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Probing the world of mycosporine like amino acids (MAAs): Prevalence, resilience, biosynthesis and beyond

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Abstract
Mycosporine-like amino acids (MAAs) are a class of fascinating compounds with diverse biological significance. These remarkable molecules, known for their natural UV-absorbing properties, have become essential players in various life forms, offering protection against the relentless assault of ultraviolet radiation. Their structural elegance and exceptional stability have not only fascinated researchers but also found applications in biotechnology and skincare. As we journey through the world of MAAs, they have significance beyond photoprotection. MAAs play pivotal roles in desiccation resistance, osmotic regulation, and potentially other cellular processes, making them versatile and resilient molecules with multifaceted functions. In this comprehensive review, we delve into the intricate world of MAAs, shedding light on their prevalence, exploring their resilience, unraveling the mysteries of biosynthesis, and venturing into the wide-ranging implications that extend beyond their initial discovery. Our exploration takes us through the captivating biosynthetic pathways of MAAs, revealing the intricate processes that govern their production. We also delve into advanced analytical techniques, such as LC-MS and multiple reaction monitoring, which enable precise quantification and characterization of these compounds. In conclusion, our comprehensive review provides a thorough and insightful examination of the multifaceted world of MAAs.

Keywords: Photoprotection, mycosporine-like amino acids (MAAs), prevalence, resilience, biosynthesis

Introduction

Mycosporine-like amino acids (MAAs) represent an aristocratic lineage comprising over 50 innate, ultraviolet (UV)-shielding virtuosos. These compounds have elegantly adapted to fend off the relentless onslaught of chronic UV radiation, and they serve as vigilant protectors across a diverse spectrum of life forms. From the venerable cyanobacteria to the botanical aristocracy encompassing algae and seaweeds, as well as the intricate invertebrates and the stately marine teleost fish, MAAs assume their role with grace. Characterized by their aqueous solubility and modest molecular stature, typically around 400 Daltons, MAAs exhibit a regal structural motif, graced by aminocyclohexenone or aminocyclohexenimine rings bedecked with nitrogen or amino alcohol [1], adornments, ensconced within the hallowed chambers of cellular cytoplasm. The origins of MAA synthesis have captivated scholars, and while conventional wisdom attributes their creation to the esteemed shikimate pathway, a captivating scientific paradox has emerged. Contrary research proposes a tale of symbiotic orchestration in cyanobacteria, where MAA production unfolds through an intricate interplay between the pentose phosphate and shikimate pathways, adding a layer of enigmatic elegance to their biosynthetic narrative. The production of mycosporines, particularly the essential compound 4-deoxygadusol, is primarily orchestrated by the enzymes 2-epi-5-epi-valiolone synthase (EVS) and an O-methyltransferase. These enzymes catalyze a significant transformation, redirecting the pentose phosphate pathway toward the critical intermediate, sedoheptulose-7-phosphate. In response to relentless ultraviolet radiation (UVR) exposure, cyanobacteria exhibit a remarkable increase in mycosporine-like amino acid (MAA) production as a strategic defense mechanism. MAAs possess notable physiological capabilities, acting as formidable protectors against UVR damage by providing robust photoprotection and antioxidant properties. They act as vigilant guardians, shielding cellular integrity from the harmful effects of UVR [2].

MAAs, however, are not limited to photoprotection; they are versatile molecules participating in various cellular processes. They play a role in enhancing resistance to desiccation, meticulous regulation of osmotic conditions, and involvement in a range of cellular functions, underscoring their biological significance. A distinctive feature of MAAs is their exceptional stability, making them an attractive and increasingly sought-after alternative to potentially harmful synthetic sunscreens and anti-aging treatments. In the realm of biotechnological product development, the employment of analytical methods and validated procedures holds paramount importance. These methodologies serve as dependable tools for overseeing the consistency, purity, quality, potency, and efficacy of active components within manufactured products. Previous research endeavors have delved into the validation processes for biotechnological products. High-performance liquid chromatography (HPLC), distinguished by its ability to compare retention periods and UV spectra, emerges as the predominant technology for the purification, identification, and quantification of Mycosporine-Like Amino Acids (MAAs). Despite the sensitivity of UV detection, attributed to the high extinction coefficients associated with MAAs, it does have a notable drawback in terms of selectivity. Biosynthetic congeners possess the capacity to interfere with the accurate quantification of MAAs. Additionally, the absence of commercially available standard compounds

poses a challenge, with only a handful of laboratories worldwide equipped to furnish a reference substance against which the structural elucidation of MAAs can be validated [3]. While capillary electrophoresis has been utilized for MAA measurement, it is not commonly employed due to its limitation in analyzing highly ionizable substances. In this context, LC- MS emerges as a more suitable option, offering heightened sensitivity and selectivity for MAA analysis. A quantitative LC-MS method has been developed for the investigation of MAAs, enabling the comprehensive characterization of all known MAAs through individual retention times, molecular weights, and UV absorption maxima. Furthermore, an LC-MS approach utilizing hydrophilic interaction chromatography (HILIC) has been applied to examine MAAs in various marine species. While this method has demonstrated strong linear correlation coefficients, it falls short in considering crucial validation aspects such as recovery and matrix effects. The method's precision and sensitivity have been significantly improved by opting for multiple reaction monitoring (MRM) instead of full scanning, allowing for the precise differentiation of isomeric molecules based on their mass [3]. We present a rapid and validated LC-MS/MS approach, which relies on individual retention times, molecular weights, and specific mass transitions acquired through MRM experiments. This methodology has demonstrated its effectiveness, even when dealing with complex biological matrices, thanks to the integration of internal standards. In a bioprospecting investigation, we assessed the applicability of this approach in evaluating MAAs produced by cyanobacteria. Cultures exposed to ultraviolet radiation were scrutinized to ascertain the potential of the suggested technology. "In this comprehensive review, the intricate world of Mycosporine-Like Amino Acids (MAAs) will be unveiled, with an exploration of their prevalence, an examination of their resilience, the unraveling of biosynthetic mysteries, and a journey into their far-reaching implications beyond their initial discovery" [4].

Prevalence of Mycosporine-Like Amino Acids (MAAs) in a Diverse Range of Organisms

Prevalence of Mycosporine-Like Amino Acids (MAAs) Across a Diverse Range of Organisms Inhabiting Marine and Freshwater Habitats is Evident. These Organisms Encompass Phytoplankton, Red Algae, Cyanobacteria, Lichens, Corals, Gorgonians, Sponges, Brine Shrimp, Sea Urchins, Starfish, Holothurids, Clams, Ascidians, and Fish. Notably, a Comprehensive Database Detailing the Prevalence of Mycosporine-Like Amino Acids Among These Organisms Has Recently Been Assembled. In general, the identification of mycosporine- like amino acids (MAAs) often relies on co-chromatography with sub-standards or the analysis of previously published UV spectrum data and HPLC retention periods. This approach, while informative, should be exercised with caution. As an illustration, the partial characterization of mycosporine-glycine-valine was recently performed using extracts from the Antarctic pteropod Limacina helicine, a common source of sub-standards. Nevertheless, upon subjecting this extract and others from Antarctic pteropods to LC-MS/MS analysis, a noteworthy revelation emerged: palythenic acid was detected instead of mycosporine-glycine-valine. A similar quandary surrounded the MAA composition reported for the Antarctic krill Euphausia superba. Furthermore, a number of MAAs remain inadequately characterized. The ongoing exploration of various organisms, coupled with ongoing methodological advancements, holds the potential to unveil novel MAAs, suggesting that their diversity and prevalence might surpass current understanding [5].

Influential factors impacting MAAs induction

Temperature, salt levels, desiccation, nutrition, photosynthetically active and UV light are among the variables that regulate the production of MAAs. Extensive research has explored the impact of these factors, particularly radiation, on a wide range of macroalgae and microalgae, including dinoflagellates, diatoms, and cyanobacteria. Environmental conditions, especially the UVR exposure experienced by these organisms, exert a notable influence on MAA production. Organisms inhabiting Arctic and Antarctic seas exhibit higher MAA levels, likely attributed to heightened UVR exposure in areas with cleaner waters and a thinner ozone layer. Furthermore, there is a significant increase in MAA concentrations within zooplankton populations across lakes at higher altitudes. Numerous studies have investigated the stimulation of MAAs under controlled laboratory conditions as a result of exposure to both photosynthetically active radiation (PAR) and ultraviolet radiation (UVR). It's worth noting that the specific parameters required to trigger significant levels of MAA production vary among different organisms. In the case of the dinoflagellate Gyrodinium dorsum, it has been demonstrated that UVB is considerably more effective than both PAR and UVA in inducing the synthesis of MAAs. Wavelengths below 300 nm, however, have been identified as potentially harmful. Scrippsiella sweeneyae, on the other hand, exhibited MAA induction after being exposed to UVR (>280 nm) as well as PAR. In the case of Antarctic diatoms, UVA and blue light proved to be more effective for MAA induction, while UVB had no discernible effect. For the prymnesiophyte Phaeocystis antarctica, a combination of UVA and UVB (>305 nm) appeared to be more suitable. In species including the coral Stylophora pistillata, exposure to UVR (>300 nm) and PAR resulted in an enhancement of MAAs levels. Exposure to UVA and PAR within the wavelength range of 350 to 490 nm resulted in an augmentation of shinorine content within the red alga Chondrus crispus [6]. Cyanobacteria, when subjected to different treatments including UVA, UVB, and PAR, exhibited inductions of MAAs, with UVB radiation (>295 nm) having the most pronounced impact, particularly on N. commune, Scytonema sp and Anabaena sp. Notably, exposure to ultraviolet radiation (UVR) can induce various effects in organisms beyond simply increasing the levels of existing MAAs. For instance, when the dinoflagellate A. tamarense encountered UVB radiation, it displayed alterations in its MAA profiles. Cells highly exposed to UVB synthesized more secondary MAAs, whereas those with less exposure favored the production of primary ones. Another intriguing case involves the conversion of mycosporine-glycine into shinorine and porphyra- 334 when Anabaena doliolum is exposed to UVB rays. Furthermore, in response to UVR exposure, C. crispus exclusively synthesized shinorine. However, when accompanied by PAR, it also produced palythine. When Porphyra leucosticta was subjected to various PAR wavelengths, it accumulated porphyra-334, palythine, and asterina-330. Notably, blue light favored the reduction of shinorine, while white, green,

yellow, and red light encouraged the accumulation of shinorine. Another influential factor affecting MAAs induction is salt stress or salt content. A study involving A. variabilis revealed that as NaCl concentrations increased, the production of MAAs also increased, both in the presence and absence of UV light. Similarly, research on the cyanobacterium Aphanothece halophytica demonstrated that higher salt concentrations, whether in the presence or absence of UVB radiation, resulted in an increase in the quantity of mycosporine-2-glycine.

The introduction of A. halophytica genes into Escherichia coli and Synechococcus strains, followed by cultivation on high-salinity media, resulted in an augmentation of MAAs production. Similarly, the dinoflagellate Gymnodinium catenatum, along with cyanobacteria like Chlorogloeopsis PCC 6912 and Scytonema sp., exhibited elevated MAA concentrations when exposed to increased salinity in their respective growth media. The impact of heat exposure on MAA biosynthesis can vary. For instance, in the case of the cyanobacterium Microcystis aeruginosa, a temperature of 45 °C had no discernible influence on MAA concentration after 4 hours of incubation. Likewise, exposure to heat (40 °C) in A. variabilis did not result in a significant increase in MAAs production. However, when combined with UVB exposure, the heat treatment led to lower MAA levels compared to UVB treatment alone. Furthermore, studies involving the dinoflagellate Peridinium aciculiferum have shown that the concentration of MAAs decreases as temperatures rise [7]. Conversely, heat stress promotes MAA accumulation in corals like Lobophytum compactum and Sinularia flexibilis. Ammonium concentration is another factor associated with MAAs levels in various species. High ammonium concentrations (300 M) significantly enhance the levels of MAAs in Porphyra columbina, especially when samples are exposed to UVA rather than UVB. Similarly, excessive ammonium concentrations (300 M) enhance the MAAs content following a 7-day incubation in Porphyra leucosticte, while Porphyra umbilicalis shows no noticeable changes. However, it's worth noting that the MAA profiles of each species vary when the ammonium concentration is altered. In A. variabilis, MAAs concentrations increased as ammonium concentrations rose. However, ammonium-treated samples exhibited reduced MAAs concentrations when exposed to 395 nm threshold filters compared to 295 threshold filters. Another element influencing MAAs regulation is desiccation, which, when coupled with UVB exposure, can enhance MAAs content in Leptolyngbya sp [8]. Phosphate deficiency is yet another factor that promotes the production of shinorine, palythine, and asterina-330 in the dinoflagellate Glenodinium foliaceum. Additionally, sulfur shortage has been associated with the bioconversion of mycosporine-glycine into palythine-serine, which represents the second MAA in A. variabilis [9]. In summary, various environmental factors play a role in controlling MAAs production. In certain instances, their combined effects may be synergistic, although this depends on the specific organism under study.

Biosynthesis pathways of primary MAAs

Elucidating the Pathways of Primary MAAs Biosynthesis- In the world of the deuteromycete Trichothecium roseum, the intricate process of mycosporine biosynthesis takes center stage, guided by the sophisticated shikimate pathway. At its core, 3-dehydroquinate (3-DHQ) assumes the pivotal role of the eminent precursor, bestowing the distinctive six-carbon ring that characterizes all fungal mycosporines. Amidst the graceful choreography of fungal mycosporine creation, 3-DHQ stands as the foundational cornerstone, while gadusol or deoxygadusol potentially act as masterful craftsmen shaping the birth of mycosporine-glycine. A profound revelation transpired with the introduction of a shikimate pathway inhibitor, the conductor known as glyphosate, manifested as N-phosphonomethyl-glycine. It was here, within the coral Stylopora pistillata, that the initial concrete evidence of MAAs synthesis in marine organisms materialized, illuminating the mysterious path they tread. To delve deeper into the specifics, it's worth noting that in the cyanobacterium Chlorogloeopsis sp. strain 6912, the central ring of MAAs is traced back to its origins within the shikimate pathway [10]. This revelation implies a striking similarity, if not outright identity, in the biosynthesis of MAAs between eukaryotic and prokaryotic species. Yet, these findings pose a challenge to the long- standing belief that MAA production involves an intermediate component within the Shikimate pathway. Interestingly, the researchers in question faced a roadblock in their attempts to observe the formation of 6-deoxygadusol. Despite subjecting the putative substrates, namely 3-dehydroquinate, to incubation alongside dehydroquinate synthase (DHQS) and O-methyltransferase (O-MT) homologues (NpR 5600 and NpR 5599, respectively) from Nostoc punctiforme (ATCC 29133), and utilizing the customary cofactors including S-adenosylmethionine (SAM), nicotinamide adenine dinucleotide (NAD+), and Co2+, the expected outcome remained elusive. Furthermore, in the presence of SAM, NAD+, and Co2+, the treatment of sedoheptulose 7-phosphate (SH7-P), an intermediate within the pentose phosphate pathway, with NpR 5600 and NpR 5599 yields a solitary product - 6-deoxygadusol [11]. Using radiolabeled amino acids, the researchers unveiled an extraordinary revelation – the exclusive synthesis of 14C-glycine and 14C-serine through the unique side chains associated with mycosporine-glycine and shinorine. This discovery firmly establishes these free amino acids as the fundamental building blocks and underscores the pivotal role of mycosporine-glycine as the direct metabolic precursor for the bisubstituted MAA, shinorine. Remarkably, similar findings emerged in the cyanobacterium Anabaena doliolum. Within this cyanobacterium, mycosporine-glycine takes center stage as a precursor for the production of shinorine and porphyra-334, triggered by exposure to UVB light. Because the inhibitory effect of DCMU on MAA biosynthesis is nullified when external fructose is supplied, it is evident that MAA synthesis in this organism relies exclusively on photosynthesis for its carbon source. This conclusion gains further credence from various lines of evidence, particularly the presence of a glycine residue in the majority of amino acid bi-substituted MAAs. Collectively, these findings support the notion that the condensation of mycosporine-glycine onto an amino acid is a widespread mechanism in the formation of amino acid bi-substituted MAAs. Notably, even in the presence of Adenosine Triphosphate (ATP) and Mg2+ cofactors, the gene Ava_3856 from Anabaena variabilis exhibits the capability to convert 6-deoxygadusol and glycine into mycosporine-glycine. Ava_3855, characterized as a nonribosomal peptide synthetase (NRPS)- like enzyme, fulfills a biosynthetic function by facilitating the ATP-

dependent conversion of mycosporine-glycine with serine, ultimately yielding shinorine. In contrast, the chemical feasibility of amino acid condensation utilizing mycosporine-taurine, another recognized oxo- carbonyl MAA, remains unexplored [12].

Regulation of biosynthesis

Increased photosynthetically active radiation (PAR) irradiance led to heightened synthesis of MAAs, and in free-living dinoflagellates, both UVA (315-400 nm) and blue light demonstrated an augmenting effect on MAA production. These findings were subsequently echoed in several dinoflagellate species, further validating the influence of light wavelengths. Notably, among dinoflagellates, the UVB wavelengths (280-315 nm) exhibited greater efficacy in inducing MAAs formation, particularly in the case of G. dorsum [13]. In Antarctic diatoms, the combined influence of UVA and blue light successfully induced MAA synthesis, whereas prymnesiophytes responded more favorably to UVB + UVA exposure. Cyanobacteria, on the other hand, exhibited the capacity to generate MAAs in response to PAR, UVA, and UVB light. Among these, UVB radiation exerted the most pronounced effect on MAA production in comparison to other wavelength bands. Corals require a combination of UVB and UVA, along with PAR, to stimulate synthesis. Interestingly, despite extensive research, no consistent induction pattern could be established for red macroalgae in Antarctica. Among the eighteen species studied, eight displayed MAA induction, revealing three distinct trends:

1. Responsiveness to the entire radiation spectrum.
2. Adaptation to PAR + UVA, with no observable effect from additional UVB.
3. A pattern similar to (2), but the introduction of additional UVB resulted in a decrease in MAAs.

These findings suggest that caution is warranted when generalizing previous research to represent the entire group. It appears that physiological acclimatization and adaptation may hold greater significance than previously assumed. The dependency of MAA synthesis on different wavelengths hints at the presence of specific photoreceptors, although these remain unidentified. Interestingly, in the case of Chondrus crispus, a marine red macroalgae, a distinct action spectrum for MAA synthesis has been observed. Scientists have proposed the existence of a previously unknown UVA-type photoreceptor, characterized by a robust absorption peak at 340 nm, a secondary peak at 320 nm, and a less prominent third peak at 400 nm. Furthermore, considering that action spectra for MAA induction demonstrate a peak at 310 nm, another potential candidate for UVB photoreceptors is a reduction in pterin levels within cyanobacteria. It's noteworthy that if the primary response to UVB radiation involves the generation of reactive oxygen species (ROS) and the subsequent induction of MAAs in response to increased ROS levels, the need for specialized UVB photoreceptors may be rendered unnecessary. Upon reaching this particular threshold, there ensues an accrual of reactive oxygen species (ROS), which subsequently wields its influence over the secondary process of MAA synthesis [14]. Beyond the purview of photosynthetically active radiation (PAR) and UV radiation, an array of additional stressors has been discerned as potent instigators of MAA production. Amongst select cyanobacteria, osmotic stress, whether in isolation or in tandem with UVB exposure, emerges as a catalyst for MAA production. In a contrasting vein, UVB radiation, when concomitantly coupled with other stressors, orchestrates the regulation of MAA accumulation by orchestrating shifts in the delicate balance between net production, excretion, and cellular division rates. An illustrative case of this phenomenon is exemplified in the realm of ultraviolet radiation (UVR), wherein a substantial reduction in cellular division unfolds within species like the diatom Thalassiosira sp. and the dinoflagellate Alexandrium catenella during the initial days of adaptation to full sun radiation. As ultraviolet radiation (UVR) failed to exert any influence on MAA production, cells exposed to full sunlight demonstrated a greater accumulation of MAAs compared to those subjected solely to photosynthetically active radiation (PAR). This observation strongly suggests that cells allocate a significant portion of their energy resources towards the synthesis of photoprotective compounds and repair mechanisms, often at the expense of carbon skeleton formation. In the case of Alexandrium tamarense, heightened exposure to ultraviolet B (BEDDNA 305nm = 2.78 kJm 2 d 1) resulted in a complete cessation of both cell division and MAA production. Intriguingly, the highest concentrations of MAAs within the cells were associated with the strain that exhibited the most conspicuous signs of stress in a recent study examining proliferation and photoprotection in two strains of A. tamarense. This response was evidently correlated with the exposure to UVB radiation. Furthermore, cells subjected to intense UVB-induced stress displayed a preference for producing secondary MAAs, such as shinorine methyl ester and palythine. In contrast, cells under less stress exhibited a relatively higher proportion of primary MAAs, notably an abundance of the antioxidant mycosporine-glycine, while maintaining elevated constitutive levels during the recovery period. The availability of nitrogen plays a pivotal role in influencing MAA synthesis [15]. The introduction of ammonium into the growth medium, in conjunction with various irradiation treatments, resulted in increased MAA concentrations concerning the dry weight of the red macroalga Porphyra columbina. Ammonium, in a concentration- dependent manner, promoted MAA production in the cyanobacterium A. variabilis PCC 7937, both in the absence and presence of UV stressors. Conversely, nitrogen deficiency led to a significant reduction in MAA synthesis among dinoflagellates like Akashiwo sanguinea along with Gymnodinium cf. instriatum. Additionally, the nitrogen status exerts a profound influence on the composition of MAAs. When cells of dinoflagellates like A. sanguinea, A. tamarense, and Karenia brevis were deprived of nitrogen, a notably higher proportion of mycosporine- glycine was observed. Interestingly, mycosporine-glycine boasts the distinction of having the lowest nitrogen content among all the identified MAAs in these dinoflagellates, yet it provides protection within the most perilous segment of the electromagnetic spectrum. Since both MAAs and toxins are considered secondary metabolites without essential functional roles, nitrogen-limited conditions prioritize the allocation of energy and intracellular nitrogen resources toward the maintenance of fundamental and critical cellular functions. Consequently, the activation of energy-intensive and nitrogen-demanding metabolic processes, such as MAAs synthesis, tends to be discouraged under these

circumstances. In poisonous strains of Alexandrium, the toxin levels diminish in parallel with additional metabolic changes during nitrogen stress. Conversely, toxin levels show a substantial increase under conditions of phosphorus limitation and at suboptimal temperatures. Regrettably, we currently lack information regarding the synthesis and accumulation of mycosporine-like amino acids (MAAs) under phosphorus-limited conditions or their development at suboptimal temperatures. Notably, it is worth emphasizing that a sulfur deficiency has been found to govern the synthesis and bioconversion of primary MAAs into secondary MAAs for the first time in the cyanobacterium A. variabilis PCC 7937 [16]. The kinetics of response seem to exhibit substantial variation among organisms. Some individuals demonstrate an immediate response, occurring within the first few hours after exposure to higher irradiances, notably observed in dinoflagellates. In contrast, others exhibit a considerably slower response, taking several days to weeks to manifest after exposure. This delayed response pattern is observed in cyanobacteria, corals, and macroalgae.

Resilience of MAAs

MAAs are conventionally considered to possess resilience as compounds with photoprotective properties against ultraviolet radiation (UVR). While pure MAA solutions do display an elevated level of photostability, there is evidence indicating their sensitivity to photosensitization when exposed to specific organic molecules like flavins. Factors such as UV exposure, extreme pH (either very acidic or highly basic), elevated temperatures (> 60 °C), and the presence of oxidizing chemicals can all diminish MAA's absorption capabilities. For instance, mycosporine-taurine (MT) significantly deteriorated after just one hour of exposure to various wavelengths of UVR. In contrast, dehydroxylusujirene and M-343 remained resilient following UVA exposure, with only moderate degradation observed after UVB and UVC irradiation. Following one hour of UVR exposure, both Shinorine and M-307 displayed reduced absorbance, signifying their resilience to both ultraviolet A and ultraviolet B irradiation. However, when subjected to UVC, the absorbances of these MAAs markedly decreased. Notably, Porphyra-334 exhibited exceptional resistance to UVA radiation, and in- depth analyses of photolyzed solutions revealed no generation of intermediate compounds, including radicals, upon radiation exposure, underscoring its impressive photostability. Experiments probing MAA degradation caused by riboflavin, rose Bengal, and exposure to seawater established that photodegradation necessitated the presence of light, oxygen, and a potent photosensitizer. Furthermore, it was observed that under intense irradiation, the components of saltwater could gradually contribute to the photodegradation of MAAs. Moreover, when exposed to stress conditions induced by 0.25% H2O2, the content analyses of palythine, asterina-330, and M-312 revealed lower values in comparison to stressors such as UVR and heat exposure. The degradation of MT and M-343 was also evident in the presence of 0.3% H2O2. Shinorine's absorbance declined notably after exposure to 0.2% H2O2, while M-307's absorbance experienced a significant decrease only when exposed to 0.4% H2O2, implying potential antioxidant activity in the latter [17].

Numerous studies have investigated the impact of elevated temperatures on MAAs. Aqueous solutions containing porphyra-334 remained stable during incubation in a dark environment at neutral pH, even at temperatures around 45 °C. Shinorine and M-307 demonstrated resilience at temperatures exceeding 40 °C, although their absorbances declined above 60 °C. Interestingly, subjecting porphyra334 and shinorine derived from Gracilaria cornea to a 6-hour heat treatment at 75 °C had no discernible effect on their presence. Shinorine, Porphyra-334, and the mycosporine-glycine-alanine extract derived from Sphaerospermopsis torques-reginae demonstrated resilience when subjected to temperatures below 50°C for 5 hours. However, significant changes in absorption were observed when exposed to 80°C. MAAs also exhibited durability when stored for ninety days at 4°C and up to 48 hours at room temperature. The pH level had diverse effects on MAAs, as evidenced by various studies. Porphyra-334 solutions remained stable across a wide pH range, from 1 to 11. They showed a decrease in absorbance under acidic conditions, which was reversed when returned to a neutral pH. However, exposure to pH 13 for less than 4 hours led to a dramatic reduction in absorbance. Conversely, mycosporine-glycine absorbance significantly declined at pH 2 but remained exceptionally resilient at pH 4, 8, and 10. However, at pH 2, there was a significant drop in absorbance. Palythene's absorption was notably diminished in acidic circumstances, as was that of its cis- isomer usujirene, as both can be converted to palythine when subjected to diluted hydrochloric acid. Palythine exhibits greater resilience and is less prone to deterioration than other MAAs, such as porphyra-334 and shinorine, especially in the presence of riboflavin. The higher level of photodegradation observed in porphyra-334 and shinorine may imply that replacement in the nitrogen atom of the cyclohexenimine unit affects the molecule's photochemical resilience. Understanding the resilience of MAAs under various stressors is a field where studies are far from comprehensive. More research is needed to understand the factors that keep MAAs resilient and whether that resilience is a result of the stressful environments in which the organisms that created them lived. At exactly the same time, a deeper understanding of their properties is required to fully realize their potential [18].

Extraction, purification, and characterization of Mycosporine-like Amino Acids (MAAs)

Extraction processes for Mycosporine-like Amino Acids (MAAs) from microorganisms, including cyanobacteria, dinoflagellates, and diatoms, exhibit notable differences. These distinctions largely stem from variations in the choice of extraction solvents, incubation durations, temperature conditions, cell disruption techniques, and solvents used for dissolving the extracted samples. Nonetheless, despite these differences, several extraction procedures involve a common sequence of steps, such as collecting and concentrating the biomass before subjecting it to extraction using a polar solvent. Following the extraction of samples containing Mycosporine-like Amino Acids (MAAs), several common post-extraction treatments are employed. These treatments include centrifugation and the subsequent collection of the supernatant, evaporation of the solvent with subsequent redissolution of the dried sample, or centrifugation followed by the collection of the supernatant after the dried sample has been redissolved. Additionally,

membrane filtration is often utilized as a final step before the sample undergoes further analysis. Given the hydrophilic nature of MAAs, the most frequently used solvents for extraction are methanol and ethanol. Ethanol concentrations in extraction solutions typically range from 50% to 80%, while methanol concentrations vary from 20% to 100%. Although less common, water can also serve as an extraction solvent, with specific procedures available for its use. The duration of solvent extraction can vary widely, spanning from just a few minutes to several hours, depending on the chosen extraction method and the specific requirements of the analysis. Throughout the extraction process, temperatures may range from near-freezing to 45 °C. Achieving a highly efficient MAAs extraction exceeding 95% can be realized by employing sample sonication in pure methanol at 4 °C, followed by subsequent filtration [19]. Interestingly, methanol-based methods using a 25% aqueous methanol solution at 45 °C have demonstrated the ability to extract up to 13 times the quantity of MAAs from specific macroalgae in comparison to traditional extraction with 100% methanol at 4 °C. It's noteworthy that extraction at elevated temperatures can potentially lead to the degradation or modification of labile MAAs. To mitigate such effects and enhance the efficacy of fresh extractions, it is advisable to directly subject samples to methanol sonication, and for lyophilized samples, a preliminary soaking step in water is recommended. The typical procedure for extracted samples involves low-pressure evaporation to remove water-insoluble components that could potentially interfere with subsequent analyses. Depending on the intended purpose, the resulting residue is then re-dissolved, either in methanol or an acidified aqueous solution. When dispersed in a solution containing formic acid and ammonium formate, MAAs display robust stability, lasting up to ninety days at 4 °C and 48 hours at room temperature. However, it's important to note that MAAs in aqueous solutions may be susceptible to bacterial degradation and should be promptly analyzed. The primary method for separating and purifying MAAs is reversed-phase high-performance liquid chromatography (RPHPLC), with commonly used columns including monomeric octylsilica C8 and octadecylsilica C18, both featuring low silanol-free groups for efficient chromatographic separation. The eluent typically comprises distilled water, methanol, and a minor amount of acetic acid, and elution is carried out under isocratic conditions. Despite being the most commonly employed technique, RP-HPLC methods have limitations when it comes to separating substances with weakly acidic and strongly acidic properties. An effective approach for resolving combinations of MAAs with varying polarities involves using trifluoroacetic acid (TFA) in conjunction with ammonium in the mobile phase on a C18 column. However, it's important to note that this method can negatively impact ESI-MS detection. To mitigate this, reducing TFA concentrations or considering an alternative like formic acid is recommended, although this may affect sensitivity. Additional MAA separation techniques include activated carbon, preparative HPLC with UV detection, capillary electrophoresis (CE), and hydrophilic interaction liquid chromatography (HILIC). In recent times, innovative methods employing HILIC and CE have emerged to address the previously mentioned constraints associated with RP-HPLC. HILIC proves to be a valuable tool for studying highly polar MAAs, albeit with the requirement of extended column equilibration times. Conversely, CE offers a cost-effective approach but is limited to highly ionizable substances. Relying solely on HPLC retention times and UV absorption spectra to determine these compounds has its limitations, including constrained selectivity due to similar or closely aligned wavelength absorption maxima found in various MAAs. Additionally, the absence of readily available commercial standards presents a challenge, making the identification and quantification of these compounds a complex endeavor. Consequently, the need arises for additional techniques like Mass Spectrometry (MS), Nuclear Magnetic Resonance (NMR), and Fourier Transform Infrared Spectroscopy (FTIR) to achieve a comprehensive characterization of MAAs [20]. Numerous methodologies have been devised to extract, separate, and elucidate the nature of MAAs. However, many of these methods are tailored for specific MAAs or are geared towards particular algal species. To achieve precise and comprehensive identification of these compounds, it becomes imperative to establish rigorously validated methodologies that can be applied universally across a diverse array of MAAs. The selection of extraction and processing solvents should be governed by a clear understanding of the ultimate analytical objectives for the sample in question.

Conclusions

In the realm of natural chemistry, Mycosporine-like amino acids (MAAs) emerge as exemplars of elegance and ingenuity. These compounds, renowned for their innate UV-absorbing prowess, have assumed roles of paramount importance across diverse life forms. Their journey from obscurity to indispensability reflects not only their structural sophistication but also their resilience in the face of nature's harshest adversary, ultraviolet radiation. The allure of MAAs lies not solely in their photoprotective abilities but also in the symphony of functions they orchestrate within the intricate framework of life. Beyond shielding against the relentless assault of ultraviolet rays, these molecules emerge as pivotal players in the theater of desiccation resistance, the orchestra of osmotic regulation, and the clandestine realms of potential cellular contributions. In the pages of our comprehensive review, we have embarked on an odyssey through the mesmerizing world of MAAs. Our expedition has encompassed the unveiling of their prevalence, the exploration of their tenacious resilience, and the unraveling of the profound mysteries of their biosynthesis. We have ventured further, delving into the far- reaching implications that extend beyond the confines of their initial discovery. As we navigated the labyrinthine biosynthetic pathways of MAAs, we were captivated by the intricate processes that govern their production. In our quest for deeper understanding, we harnessed advanced analytical techniques, such as LC-MS and multiple reaction monitoring, illuminating these compounds with precision and clarity. MAAs that transcend the boundaries of scientific curiosity, their applications span a spectrum, from biotechnological innovations to the luxurious realms of skincare. MAAs, with their versatility and steadfast stability, emerge as beacons of hope and inspiration for future endeavors in sustainable solutions. In the grand tapestry of nature, Mycosporine-like amino acids (MAAs) stand as gems of unparalleled brilliance, casting their radiant light upon the intricacies of life [21]. Our comprehensive

review has served as a torchbearer, shedding light upon the multifaceted world of MAAs and their far- reaching implications. Through elegance, resilience, and unwavering utility, MAAs transcend the boundaries of science, carving a path toward a future where nature's secrets continue to inspire and amaze.

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