can be used in metabolic engineering, producing an heterologous host through genetic engineering, using plasmids or recombinant systems using interspaced palindromic repeat, one of the most recent techniques applied in genetic engineering [115].

Another trend also used on unculturable microorganisms is the discovery of environmental DNA coupled to cosmids for gene expression, which have also been used for the selective isolation of biologically active natural products [116].

Search of ideal media and culture conditions have also been a major challenge in optimizing the amount of metabolite present on the microorganism, which have been developed by either trial and error or statistical design [116]; an example is the presence of metallic salts to activate enzymes involved in the biosynthetic route or by manipulating temperature, light, aeration and pH [117] as we saw in the obtention throughout the chapter [117].

Another technique widely observed along the chapter was co-cultivation technique between bacteria, fungi or in combination to improve metabolite production.

Conventionally organic solvents are often used for natural product extraction, which is an important step for industrial scale-up [118, 119]. However, due to the health and environmental hazards, alternative extraction techniques have been searched for with the purpose of reducing residues and thus environmental impact [119, 120]. Among the alternative extraction techniques are ultrasound, microwave, enzyme and pulse electric field. The latter techniques have been widely explored on plants; nevertheless, on microorganisms, they have been poorly explored, thus representing a critical challenge in natural product research [121, 122].

The possibility to expand the research in this regard, is also the search of alternative solvents such as supercritical fluids, [119, 120], ionic liquids, gas-expanded liquids and vegetable oils [121–123].

8. Conclusions

Microbial natural products are a wide research field with much potential to be explored, the main goals being:

a. Isolating new microorganisms, being successful in marine as well as extreme environments, including genetic diversity studies for unculturable microorganisms.

b. Screening of isolates with potential biological activity, by performing extractions of different polarity to begin the selectivity of compounds.

c. Extraction and purification for identification of active compounds, where new extraction techniques can be explored such as supercritical fluids, microwave, enzyme, among others to make the discovery process more eco-friendly.

d. Elucidation of action mechanisms of new active compounds through in silico studies, for considering the possibility of improving the activity.

e. Improvement of natural compounds production for industrial scale-up, as it has already been seen that these are the main challenges and trends through alternative techniques such as co-cultivation, genome mining and media formulation, the last one being the first approach for production enhancement.

f. Preclinical and clinical trials of microbial natural products with already discovered potential activity, to determine biocompatibility and innocuousness of compounds such as EPs and EPSs for antitumoral activity as well as tissue engineering.

As it can be seen, there is a long way ahead in natural product discovery that could solve many health and environmental issues such as antibiotic resistance, cancer, soil and water contamination, tissue engineering, among other contributions.

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