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REVIEW PAPER

Ceramide's Role and Biosynthesis: A Brief Review

Lita Amalia and Shen-Long Tsai

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Abstract The utilization of ceramides, which are members of the sphingolipid family, has been widely acknowledged in the cosmetic and pharmaceutical industries, along with various other applications as therapeutic agents. Most ceramides currently available on the market are synthetic ceramides created through chemical reactions with precursors resembling the natural precursor of sphingolipid production by living organisms. In fact, many organisms ranging from microbes to higher-order mammals natively use metabolism to produce sphingolipids, including ceramides and their derivatives, to support cell molecular functions. Sphingolipids, for instance, are present in the cell membranes of mammals, plants, and yeast to maintain membrane morphology. As a green alternative to the chemical synthesis method, many studies have been carried out to reveal the diversity and characteristics of biologically produced ceramide derivatives. In this review, we summarized the most important aspects of ceramide biosynthesis in general and provide a quick overview of the common organisms producing ceramides. In addition, a brief discussion regarding the role of ceramides in cells and their risks was included. As the biosynthesis of ceramides is an attractive alternative to current commercial methods, the advances reviewed herein demonstrate the untapped potential for the further development of synthetic pathways to enhance biobased-ceramide production.

Keywords: sphingolipid, ceramide, skin-barrier, biosynthesis, characterization, apoptotic

Lita Amalia, Shen-Long Tsai*
Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan
Tel: +886-2-2737-6628; Fax: +886-2-2737-6644
E-mail: stsai@mail.ntust.edu.tw

1. Introduction

Ceramides are the basic building blocks of all sphingolipids and are commonly present in the cell membranes of plants, animals, fungi, and some prokaryotic viruses. Ceramides are composed of long-chain fatty acids linked to the backbone of sphingoid bases by amide bonds, which is the condensation result of palmitoyl-CoA and serine [1,2]. Consequently, the term "ceramide" technically refers to an entire family of molecules with different fatty acid chain lengths, sphingoid base lengths, or moieties that are capable of performing a variety of biological roles. Correspondingly, different types of ceramides are found on the outermost layer of skin which also identified that they are composed of different sphingoid bases and fatty acid chains (Table 1). Ceramides have grown in popularity as their functions have been gradually revealed (Fig. 1). For example, they are crucial in retaining moisture and strengthening the skin barrier along with other lipids found in the stratum corneum (the outermost layer of skin) [3-5]. In addition, ceramides are known to inhibit melanin synthesis [6].

The deficiency of ceramides or altered patterns of ceramides, the main constituents of highly organized lamellar sheets, can impair the skin barrier and lead to various skin problems with age. The appearance of the skin can be improved by topical application of ceramides or other treatments to enhance the ability of the epidermis to synthesize lipids. That is what makes skincare and even haircare products containing ceramides the latest craze in the cosmetics industry. The benefit of ceramides is that they are active at relatively low doses, making them a valuable cosmetic ingredient. The ceramides can provide long-lasting hydration to the skin and hair, preventing dryness and moisturizing them. Ceramides also have a protective effect as they help reduce irritation from synthetic surfactants. Ceramides presented in haircare products such as shampoo, conditioner, and hair serum, are capable of overcoming

Table 1. Different types of ceramides found in the human stratum corneum [17]

Sphingoid base moiety	Fatty acid moiety		
	Non-hydroxyl fatty acid [N]	α-hydroxyl fatty acid [A]	Esterified ω-hydroxyl fatty acid [EO]
Sphingosine [S]	[NS, Cer 2]	[AS, Cer 5]	[EOS, Cer 1]
Phytosphingosine [P]	[NP, Cer 3]	[AP, Cer 6]	[EOP, Cer 9]
6-hydroxy sphingosine [H]	[NH, Cer 8]	[AH, Cer 7]	[EOH, Cer 4]
Dihydrosphingosine [dS]	[NdS, Cer 10]	[AdS, Cer 11]	[EOdS, Cer 12]

Modified from the article of Cha *et al*. (2016) [17].
Cer: ceramide.

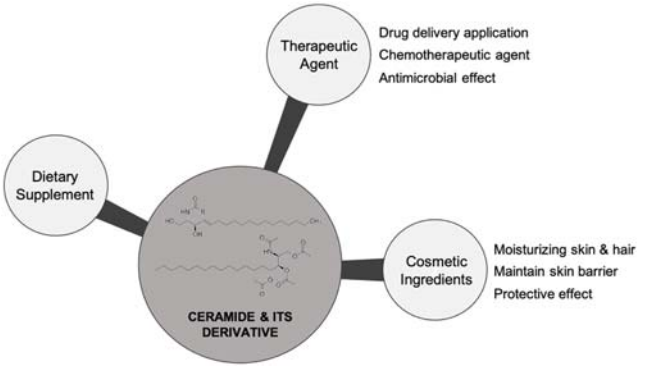

Fig. 1. Useful applications of ceramides and their derivatives.

hair damage by acting as a barrier to prevent protein loss in the hair strands and strengthen the cuticle cohesion [7,8].

In addition to its usefulness as an active ingredient in a variety of cosmetic products, a significant amount of research is currently being conducted to investigate the possibility of employing ceramide as an active ingredient for pharmaceuticals or other pharmaceutical uses. Short-chain C_6-ceramides are known to be effective as an antibacterial agent against the pathogen *Neisseria meningitidis* and related species *Neisseria gonorrhoeae*, although it is ineffective against *Escherichia coli* and *Staphylococcus aureus* [9]. Ceramide dimers can also be used in the manufacture of pharmaceuticals [10] and other inventions have documented many potential applications of ceramide-based formulations for drug delivery [11]. The fact that ceramides can mediate cell death by triggering apoptosis enhances their utility as a chemotherapeutic agent in cancer treatment [12,13]. In addition, foods containing adequate amounts of ceramides have also been studied for their potential health benefits [14-16].

As mentioned above, a variety of organisms manufacture numerous forms of ceramides with various functions. In the following, we discuss some of these sources of ceramide synthesis along with a brief discussion of the metabolic pathways in comparable microorganisms that are generally similar but differ in the stages of ceramide complex formation. In addition, we provide information on common ceramide derivatives produced by specific organisms. Accordingly, perspectives and perceptions that may be applied to the requirements of large-scale ceramide production will be developed.

2. Ceramide from Living Organisms

In the vast majority of organisms, ceramide production is

initiated by serine palmitoyl transferase (SPT), which catalyzes the reaction of palmitoyl CoA and serine to obtain 3-ketosphinganine, which is further reduced to be sphinganine or dihydrosphingosine (DHS). 3-ketosphinganine reductase is the enzyme responsible for the reduction. Thus, it is clear that the initial precursors of sphingolipid metabolism are identical for all organisms, as well as the two aforementioned biomolecular intermediates, which are quite prevalent in nearly all organisms capable of metabolizing sphingolipids (shown in bold font in Fig. 2). Enzymes involved in the subsequent steps significantly influence the ceramide compounds that will be formed. In higher animals, DHS is often converted to dihydroceramide by ceramide synthase (CERS). Ceramide is then synthesized from dihydroceramide by the enzyme dihydroceramide 4-desaturase (DES), which catalyzes the C-4 oxidation of the substrate. The ceramides referred to here are compounds with a sphingoid base and various fatty acid chains, possibly as shown in Table 1. Although not a major pathway, mammals have also been reported to produce phytosphingosine from DHS. As a sphingoid base, phytosphingosine is a precursor for phytoceramides mainly found in plants [18] and yeast [3]. However, mammalian cells have also been observed to contain phytoceramides [19]. In contrast to phytoceramide, sphingomyelin, which has never been identified in plants, is found in abundance in mammals [20]. The sphingomyelin synthase enzyme converts ceramide into sphingomyelin. Sphingomyelinase is responsible for the inverse reaction, which is the breakdown of sphingomyelin into ceramide. Not only does ceramide serve as a precursor to sphingomyelin, but it also serves as a precursor to sphingosine when processed by ceramidase, which cleaves fatty acids from

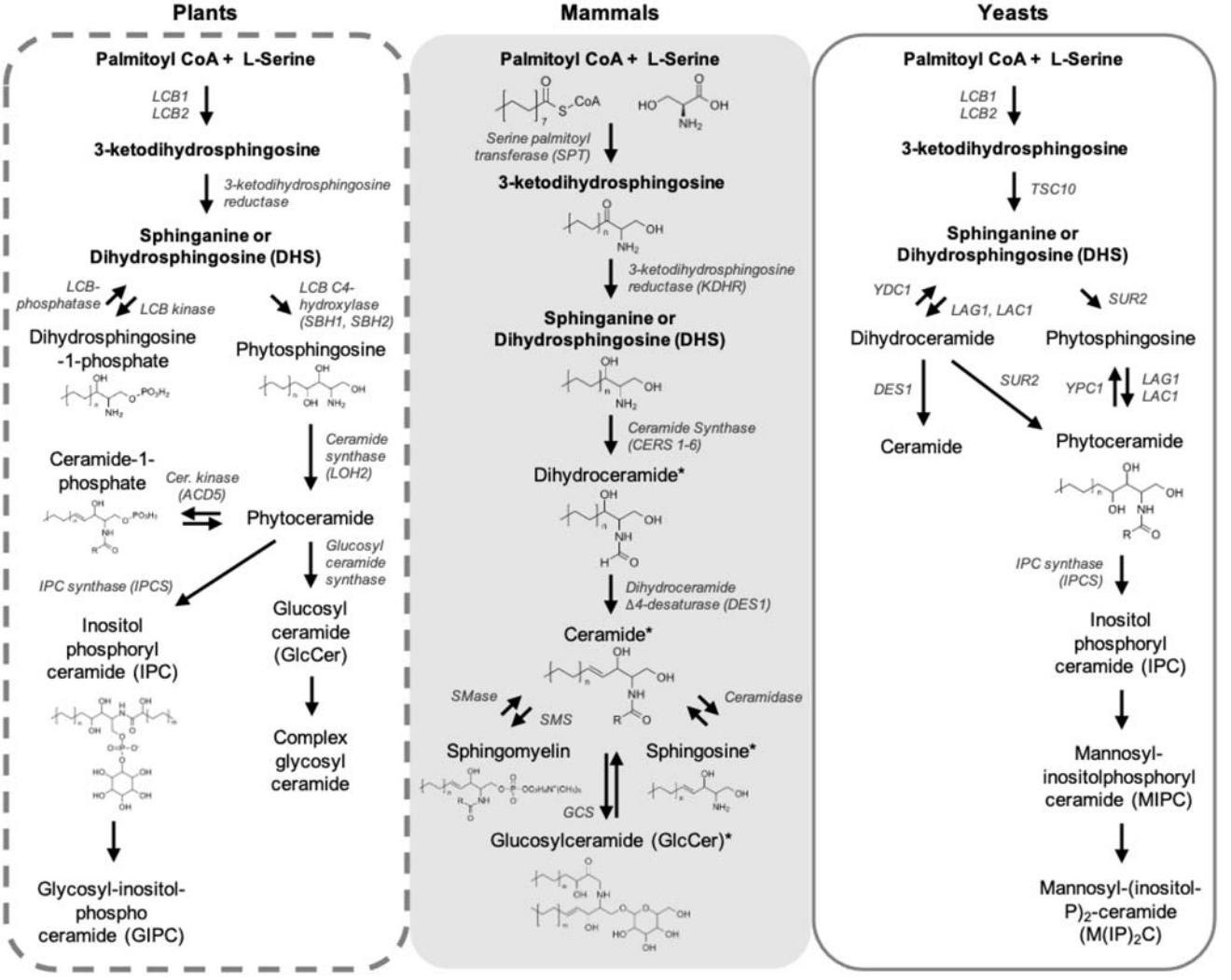

Fig. 2. Major metabolic pathway of sphingolipid in different organism. Metabolic pathway in yeasts and plants may also involve those indicated in asterisk. Several structures not shown in 'plants' and 'yeasts' columns are available in the 'mammals' column. The structure of complex sphingolipid compound is not provided. LCB: long-chain base.

ceramides. Glucosylceramide (also written as GlcCer) is another ceramide derivative that acts as a precursor for a wide range of other complex ceramide in mammals.

Plants, mammals, and yeast all share the same initial three steps in their sphingolipid metabolic pathways. The enzymes involved in the process of converting biomolecules are also similar. What sets them apart is the gene that encodes the enzyme. The major product of DHS in plants is phytosphingosine via a reaction catalyzed by long-chain base (LCB) C4-hydroxylase, in contrast to mammals, where it is more likely to be converted to dihydroceramide. DHS is changed into DHS-1-phosphate by an additional enzyme called LCB kinase. Ceramides present in plants are typically synthesized from phytosphingosine by ceramide synthase [21]. Hence they are often referred to as phytoceramides: a plant-based ceramide. Furthermore, phytoceramides are used by plants to produce ceramide-1-phosphate and complex ceramides such as inositol phosphoryl ceramide, GlcCer, and other ceramide derivatives, assisted by related enzymes according to Fig. 2. The metabolic pathways of yeast are comparable to those of plants and animals combined. In addition to manufacturing phytosphingosine by the sphinganine C4-hydroxylase (SUR2) enzyme, yeast is known to convert DHS to dihydroceramide, which intern converts ceramide molecules into ceramide complexes along with further metabolic pathways [22]. The enzyme responsible for the conversion of DHS to dihydroceramide is homologous to CERS in mammals. In yeast, it is known as LAG1, and its homolog is LAC1. The same enzyme participates in the reaction that produces phytoceramide from phytosphingosine. Similarly, phytoceramide in yeast serves as a precursor for complex compounds made from inositol ceramide. In addition to the major metabolic pathways of sphingolipid listed in Fig. 2, other ceramide derivatives may be present; however, they are not typically the most abundant biomolecules.

2.1. Mammalian ceramide synthase

Ceramide synthase is an essential enzyme that determines the diversity of sphingolipid compounds by catalyzing the addition of fatty acid chains to the sphingoid base [23]. In mammals, a family of six ceramide synthases (CERS1-6) generates ceramides with different acyl chain lengths. CERS2 is able to catalyze the formation of C_{22}, C_{24}, and C_{26}-ceramides; CERS3 exclusively produces C_{18} and C_{24}-ceramides, while CERS4 can generate C_{18} and C_{20}-ceramides. In addition, CERS5 and CERS6 are involved in the synthesis of C_{14} and C_{16} medium-chain ceramides. However, some references differ in the types of ceramides that each CERS can synthesize. Nevertheless, they all agree that CERS1 is the most specific ceramide synthase, targeting only C_{18}-ceramide [24,25]. Each CERS serves a distinct role in numerous biological processes, including cancer suppression, chemotherapeutic agents, and neurological disorders. Furthermore, they share several biological properties, including their catalytic function, intracellular structure, and expression location such as in the endoplasmic reticulum or Golgi [24]. In order to satisfy the market need for ceramide, extraction and isolation of ceramide from animal sources is the conventional method used. Among all mammals, bovines are the primary source of natural ceramides used in skin care products [26].

2.2. Plant-derived ceramides and their roles

Since animals can transmit zoonotic diseases, the production of ceramides from animal sources has been drastically reduced. As a result, an alternative method of preparing ceramides from plants has been developed, which is then known as phytoceramides. Plant-derived ceramides are predominantly glycosylated components of the plasma membrane and tonoplast [27]. They can be categorized into four groups: glycosylceramides, glycosylinositol phosphorceramides, ceramides, and free LCBs, while mono-GlcCers are the most prevalent type of sphingolipids in plant tissues [28]. Collectively, they play important roles in membrane integrity and permeability, signaling, cell regulation, and cell-to-cell interactions [29,30].

Although their chemical structures are comparable, there are differences in chain length and hydroxylation patterns between plant-derived and skin-derived ceramides [27]. The diversity of sphingoid bases in plants is greater than in mammals. However, plant-derived ceramides typically contain 3 to 4 hydroxyl groups, which share similarities with mammalian ceramides such as ceramide NP and ceramide AP (see Table 1). This enables the use of phytoceramides to enhance the skin barrier function of aging skin. In general, plants commonly used for phytoceramide production include rice (*Oryza sativa*), wheat (*Triticum aestivum* L.), potato (*Solanum tuberosum* L.), sweet potato (*Ipomoea batatas*.), konjac (*Amorphophallus konjac*), and maize (*Zea mays*) [28]. Although numerous variants of ceramide derivatives are available from a range of plants, difficulties in extracting the compounds from plants and poor ceramide yields make this source unsuitable for large-scale applications.

2.3. Sphingolipid production through yeast engineering

Yeasts have also been used to investigate the possibility of making ceramides due to their rapid growth, nontoxicity, and transgenic capacity. The metabolic pathways of sphingolipids are nearly identical in all yeasts, with an emphasis on ceramide synthase, usually represented by the LAG1 gene (see Fig. 2). *Wickerhamomyces ciferrii* (formerly known as *Pichia ciferrii*) is known to produce ceramide derivatives via the catalysis of the ceramide synthase enzymes *Pc*LAG1

and *Pc*LAF1. *W. ciferrii* also can secrete huge quantities of phytosphingosine in the form of tetraacetyl phytosphingosine (TAPS) with the aid of additional enzymes. Notably, wild-type *W. ciferrii* did not produce measurable levels of sphingosine. However, several genetically modified strains have been shown to produce sphingoid bases, a ceramide precursor that could be screened using liquid chromatography-electrospray ionization- mass spectrometry (LC-ESI-MS) and Nuclear magnetic resonance (NMR) spectroscopy [31]. In addition, some techniques for the manufacture of sphingoid bases utilizing *W. Ciferri* have been patented, notably by Evonik Industries AG [32].

Another study described the generation and secretion of TAPS utilizing engineered *Yarrowia lipolytica*. By disrupting the *LCB4* gene to inhibit the sphingolipid degradation pathway, phytosphingosine accumulation could be elevated. Phytosphingosine then becomes a precursor that is transformed to TAPS by *SLI1* and *ATF2* [33], encoding phytosphingosine acetyl-transferase enzyme from *W. ciferrii* that is naturally capable of secreting large amounts of TAPS. By irradiating *W. ciferrii* with γ-ray, another team was able to create a mutant referred to as mutant 736. Although Sanger sequencing revealed that the sequences of eight genes involved in TAPS metabolism were identical to the wild type, mRNA expression analysis revealed that the TCS10 gene was expressed 30% greater than expected (see Fig. 2 for more details on TSC10). During batch fermentation, mutant 736 was able to produce ~5.3 folds TAPS titer than wild-type *W. ciferrii*. Hence it was believed that this strain would be more suitable for commercial TAPS production [34]. This ceramide derivative compound is intended for use as an eye cream ingredient. Moreover, a recent TAPS-related study involved the development of a fluorescence-activated cell sorting (FACS) reporter system for the measurement of TAPS content in the *W. ciferrii* mutant library using BODIPY 505/515 stain. Using this technique, the M40 strain resulting from random mutagenesis of *W. ciferrii* was determined to have a 60% greater TAPS volumetric productivity than wild-type cells [35].

Saccharomyces cerevisiae is the most extensively studied yeast associated with ceramide production. In one investigation, the KCCM 50515 strain that produced the most ceramides was chosen and optimized for ceramide production using batch fermentation. The amount of ceramide obtained from *S. cerevisiae* increased 5.9-fold when the cells were subjected to heat shock. In addition, it has been demonstrated that ceramide synthesis increases during the stationary phase of cell growth [36]. Despite numerous investigations, *S. cerevisiae* does not produce sphingosine-containing sphingolipids analogous to human ceramides, but rather generates phytosphingosine-containing sphingolipids. Murakami and his team [3] have patented a method of modifying the sphingolipid metabolic pathways of *S. cerevisiae* to efficiently produce human ceramides often utilized as active components in cosmetics. They introduced the heterogeneous human DES1 gene that encodes a sphingolipid Δ4-desaturase for the production of sphingosine from the intermediate compound, DHS. Furthermore, they reported that heterogeneous gene expression may affect the location of related protein expression [3].

2.4. Ceramide-producing prokaryotes

Ceramide-producing microorganisms are not limited to yeast. *Caulobacter crescentus* is one of the few bacteria known to produce sphingolipids in its membranes. The wild-type *C. crescentus* produces a variety of dihydroceramide molecules. The acyl carrier protein, acyl-ACP synthetase, SPT, ketosphinganine reductase, and *N*-acyltransferase are the five structural genes required for ceramide synthesis and *C. crescentus* survival [37]. In addition to *Caulobacter* species, the genus *Clostridium* has also been recognized for its ability to create ceramide derivatives by breaking down GlcCers. In addition, *Pseudomonas* is believed to produce ceramidases that regulate levels of biologically active lipids, particularly ceramides, sphingosine, and sphingosine-1-phosphate. The latter two bacteria are known to have patented technologies for producing enzymes or related substances. In humans, several bacterial sphingolipid metabolites were detected, indicating that *Bacteroides* species in the gut microbiome produce sphingolipids [38,39].

3. Industrial Ceramide Production

Even though plants and animals are rich in ceramides, they are not a reliable source because extraction methods are tedious and expensive. Therefore, most of the ceramides used in the cosmetics industry are synthetic. Consequently, the market is flooded with new ceramide derivatives. The manufacture of ceramides can be carried out by a variety of synthetic methods, most of which involve the reaction between fatty acids and sphingoid bases through amide bonds [40]. This can be achieved by the reaction of sphingoid bases with acyl chlorides, although the results are not selective due to simultaneous esterification and amidation. However, esters can be eliminated by selective mild alkaline hydrolysis. Alternatively, ceramides can be synthesized without esterification through the activation of fatty acids with carbodiimides. For example, the reaction between (2S,3R)-sphinganine and methyl stearate yields ceramide NS and other contaminants. At the same time, glycine ester derivatives react with activated palmitic acid and then with stearoyl chloride to generate pure ceramide NS [41]. Some companies produce ceramides via fermentation

techniques utilizing yeasts such as *W. ciferrii*, *S. cerevisiae* KCCM 50515, and *Y. lipolytica*. Evonik Industries AG Personal Care Products sells *comoferm*, a ceramide solution that utilizes a patented fermentation process to produce phytosphingosine, which can be converted into a variety of ceramides identical to human skin. Additionally, a genetically engineered yeast strain with enhanced sphingoid base production was described [36,42]. Previously, Unilever Rotterdam and Unilever London once held a patent for producing phytosphingosine-containing ceramides. The process involves the manufacture of TAPS via the combination of microbial fermentation and chemical techniques. TAPS was initially created by the F-60-10 mating-type strain of *Hansenula ciferrii* (now *W. ciferrii*). TAPS was deacetylated subsequently to yield phytosphingosine. The amino group of phytosphingosine is then chemically acylated with fatty acids to form phytosphingosine-containing ceramide [43].

4. Ceramide Diversity and Characterization

Major sphingolipid metabolisms have been identified, but their metabolic details in many organisms have not been widely reported, especially in plants where sphingolipid metabolic pathways are poorly documented. This requires some labeling techniques to track the related metabolites. For example, to trace sphingolipid metabolism in budding and fission yeasts, tritium radiolabelling was utilized [3,44]. Whereas, fluorophore labels have been used to track sphingolipid trafficking across microbial and mammalian cell membranes [45].

The structural diversity of sphingolipids is due to variations in sphingoid bases, fatty acyl chains, and polar head groups. Due to the complex nature of the structures, their qualitative and quantitative analysis is also a big challenge. Sphingoid or LCBs and fatty acyl chains are typically analyzed by high-performance liquid chromatography (HPLC). Using HPLC, mass spectrometer (MS), and electrospray ionisation mass spectrometry (ESI-MS), a study differentiated 8 LCBs with 10 fatty acyl chains [46]. Others utilized ultra-performance liquid chromatography coupled with a MS detector to detect sphingolipids and other lipids in plant lipids and successfully identified 31 varieties of ceramides [47]. A separate team was also able to detect 100 sphingolipids with odd-numbered fatty acid chains using liquid chromatography with tandem mass spectrometry (LC-MS/MS) [48]. Gas chromatography- mass spectrometry (GC-MS) is also frequently used to detect sphingolipids [49]. Matrix-assisted laser desorption/ionization- mass spectrometry (MALDI-MS) or matrix-assisted laser desorption/ionization-time of flight- mass spectrometry (MALDI-TOF-MS) can be used to differentiate the head groups of glycosyl inositol phosphoceramides which is one of the ceramide complexes [45,49,50]. In addition, infrared (IR) spectroscopy, ultraviolet (UV) spectroscopy, and NMR characterization can effectively identify novel phytosphingolipid molecules [51].

5. Advantages and Limitations of Ceramide Overexpression

Organisms express ceramides with unique functions, especially as membrane components. Ceramides and their sphingolipid derivatives are essential for maintaining membrane morphology and modulating membrane function in plants, animals, and yeast. Similarly, in bacteria, sphingolipids, such as dihydroceramide and ceramides, are required for outer membrane stability, making them beneficial for bacterial survival [37]. Sphingolipids containing sugar head groups can act as surface receptors to sense toxins and salts in plants. Animal glycosphingolipids serve a similar purpose. In addition, sphingolipids act as signaling molecules responsible for regulating cell growth and stress responses [45].

On the other hand, the expression of sphingolipids is also associated with organ dysfunction due to disease-induced cell death, which is often called lipotoxicity. The expression of ceramide synthase is closely related to apoptosis in mammals and yeast, leading to the accumulation of certain ceramides [24,25]. Fatty acid length is believed to correlate with the effects of apoptosis and cell death. In this regard, shorter chain lengths and higher degrees of unsaturation are associated with greater toxicity. Like medium saturated fatty acids (SFA), short-chain fatty acids have been found to induce apoptotic cell death in yeast cells. On the other hand, long-chain SFA is harmless to wild-type cells [22]. Petschnigg *et al*. [52] demonstrated that C16:0 and C18:0 SFA do not affect the growth of either wild-type or mutant strains in their study. SFA undergoes elongation and/or desaturation, resulting in a fatty acid distribution that mimics the natural spectrum found in yeast membrane lipids.

6. Conclusion and Future Perspective

In the past few decades, significant progress has been made in unraveling sphingolipid biosynthesis. Nonetheless, given the large number of sphingolipid derivatives, many obstacles and challenges remained to be overcome to fully understand sphingolipids. In this review, we have highlighted several organisms that can be used as sources for the extraction of different sphingolipids. The high diversity of sphingolipids in mammals, plants, and microbes suggests that sphingolipids have multiple functions, even though alterations in

sphingolipid concentration and structure are associated with cell death. Aside from its employment in the pharmaceutical and cosmetics sectors, the expression of sphingolipid derivatives has balanced advantages and disadvantages, such as regulation of cell survival or cell death, as well as signaling processes and stress responses. It all depends on the composition and expression levels of ceramides. We suggest that optimal utilization of ceramides may be achieved by metabolic modifications to maximize ceramide expression within the safe range of the organism. Or perhaps by heterologous expression of genes from different organisms to reduce negative effects on host cells. For instance, if a gene involved in the synthesis of a particular ceramide derivative is known to be harmful and induces apoptosis, it might be replaced by a homologous gene from another organism with a lower risk, based on existing studies. Thus, this perspective holds future potential for the synthesis of safe, high-yield, organism-based commercial ceramides.

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Ethical Statements

The authors declare there is no conflict of interest.
Neither ethical approval nor informed consent was required for this study.

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