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# From Targeted Quantification to Untargeted Metabolomics

Veronica Lelli, Antonio Belardo and Anna Maria Timperio

## Abstract

Metabolomics is an emerging and rapidly evolving technology tool, which involves quantitative and qualitative metabolite assessments science. It offers tremendous promise for different applications in various fields such as medical, environmental, nutrition, and agricultural sciences. Metabolomic approach is based on global identification of a high number of metabolites present in a biological fluid. This allows to characterize the metabolic profile of a given condition and to identify which metabolites or metabolite patterns may be useful in the discrimination between different groups. The use of one mass spectrometry (MS) platform from targeted quantification to untargeted metabolomics will make more efficient workflows in many fields and should allow projects to be more easily undertaken and realized. Metabolomics can be divided into *non-targeted* and *targeted*. The first one can analyze metabolites derived from the organisms comprehensively and systematically, so it is an unbiased metabolomics analysis that can discover new biomarkers. Targeted metabolomics, on the other hand, is the study and analysis of specific metabolites. Both have their own advantages and disadvantages, and are often used in combination for discovery and accurate weight determination of differential metabolites, and allow in-depth research and analysis of subsequent metabolic molecular markers. Targeted and non-targeted metabolomics are involved in food identification, disease research, animal model verification, biomarker discovery, disease diagnosis, drug development, drug screening, drug evaluation, clinical plant metabolism and microbial metabolism research. The aim of this chapter is to highlight the versatility of metabolomic analysis due to both the enormous variety of samples and the no strict barriers between quantitative and qualitative analysis. For this purpose, two examples from our group will be considered. Using non-targeted metabolomics in opposite Antarctic *cryptoendolytic* communities exposed to the sun, we revealed specific adaptations. Instead, through the targeted metabolomics applied to the urine during childbirth, we identified a different distribution of specific metabolites and the metabolic differences allowed us to discriminate between the two phases of labor, highlighting the metabolites most involved in the discrimination. The choice of these two approaches is to highlight that metabolomic analysis can be applied to any sample, even physiologically and metabolically very distant, as can be microorganisms living on Antarctic rocks and biological fluids such as urine.

**Keywords:** urine, metabolite, HPLC–MS, fungus, biomarkers, system biology

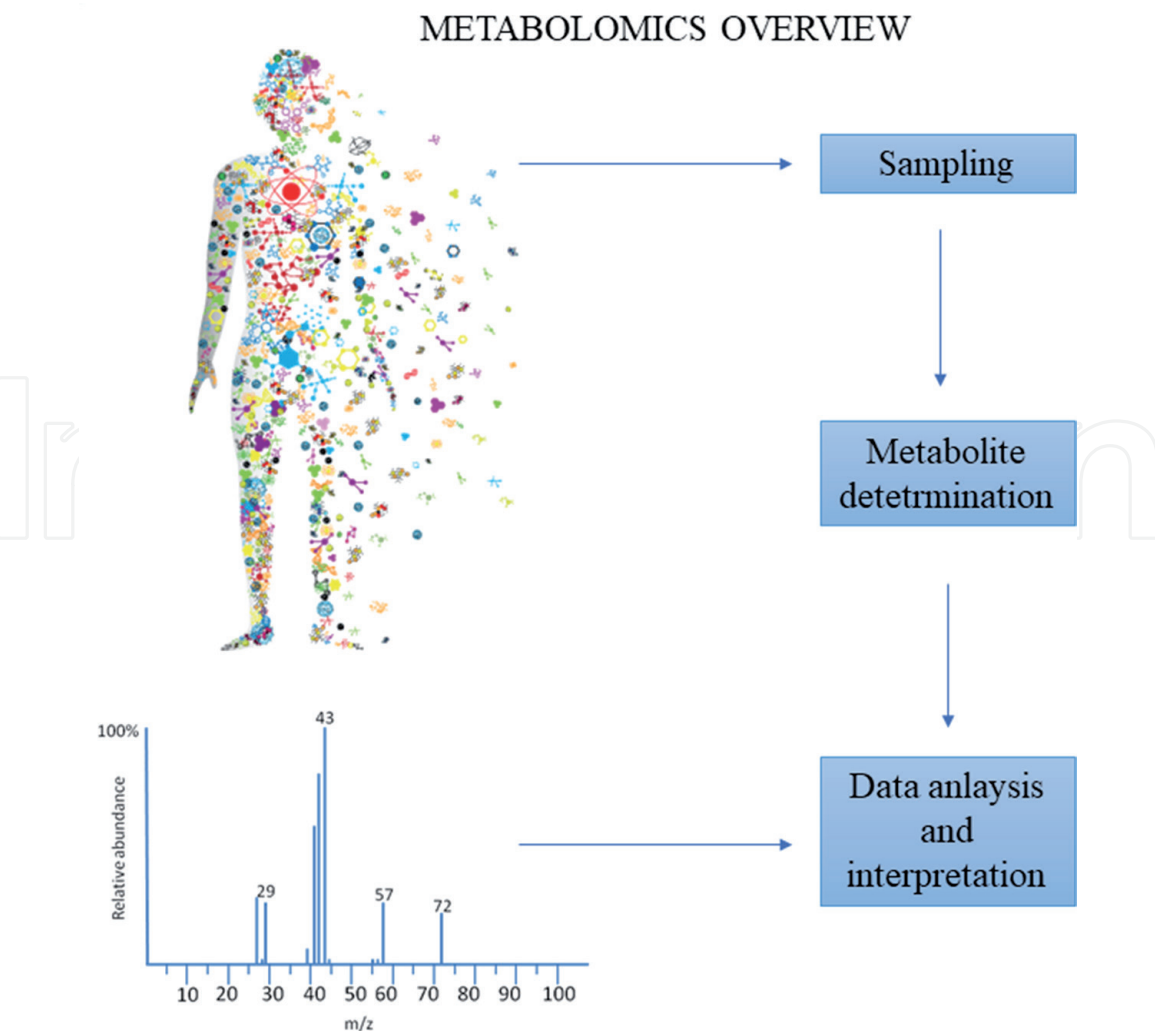
# 1. Introduction

## 1.1 Metabolomics: the medicine of the third millennium

Metabolomics is a discipline thanks to whose analysis it is possible to ascertain the presence of biochemical imbalances caused by the lack of nutrients that are the basis of the functions of our body. With this research it is therefore possible to identify the true causes of any chronic disease and restore the biochemical balance of our body.

The use of “omics” sciences, especially metabolomics, has been having positive implications in recent years in the main actions of daily life, as monitoring the metabolism helps to keep energy levels, sleep and body weight under control. The operation is very simple: by measuring the metabolites present in the body, problems are identified and action is taken in a targeted and relevant way. An action of extraordinary effectiveness if you think about the current situation, for example in the food sector; nutrition today is very rich in calories but poor in nutrients, with the real risk of being overfed, but undernourished (**Figure 1**).

It is essential to remember that this is not an alternative medicine, but is complementary to other disciplines: in the face of even important pathologies, such as neoplasms, it will support the oncologist, improving the responses to cancer treatment and helping to defeat the disease without interfering with the pathways of oncological treatment. A normal blood draw or a simple urine sample is sufficient to check the metabolites. An extraordinarily precise picture of the situation will emerge, a sort of fingerprint of our body and of how many external factors,



**Figure 1.**  
*Metabolomic workflow.*

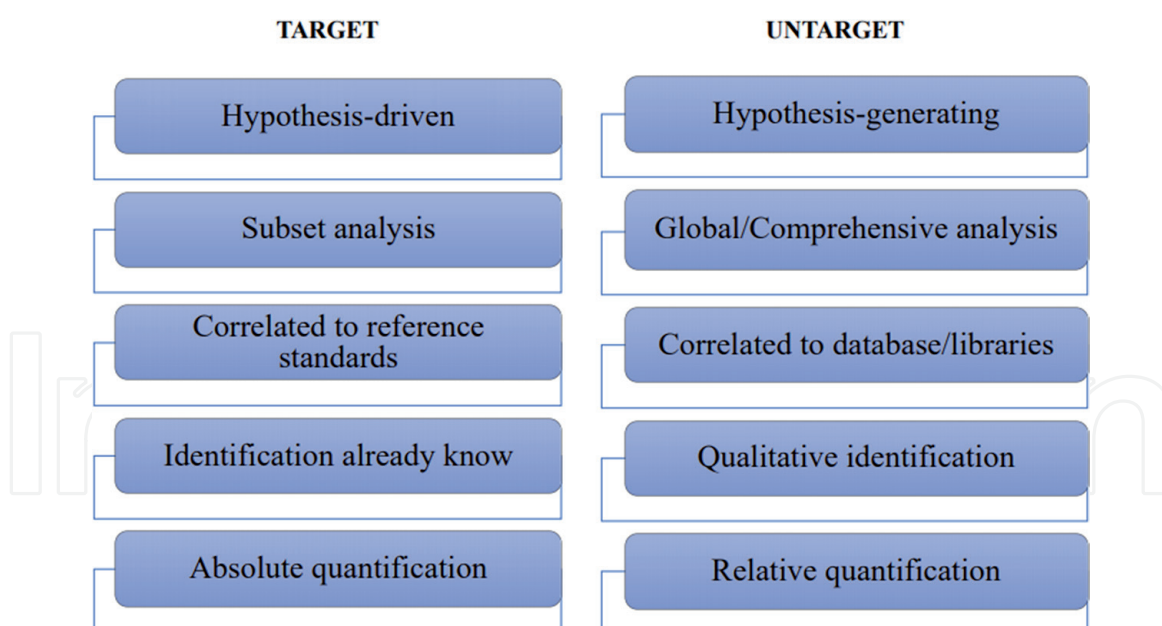
which we can define as part of the so-called epigenetics, can directly influence the chemical aspect. The ultimate goal is to bring the body back to perfect efficiency, taking into account that the body tends by itself, by its nature, to the best possible state of health. However, when the body has difficulty repairing the damage or is facing a progressive problem, which does not have time to fix or lacks adequate resources, metabolomics comes to its rescue. Our body must be considered like a car: to function at its best, the best fuel is needed together with winning strategies of suitable equipment. This discipline, which is progressively affirming itself, has set itself the task of identifying the optimal conditions to support the architrave of human existence.

## **2. Targeted and untargeted metabolomics**

The metabolome is the final downstream product of the genome and consists of all low molecular weight molecules (metabolites) in a cell, tissue or organism [1, 2]. The metabolic profile can provide a complete picture of that cell's physiology. As emerging data suggest an important role for the microbiome and its metabolic products, the potential size of the metabolome is often highly controversial. Given the variety of chemical classes and physical properties that characterize metabolites and the dynamic range of metabolite concentrations over large orders of magnitude, a wide range of analytical techniques are required for metabolomics research. Metabolomics aims to identify and quantify multiple molecules in the context of physiological stimuli or in disease states. The “omics” revolution of the 1980s and 1990s provided new methodologies for the study of interactions on a global level and offered an alternative means of investigation to the more reductionist one in molecular biology. Omics is a field that aims to study the abundance and/or structural characterization of a wide range of molecules in organisms in distinct scenarios. In the clinical field, high-throughput omics techniques are used for disease characterization to better predict the clinical course of organisms and to evaluate the efficacy of existing or developing therapies [3]. In food science, for example, omics plays a significant role in trying to improve human nutrition [4]. On the other hand, concerning the environment, omics studies aim to evaluate the alterations that organisms could undergo after exposure to environmental stressors [5, 6]. In recent years, a variety of omics subdisciplines have emerged (eg Fluxomics, lipidomics, glycomics, foodomics, interactomics and metalomics), demonstrating that omics is a continuously evolving discipline and among all these platforms, metabolomics is becoming increasingly popular [7].

The first definition of metabolomics dates back to the 1990s, describing techniques aimed at identifying existing metabolites within a cell, tissue or organism during a genetic alteration or physiological stimulus [8, 9]. Metabolomics has been shown to be complementary to other omics techniques, thus identifying - called silent phenotypes - genes that when perturbed have no apparent influence on physical characteristics or behavior [10]. The metabolomic approach can be conducted in two distinct ways; non-targeted approach and targeted approach [11].

The reason for this differentiation is due to the different types of data generated in these two approaches, which must be handled accordingly (**Figure 2**). Targeted studies focus research on a number of known metabolites, while non-targeted studies allow for a more comprehensive evaluation of metabolomic profiles. Most of the methodologies used in the first targeted studies only allowed for the identification of a limited number of metabolites. However, recent targeted methodologies allow for the creation of large-scale metabolic profiles, including hundreds of compounds. However, the number of compounds analyzed in non-targeted studies is even greater. This is because entire datasets, including thousands of metabolic signals, need to be processed, and of these, few are finally identified as candidate biomarkers.



**Figure 2.**  
*Targeted and untargeted approach.*

### 3. Untargeted approaches

Untargeted approaches provide the most correct path to detect unexpected changes in metabolite concentrations. The goal is to maximize the number of metabolites detected and thus provide the opportunity to observe unexpected changes. However, a single analytical method cannot detect all metabolites in a biological system. It is therefore necessary to combine multiple analytical approaches (such as complementary HPLC methods) to maximize the number of metabolites detected and improve metabolome coverage.

Sample preparation in non-targeted studies consists of extracting the metabolites from the biological sample in a suitable solvent for analytical analysis. The extracted sample is analyzed with an appropriate analytical method (for example, LC-MS). The result of the mass spectrometry analysis is a chromatogram and the peak area of each metabolite is used as a parameter in the statistical analysis to define the concentration differences between the different biological samples measured. This is called relative quantification as there is no comparison with calibration curves constructed from chemical standards. The use of calibration curves is indispensable for full quantification. The biological significance of each metabolite is determined during data analysis and metabolite identification, and biological interpretation is performed at the end of the experimental pipeline. Currently, one of the main limitations in non-targeted approaches is the identification of metabolites. It may not be possible to identify the metabolites highlighted in the statistical analysis as significant changes between biological classes in the study. The identification of metabolites is currently one of the hot topics of metabolomics.

### 4. Targeted approaches

Targeted studies investigate a relatively limited and specific number of metabolites. At the start of the study, before data acquisition is performed, the metabolites are chemically characterized and biochemically annotated. Targeted methods have greater selectivity and sensitivity than non-targeted methods. A targeted study



can only be performed if a genuine chemical standard of the metabolite is available. The quantification of metabolites is performed using internal and chemical standards to construct calibration curves for each of the metabolites under study. Sample preparation in targeted studies applies methods that can be optimized to retain metabolites of interest and to remove other biological species and analytical artifacts that are not performed via downstream analysis.

Therefore, data analysis strategies for non-targeted studies require very extensive chromatogram processing. A large number of data analysis strategies are found in the literature, but none of them can be considered the optimal choice in all cases, which makes data analysis an open task in bioinformatics research. In fact, the field of MS-based metabolomics is quite young and new methods, software and platforms are regularly published or updated.

The data are produced by chemical–physical investigation techniques such as magnetic resonance spectroscopy, chromatography and mass spectrometry applied to biofluid samples or suitably selected solid tissues. These methodologies find today numerous possibilities of use in the field of medical sciences where there are numerous variables detectable on human and animal subjects that present a specific pathology. This approach is valid both for the description of existing pathologies and for the identification of pre-pathological stages. The use of metabolomic methods can help provide an overall - holistic - view of the problem, highlighting the relationships between variables and their relative importance, and can also highlight differences and similarities between samples. Considering individual biological processes as isolated processes expresses a reductionist view of vital functions, an abstraction that at times makes it possible to considerably simplify the problem under consideration but which inevitably leads to models of limited value.

## **5. Metabolomics techniques**

There is a range of analytical chemistry tools applied in metabolomics research.

Each instrument has advantages and limitations, and no single instrument or instrumental method can detect all of the metabolites present in a metabolome, but multiple instrumental methods or multiple different instruments are required to provide the largest number of metabolites detected.

Several analytical techniques have been developed for each of the omics platforms, including techniques based on DNA microarray and RNA sequencing [12], nuclear magnetic resonance (NMR) spectroscopy [13, 14] and mass spectrometry (MS) [15, 16]. NMR and MS are the most used in the field of metabolomics. High resolution proton NMR spectroscopy (<sup>1</sup>H-NMR) has proven to be one of the best technologies for examining biofluids and studying intact tissues, as the result is a complete signal profile of metabolites without separation, derivatization and pre-selected measurement parameters [17, 18]. On the other hand, MS methods, both by direct injection [19] and coupled with chromatographic techniques [20], have also evolved into an excellent technology for metabolomics due to their ability to analyze low molecular weight compounds in biological systems. These two approaches (NMR and MS) are complementary and the integration of both technologies can provide more comprehensive information in the field of metabolomics.

Applications of metabolomics have expanded in line with genomics, proteomics and transcriptomics with the aim of determining gene function in microbes [21], plants [22] and animals [23]. Many different applications are also used today, such as the determination of metabolic biomarkers that change as an indicator of the presence of a disease or in response to a pharmacological intervention or the determination of the effect of biochemical or environmental stress on plants or

microbes, genetically modified plants [24], bacterial characterization [25], health status assessments [26] and metabolic engineering [27].

### **5.1 Gas chromatography–mass spectrometry (GC–MS)**

GC–MS is a combined system in which volatile and thermally stable compounds are first separated from the GC and then the eluted compounds are detected by electron impact mass spectrometers. During the run, aliquots of derivatized samples (injection volumes of 1  $\mu$ l or less) are analyzed using split and splitless techniques on different polarity GC columns (DB-5 or DB-50 or similar capillary columns in stationary phase are most commonly used). These provide both high chromatographic resolution of compounds and high sensitivity (typical limits of detection are concentrations of pmol or nmol). Quantification is provided by external calibration or response ratio (metabolite peak area/internal standard peak area). The coverage of the metabolome is largely characterized by the volatility of the non-derivatized or derivatized sample components. The identification of metabolites is provided by matching the retention time and mass spectrum of the sample peak with those of a pure compound previously tested on the same or a different instrument under identical instrumental conditions [28]. Since the electron impact mass spectrometer provides the standard fragmentation of molecular ions during ionization, structural identification can be performed through the interpretation of fragment ions and fragmentation patterns.

### **5.2 Liquid chromatography-mass spectrometry (LC–MS)**

LC–MS provides separation of metabolites by liquid chromatography followed by electrospray ionization (ESI) or, less typically, atmospheric pressure chemical ionization (APCI) [29]. This technique differs from GC–MS for several reasons (lower assay temperatures and unsolicited sample volatility) and this simplifies sample preparation. In most non-pharmaceutical applications, samples are prepared after intracellular extraction and/or protein precipitation by dilution in an appropriate solvent. The chemistry and HPLC column size used will affect chromatographic resolution and sensitivity. Analytical columns do not provide the chromatographic resolving power to separate these complex samples and run times of 10 minutes followed by chemometric instruments are used to extract the chromatographically unsolved data and classify the differences between the samples [30]. The application of very high-pressure chromatography systems can improve the chromatographic resolution. The most common column chemicals used today are reverse phase C 18 or C 8 columns. However, for polar metabolites injected on these columns, the retention of these metabolites is minimal, thus reducing the volume of interpretive data. To overcome this problem, other chemical columns are needed, such as HILIC [31] and other weak ion exchange chemicals. The sample then, once the chromatographic separation has been carried out, reaches the source of the mass spectrometer. Electrospray instrumentation operates in positive and negative ion modes (as separate experiments or by polarity switching during analyses) and detects only those metabolites that can be ionized by adding or removing a proton or adding another species of ions. Metabolites are generally detected in one but not both ion modalities, so broader metabolic coverage can be achieved by analysis in both modalities. Quantification is performed by external calibration. ESI does not cause molecular ion fragmentation as observed in electron impact mass spectrometers, thus it does not allow direct identification of metabolites by comparing ESI mass spectra, as ESI mass spectral libraries are not commonly available (as in the case by GC–MS). However, with the use of accurate mass measurements

and/or tandem MS (MS/MS) to provide collision induced dissociation (CID) and correlated mass spectra (MS/MS), correct identification of metabolites can be achieved [30].

### **5.3 Direct injection mass spectrometry (DIMS)**

DIMS is shown as a high-throughput screening tool (hundreds of samples per day with a run time generally of 1 minute). The extracts of the crude sample are injected or infused into an electrospray mass spectrometer and results in a mass spectrum per sample, which is representative of the composition of the sample. The ionization capacity of the metabolite determines metabolic coverage, as for LC-MS. The applications of DIMS are mainly concentrated in the microbial [32] and plant areas.

### **5.4 Fourier transform infrared spectroscopy (FT-IR)**

Vibrational spectroscopy techniques such as Fourier transform infrared spectroscopy (FT-IR) and Raman spectroscopy have been used to analyze metabolic changes in biological samples. The methodologies consist of passing ultraviolet or infrared light through a sample before it is detected. The vibrations and rotations of the bonds relating to different chemical groups resulting from the interaction of the sample with ultraviolet or infrared light are mainly measured.

The problem with these techniques is the inability to detect each metabolite separately; instead, it is a specific technique for single molecules that will absorb ultraviolet or infrared light at specific wavelengths. In FT-IR, a metabolic fingerprint is taken with a single absorption spectrum collected for each sample and consisting of information for many metabolites. The result is similar to data produced by direct infusion mass spectrometry where a single mass spectrum is collected rather than an absorption spectrum for each sample. The metabolic fingerprints produced in these approaches lack the sensitivity of mass spectrometry but are a useful tool for high throughput screening since the FT-IR analysis time is approximately one minute per sample. To date, the vast majority of metabolomic studies undertaken using vibrational spectroscopy have been performed with FT-IR spectroscopy. However, the work was done using Raman and, in terms of metabolomics, this is an emerging technology with significant potential for metabolite monitoring [33].

### **5.5 Nuclear magnetic resonance (NMR)**

Nuclear magnetic resonance (NMR) spectroscopy applies the magnetic properties of atomic nuclei in a metabolite. Only some atoms are active NMRs and include  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{31}\text{P}$ . Proton ( $^1\text{H}$ ) NMR spectroscopy is the most frequently applied in metabolomics. The technique works by inserting a liquid sample into a small internal diameter tube (about 5 mm), where it is pulsed with a range of radio frequencies that cover all possible energies required to excite the selected type of nuclei. Nuclei absorb energy at different radio frequencies depending on their chemical environment and then the release of this energy is measured, forming what is called a free induction decay (FID). This FID is converted from a time domain data set to a frequency domain - using a Fourier transformation - and an NMR spectrum is constructed as the absorption energy plotted against the peak intensity. The NMR spectrum (in particular the chemical shift) depends on the effect of the shielding by the electrons orbiting the nucleus. The chemical shift for  $^1\text{H}$  NMR is determined as the difference (in ppm) between the resonance frequency of the observed proton and that of a reference proton present in a reference compound (for  $^1\text{H}$  NMR experiments, tetramethyl-silane in solution, fixed at 0 ppm).



The intensity of the signal depends on the number of identical nuclei and the presence of complex samples does not interfere with the measured intensity, as does the suppression of ionization with electrospray ionization. NMR spectroscopy is a high-speed fingerprinting technique. Raw samples are mixed with a solution of the reference compound added to an NMR probe (usually less than 2 mL), inserted into the instrument and analyzed. NMR probes are generally based on a large volume of  $\mu\text{l}$  and this adds constraints to the required sample volume. However, the introduction of 1 mm  $\mu\text{l}$  probes allowed to analyze volumes of 2  $\mu\text{l}$ , thus enabling invasive sampling of smaller volumes of study subjects, which is important for small animal studies [10]. Spectra are complex and contain thousands of metabolic signals. For data processing, the spectrum is generally divided into chemical shift ranges with widths of 0.02–0.04 ppm. All signals in this bucket are added together. Chemical changes can be assigned to specific metabolites and the pure metabolite can be added for further clarification. However, the spectrum model is generally used in sample classification, similar to that used for FT-IR and DIMS.

## 6. Examples of metabolomics studies

### 6.1 Urine metabolomics

The metabolic profile of biofluids has emerged as an important tool in the diagnosis of numerous diseases that remain silent until late progression of the disease [34]. Because urine is such a rich source of biomarkers, the metabolic profile is a promising tool for assessing therapeutic efficacy. Urine collection is also non-invasive, does not require patient preparation and substantially improves compliance. Recent results clearly demonstrate the potential of urine metabolomics in diagnosis by providing new insights into the biochemistry of its pathophysiology [35]. The qualitative/quantitative analysis on the urine of pregnant women between two different stages of labor called OL (out of labor) and IL-DP (in labor in the dilation phase), using as a technique the ultra-performance liquid chromatography of hydrophilic interaction coupled with mass spectrometry (HILIC-UPLC–MS), a highly sensitive, accurate and unbiased approach, are an example [36]. The list of metabolites is shown in **Table 1**. The urinary metabolites showing the greatest differences belong to the steroid hormone, in particular conjugated estrogens and amino acids, much of this difference being determined by fetal contribution. The increased excretion of conjugated estrogens in the DP stage may confirm the coordinated role played between fetus, mother and placenta during labor. It is reasonable to consider this terminal phase of pregnancy not only as a mechanical event linked to the increase in uterine contractions, but as a more complex process. **Figure 3** shows the major compounds excreted in the urine (such as Estradiol Glucuronide, Estrone 3-Sulphate and Estriol Glucuronate) which are downstream of a more intricate process. These compounds originate from an interconnected metabolism between mother-fetus and placenta involving steroid hormones. As seen in **Figure 3**, the metabolites excreted can come directly from the mother, or with the contribution of both the placenta and the fetus. In the latter case, the excretion of metabolites occurs through the degradation of intermediates, in particular the hormone pregnenolone.

### 6.2 Antarctic cryptoendolithic communities

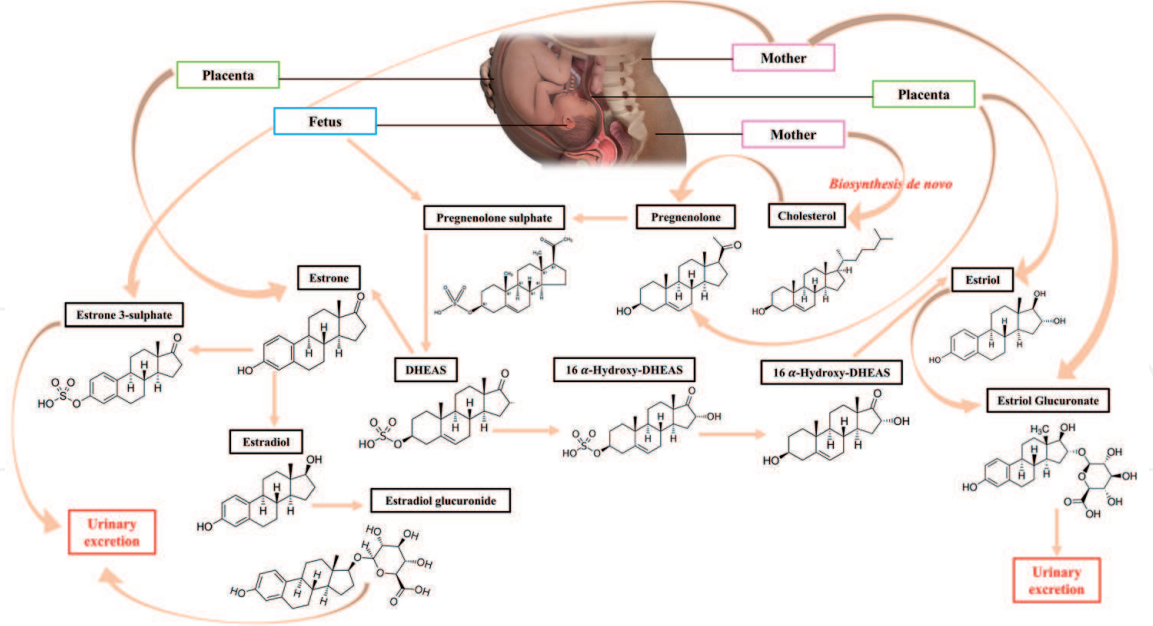
Antarctic *cryptoendolite* communities are microbial ecosystems that dominate the biology of most ice-free areas in mainland Antarctica. These are complex and

Compounds	Molecular weight	Percentage
3 hydroxy2-methyl-1H-quinolin-one	175.05	3% - up
19 chloro19-Chloro-3beta-hydroxyandrost-5-en-17-one = dehydroepiandrosterone	365.1695	95% - up
Androst-5-ene-3beta,17beta-diol = androsterone	290.1736	21% - down
Androsterone	290.1736	21% - down
Dehydroepiandrosterone Sulfate	369.0992	92% - down
Dehydroepiandrosterone	288.1873	3% - down
Pregnanediol	321.2145	25% - down
3-Hydroxy-1-methylestra-1,3,5(10),6-tetraen-17-one	283.1551	63% - up
Tetrahydrocortisone	365.1592	88% - down
Estrone 3 sulfate	351.1085	>100% - up
Estrone gluconoride	447.1790	87% - down
Estradiol 17 beta 3 gluconoride	449.1945	42% - up
Ser	105.09	59% - down
Val	117.15	35% - up
His	155.15	23% - up
Arg	174.20	11% - up
Cys	121.16	47% - up
Ala	89.09	47% - down
Glu	147.13	85% - up
Gln	146.14	25% - up
Leu	131.17	38% - up
Lys	146.19	60% - up
Ile	131.17	38% - up
Thr	119.12	18% - up
Phe	165.19	11% - down
Tyr	181.19	12% - down

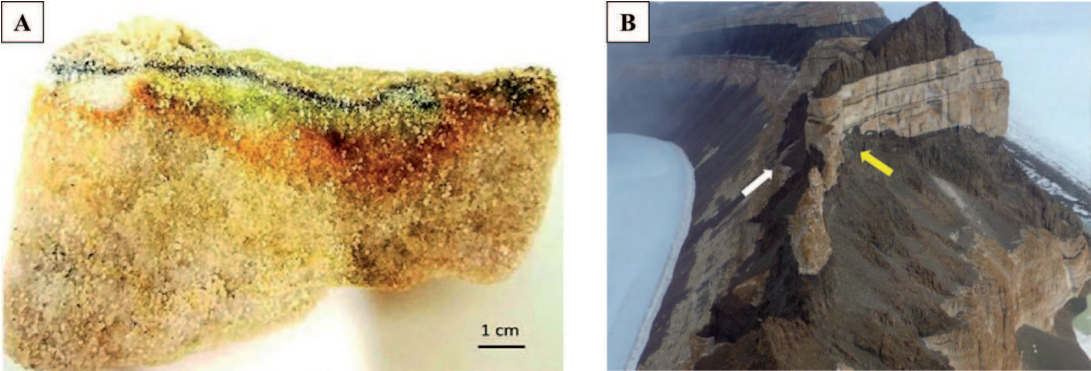
**Table 1.**  
*Estrogen and amino acid amount extracted from the urine in the two stages of pregnancy (OL and IL-DP). The table refers to the relative concentration of the metabolites calculating as a percentage of the total compounds by comparing the intensity of deconvolution of each compound.*

self-supporting assemblies formed by the association of autotrophic and heterotrophic microorganisms such as Bacteria Chlorophyta and Fungi, which live at the limit of their physiological adaptability and this represent the only possibility of survival before extinction. They live inside the pores of the rocks creating an environment that protects them from environmental stress, they are extremely tolerant and remarkably resistant to stress and finally they adapt perfectly to the lithic life (**Figure 4A**).

The study was conducted on the basis of the different solar exposure, verifying how this factor influences the production of key metabolites and therefore on the adaptation strategies implemented by these microorganisms to survive in extreme conditions (**Figure 4B**). The result is the presence of 331 altered and differentially expressed metabolites [37]. All intermediates of melanogenesis are found in the highest concentration in south facing rocks (**Table 2**). Organisms have developed



**Figure 3.**  
A coordinated cycle between mother fetus and placenta through the biosynthesis of steroid hormones.



**Figure 4.**  
A - Communities stratified on rocks. B - Antarctic cryptoendolite communities. The yellow arrow indicates the north-facing surface, while the white arrow indicates the south-facing surface. (Figure 4B comes from Coleine et al. [37].)

Compound	Molecular weight	Regulation
Gentisyl alcohol	140.14	Down
Hypoxanthine	136.11	Down
6- Methoxymellein	208.21	Down
Allantoin	158.12	Down
DOPA	197.18	Up
5,6-Hydroxy indole	133.15	Up
Anthocyanin 3-O- $\beta$ -D glucoside	449.38	Up
Plastoquinone	749.2	Up
Xanthine	152.11	Down
Uric acid	168.11	Down
Tyrosine	181.19	Up

Compound	Molecular weight	Regulation
Dopaquinone	195.17	Up
Cysteinyl dopa	316.33	Down
Dopachrome	193.16	Up
5,6-Dihydroxyindole	149.15	Up
Indole 5,6- quinone	147.13	Up

**Table 2.**  
*Regulation of metabolites in Antarctic cryptoendolithic communities exposed to the south in the absence of sun compared to the north in the presence of minimal solar radiation.*

metabolic profiles responding to the condition of deprivation of sunlight, in fact in fungi, melanin performs functions such as photoprotection, energy conversion, protection from thermal stress, metal chelation, resistance to drying and cell strengthening. Therefore, every organism that lives in non-optimal conditions has the ability to modify its biological functions and its structures according to external dynamics. The adaptation analysis allowed to demonstrate how the environment, and in particular the solar exposure, can strongly influence the olic phenotype of these microbial communities, demonstrating how the direct incidence of sunlight in one case, or their absence in the other, it has determined the acquisition of various adaptations by the colonizing species aimed at favoring their growth and guaranteeing their survival in such a hostile environment.

## 7. Conclusion

Starting from a holistic approach, with a high-throughput non-targeted metabolomics without prior knowledge of the metabolome, it is possible to find biologically relevant metabolites (potential biomarkers), characteristic of targeted metabolomics as shown by the two examples described in this chapter. In the case of urinary metabolites hundreds of metabolites are represented but the major differences between OL and IL-DP belong to the steroid hormone, in particular to conjugated estrogens and amino acids. These compounds could predict the time to delivery in pregnant women.

As for the Antarctic *cryptoendolytic* communities, among the candidates to be considered biomarkers, the precursor metabolites of the melanin and allantoin pathways emerged as these were the most affected by exposure to the sun. These paths can be considered directly involved in the response to environmental pressure. In conclusion, both targeted and non-targeted metabolomics are then used in combination for the detection and accurate weighting of differential metabolites. Furthermore, the types of metabolites that are recovered are influenced by the extraction and the analytical method chosen. In this chapter the main devices used have been summarized, subsequently the results will require computational tools to identify and correlate the metabolites among the samples to examine their connection in the metabolic pathways in relation to the phenotype.

## Conflict of interest

The authors declare no conflict of interest.



Abbreviations

CID	Collision induced dissociation
DIMS	Direct Injection Mass Spectrometry
FID	Free induction decay
FT-IR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
HILIC	Hydrophilic interaction chromatography
HPLC	High performance liquid chromatography
IL-DP	In labor in the dilation phase
LC-MS	Liquid Chromatography-Mass Spectrometry
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
OL	Out of labor

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