Bioprospecting antioxidants from endophytic bacteria
A review of the growth, extraction and identification methods

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Background

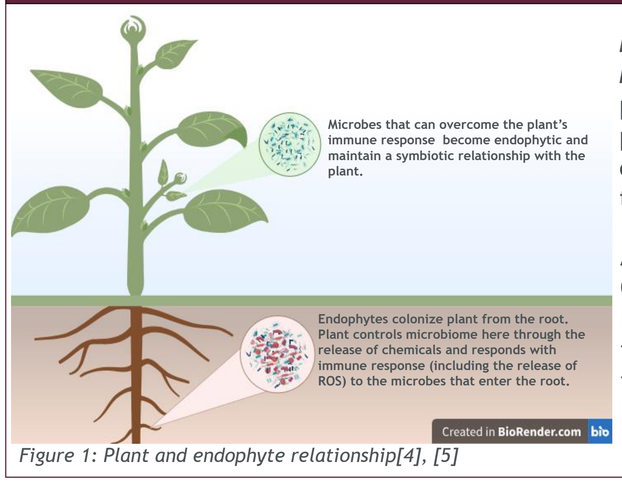

Figure 1: Plant and endophyte relationship[4], [5]

Medicinal plants and endophytic bacteria

Medicinal plants can treat certain medical conditions due to their production of bioactive phytochemicals. Endophytes promote their hosts' survival and have been proven to produce bioactive chemicals which have been attributed to their host plant. Thus, endophytes are potentially a sustainable source of medicinal bioactive compounds for the pharmaceutical industry. [1]-[3]

Antioxidant phytochemicals

Oxidative stress has been linked to several diseases as excess reactive oxygen species (ROS) cause changes in DNA, protein, lipids and sugars[6], [7]. Antioxidants counteract this by reacting with ROS (or its precursors), in either hydrophilic or lipophilic conditions, to less reactive products. Numerous phytochemicals exhibit antioxidant activity (Fig 2). [8]

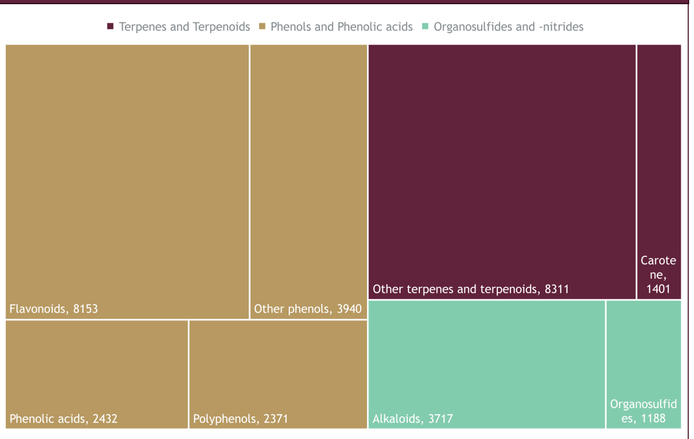

Figure 2: Articles citing phytochemicals with antioxidant activity [9]

Aims and Objectives

The aim of this study is to establish a framework for the investigation of antioxidant bioactive compounds from endophytic bacteria

Objectives

- Investigate extraction processes from microbes and medicinal plants; antioxidant testing methods, and compound analysis methods.
- Synthesize appropriate procedures for the purpose of identifying antioxidant metabolites from endophytic bacteria.

Materials and Methods

This desktop study investigates the existing knowledge on the extraction processes, antioxidant testing methods, and compound analysis methods using journal articles and handbooks in the field of natural products, biotechnology and bioprocess engineering.

Results and Discussion

Microbial growth
- Figure 4 shows the growth process
- Grown in generic nutrient broth, in aerobic conditions
- Final volume is generally one to five litres
- Nutrient broth is harvested after seven days

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Extraction of bioactive compounds
- Figure 5 shows general extraction process from the nutrient broth
- Resin and solvents and which extract weakly polar compounds
- Extract is concentrated in evaporator

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Antioxidant testing
- Hydrogen atom transfer (HAT) assays: ORAC, HORAC, TRAP assays
- Single electron transfer (SET) assays : CUPRAC, FRAP, FC assays
- Mixed mode tests: ABTS, DPPH

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Compound identification
- Chromatography – mass spectrometry
  - Gas chromatography for volatiles
  - Liquid chromatography for non-volatiles

Figure 3: Process for bioprospecting antioxidant compounds from endophytic bacteria [Note: Oxygen radical absorption capacity (ORAC), hydroxyl radical antioxidant capacity (HORAC), trapping antioxidant parameter (trap);cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant capacity (FRAP), and the folin-ciocalteu (FC); 2,2'-azinobis-3-ethylbenzthyazolin-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazil (DPPH)]

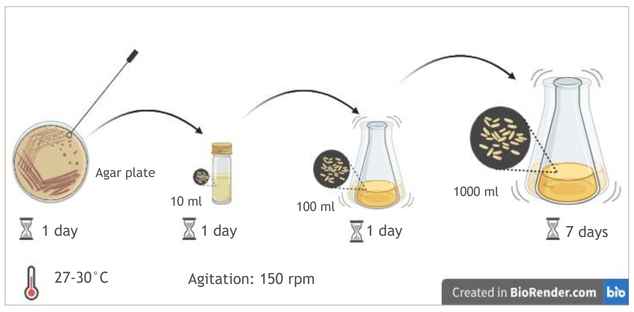

Figure 4: Growth of endophytes in nutrient broth [10],[11]

Growth
- The goal of microbial growth is to reach the stationary phase as this is when secondary metabolites are produced.
- Metabolic activity is generally not arrested when harvesting the broth as changes in concentrations are assumed to be negligible
- No headspace analysis is conducted, excluding these volatile compounds from the investigation, which may exhibit antioxidant activity.

Extraction
- It is assumed that the secondary metabolites are extracellular since the cell excretes metabolites which it does not consume. It is also accepted that secondary metabolites are not reabsorbed into the cell as they are not a suitable carbon source [12], [13].
- The use of resin and solvents and which extract weakly polar compounds are common in literature while no justification is provided for their use. This limits the range of bioactives that can be assessed for their antioxidant activities [10],[11].
- Bioprospecting phytochemicals from plant matter, however, generally involves cascades or separate extractions targeting compounds across the polarity scale [14]. Figure 6 synthesizes the information on solvents along the polarity gradient, and their extractable phytochemicals. This can be applied to the extraction from microbes.

Antioxidant testing and identification
- HAT assays assess scavenging activity of the antioxidant by reacting with oxygen radicals. All mentioned assays are biologically relevant as they occur at normal body temperatures and pH close to 7 . ORAC and TRAP are applicable for both lipophilic and hydrophilic conditions if appropriate reagents are used.
- SET assays assess the reducing ability of the antioxidants. CUPRAC and FRAP occur at biologically relevant conditions and can be modified to suit both lipophilic and hydrophilic conditions.
- Mixed mode tests make use of synthetic radicals and provide insights on the radical scavenging ability of the antioxidant irrespective of the mechanism. They have simpler protocols compared to HAT assays.
- Modifications for different polarities make it difficult to compare between extracts [15], [16].

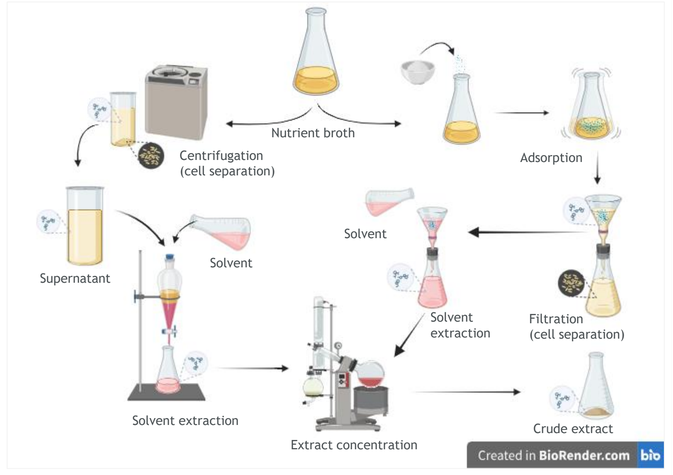

Figure 4: Product extraction methods from nutrient broth

Antioxidant testing and identification (cont.)
- To avoid modification, assays can be tested using known standards across the polarity gradient for better comparability between extracts.
- Gas chromatography is appropriate for volatile compounds and inappropriate for thermally sensitive compounds. Helium is used as the stationary phase, and the liquid is stationary. Temperature is gradually changed from 40-70°C to 240-290°C [17],[18].
- Liquid chromatography separates along the polarity gradient and appropriate for thermally sensitive compounds. Appropriate stationary phases include aluminium (phenols, terpenes and organonitrides and -sulfides), silica gel (alkaloids), and celite (steroids). [19]

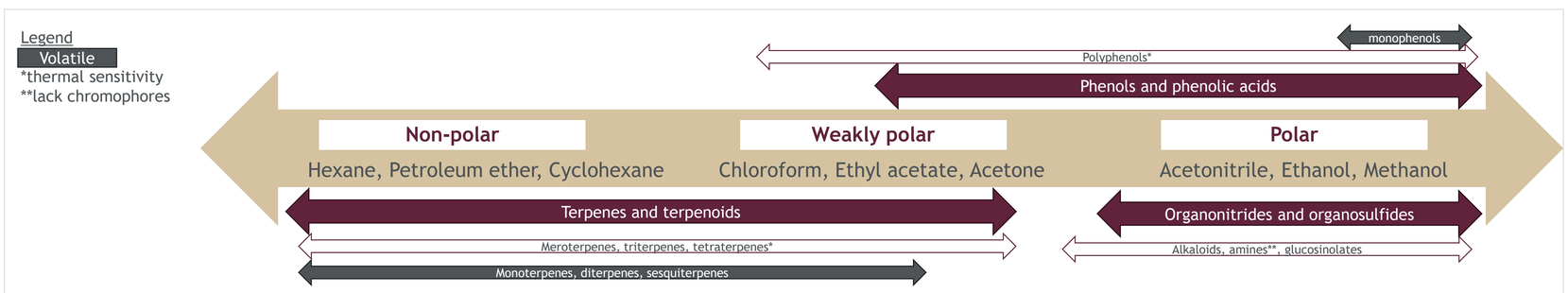

Figure 6: Solvents along the polarity gradient and extractable phytochemicals [14]

Conclusions and Recommendations

The knowledge on bioprospecting medicinal compounds from endophytic bacteria is novel, and the best methods are not clearly defined in literature. This study has shown that by combining methods intrinsic to the processing of bacteria and the downstream processing of medicinal plants, the bioprospecting of antioxidants compounds may yield a wider range of bioactive compounds. Moreover, it may support or reject the commonly adopted approaches of bioprospecting primarily polar or weakly polar antioxidant compounds only.

It is recommended that more studies make use of the aforementioned framework to establish the best protocols for bioprospecting secondary metabolites from endophytic bacteria.

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