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EXPLORING SOIL ACTINOMYCETES AS A SOURCE OF MELANIN FOR SUSTAINABLE SUNSCREEN APPLICATION

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ABSTRACT: A significant source of metabolites with pharmacological activity is now microorganisms. It is a significant component of natural products that include chemicals that are antiviral, anticancer, antibiotic, and secondary metabolites. A global search for new natural products has been centered on marine species for the past few decades. Among the new bioactive producers are actinomycetes found in the marine environment. The term "melanin" and "melanin-like" have been used to describe the brown-black pigment. It has been demonstrated to shield microorganisms against oxidants, enzymatic lysis, ultraviolet light, and alveolar macrophage death. Although melanin has a wide range of biotechnological uses, little is known about its significance and uniqueness in marine actinomycetes. Actinomycetes isolated from soil and marine environments produce melanin, a pigment with antioxidant, antimicrobial, and anticancer properties. This study characterizes melanin-producing Actinomycetes, optimizing culture conditions and evaluating their potential applications in pharmaceutical, agricultural, and environmental fields.

Index Terms *– Actinomycetes; Antimicrobial; melanin; pigmentation; Soil microbes.*

Introduction: -

Actinomycetes are saprophytic, free- living bacteria that are generally set up in soil, water, and colonizing shops. Actinomycetes are one of the main groups of soil organisms that might change depending on the type of soil (Al- Qadisiyah et al., 2013). Actinomycetes from the marine terrain have been shown to be largely productive and cache interesting mixes compare to terrestrial counterparts. Marine actinomycetes not only live in the water and deposit of abysms, but they are also considerably associated with in different marine ecosystems like estuarine homes, ocean meadows, fishes, Phyto plankton, tar marsh shops, bloodsuckers, ocean imps, mangrove, coral reefs etc., The exploitation of marine actinomycetes as a source of new secondary metabolites is in its childhood. Actinomycetes, one of the most colorful microbes are characterized by the product of various colors on different natural and synthetic media. The color product from microbes has advantages because they can produce colors on an economically realizable medium and they have fast growth. It's an arising field of disquisition which has various artificial operations. Actinomycetes are significant microorganisms in biotechnology that give a variety of colors used in sedulity (Vasanth Bharathi et al., 2011). Melanin are the macromolecules synthesized through oxidative polymerization and hydroxylation of indole or phenolic mixes. ultimate of the living organisms including shops, brutes, and microbes (prokaryotes to eukaryotes) like bacteria, fungi, and actinomycetes cortege melanin product. There are mainly three types of melanin do in nature videlicet, pheomelanin or cysteinyl DOPA polymers, eumelanin or DOPA (o-dihydroxyphenylalanine) polymers, and all melanin or DHN (di- hydroxynaphthalene) polymers. Melanin is mainly set up in mortal hairs, skin, and in the microbes present in their cell walls. The natural emulsion of melanin was started from L- tyrosine over an array of enzymatic and nonenzymatic responses from the enzyme tyrosinase. Melanin attains a major part in humans, and the lack of this color cause multitudinous abnormalities and conditions. dropped position of melanin in the neurons leads to Parkinson’s complaint. Melanin was

reported for its wide range of operations like photoprotection, substance chelation, thermoregulation, resistance to mechanical and chemical stress, energy harvesting, desiccation, antioxidant, cell development, etc. (CV Dilip et al., 2013).

1. ACTINOMYCETES: -

1.1 CHARACTERISTICS OF ACTINOMYCETES: -

The nature of actinomycetes is heterotrophic. While some have parasitic or mutualistic relationships with plants and animals, the majority are strict saprophytes. It is generally accepted that actinomycetes play a part in nutrient recycling. Some, like Actinomyces, are anaerobic, while others are aerobic. Particularly specific growth media and incubation conditions are needed for species such as Frankia (CV Dilip *et al.,2013*). Based on growth intensity, substrate color, and aerial mycelia on SCA media, the MR28 isolate's morphological characteristics were documented. Spore morphology was examined using a SEM device (JSM-IT500LA, Tokyo, Japan). The mycelial spores were loaded onto a carbon stub, followed by gold sputtering, and imaging was done up to a magnification of 10 µm (M Rudrappa *et al.,2022*). Using both macroscopic and microscopic methods, the morphological traits of the selected isolate (ACT3) were examined. Using the International Streptomyces Project (ISP) medium types ISP-2, ISP-3, ISP-4, and ISP-6, the isolate was first characterized macroscopically. The synthesis of diffusible pigments, color, and mycelia in the air and substrate were all visually examined(NA El-Zawawi *et al.,2024).*

1.2 SECONDARY METABOLITES OF ACTINOMYCETES: -

Drug development used to heavily rely on natural ingredients. Many novel metabolites have been identified in recent years, despite the fact that the use of marine actinomycetes as a source for the discovery of novel secondary metabolites is still in its infancy. Examples of novel secondary metabolites that were isolated from marine actinomycetes between 2005 and 2010 are displayed. Although this list is impressive and shows the wide variety of structures with documented biological activity, it is by no means a comprehensive search of all novel secondary metabolites produced by marine actinomycetes during this 5-year period. Among these, some substances noteworthy, including staurosporinone, salinosporamide A, lodopyridone, arenimycin, marinomycins, and proximicins *(*R Subramani *et al., 2012*)

COMPOUNDS	**SOURCE**
Chinikomycins	*Streptomyces* sp. (Anticancer)
Chloro-hydroquinone	*Novel actinomycete* (Antibacterial; anticancer)
Glaciapyrroles	*Streptomyces* sp. (Antibacterial)
Frigocyclinone	*Streptomyces griseus* (Antibacterial)
Lajollamycin	*Streptomyces nodosus* (Antibacterial)
Mechercharmycins	*Thermoactinomyces* sp. (Anticancer)
Salinosporamide A	*Salinispora tropica* (Anticancer; Antimalarial)
Sporolide A	*Salinispora tropica* (Unknown)
Salinosporamides B&C	*Salinispora tropica* (Cytotoxicity)
2-Allyloxyphenol	*Streptomyces* sp. (Antimicrobial, food preservative)
Saliniketal	*Salinispora arenicola* (Cancer chemoprevention)
Marinomycins A-D	*Marinispora* (Antimicrobial; anticancer)

BIOLOGICAL ACTIVITIES: -

1.Anticancer: Chinikomycins, Chloro-dihydroquinones, Mechercharmycins, Salinosporamide A, Marinomycins A-D
2.Antibacterial: Chloro-dihydroquinones, Glaciapyrroles, Frigocyclinone, Lajollamycin, 2-Allyloxyphenol, Marinomycins A-D
3.Antimalarial: Salinosporamide A
4.Antimicrobial: 2-Allyloxyphenol
5.Cancer chemoprevention: Saliniketal
6.Cytotoxicity: Salinosporamides B & C.

1.3 ORIGIN: -

Respiration, fungal growths, and staphylococci bacteria are all classified as actinomycetes. According to DNA-rRNA combination study results and 16S ribosomal cataloging, their DNA displayed a high (G+C) ratio (>55%). The 18 major bacterial lineages that we now know about include this one as one of their primary taxonomic categories. Originating from the Greek word's "attacks" (a ray) and "makes" (fungus), actinomycetes are thought to have characteristics of both bacteria and fungi. Among these are antibiotics, immunosuppressive drugs, anticancer drugs, and enzymes also, these metabolites have been shown to possess antibacterial, antioxidant, antifungal, anticancer, neurogenic, antialgal, antimalarial, anti-inflammatory, and antihelmintic qualities (GA Nasir *et al*.,2022).

1.4 PROPERTIES: -

In every instance, growth and enzyme production were connected, and product analysis showed that endoxylanase activity was the cause of degradation. Xylobiose was likely the end-product that prevented this, although cellobiose and monomeric sugars did not (As bell *et al.*,1989). Actinomycetes are now understood to be procaryotic creatures that are closely linked to bacteria, but they were formerly thought to be connected to both fungi and bacteria. Use of any antibacterial antibiotic inevitably suppresses certain actinomycetes; nevertheless, populations of bacteria on plates can be lowered by adding sodium benzyl penicillin and polymyxin B sulfate from sterile solutions to the sterilized medium. Actinomycetes' colonies are denser and more highly pigmented than those on chitin agar, while bacteria grow more easily on starch-casein, which permits the development of the same genera as chitin (ST Williams *et al*.,1982). Compounds that can be applied to different industrial settings, such the creation of plastics, have been found during the hunt for actinomycete secondary metabolites. In one study, S. lividans was modified to produce 4-vinylphenol, a plastics additive, from cellulose by introducing phenolic acid decarboxylase from other species. Another work improved Streptomyces maritimus benzoate synthesis, which enabled it to directly generate benzoic acid from cellulose using a new method. Benzoic acid has numerous applications in pharmaceuticals, food preservation, cosmetics, and hygiene goods (VS Jaganathan *et al*.,2021).

2. MELANIN: -

Because of its medicinal properties and wide range of uses in different disciplines, melanin has been generated and extracted from a variety of microorganisms (M Rudrappa *et al.*,2022). Animals, plants, and the majority of microbes contain dark-colored pigments called melanins. These colors are widely used in pharmacology, medicine, and cosmetic preparations. Melanins are negatively charged substances made up of polyphenolic chemicals and multifunctional polymers that are created by fermentative oxidation in a variety of microorganisms. Additionally, they possess antioxidant and radioprotective qualities that can successfully shield living things from UV rays. The industry finds melanin's capacity for polymerization intriguing because of its applications in nanotechnology for bio-plastics and biopolymers (p gupta *et al*.,2022). Microorganisms' ability to produce melanin has gained popularity recently as a cost-effective and environmentally beneficial substitute for chemicals. The biotechnologically useful bacteria known as actinomycetes are highly utilized for their secondary metabolites. They can naturally produce and expel melanins, also known as melanoid pigments, which are dark soluble pigments(NEA El-Naggar *et al*.,2017).

2.1 BIOSYNTHESIS OF MELANIN: -

Similar to larger creatures, microbes use distinct routes for the manufacture of melanin pigment. Tyrosinase can be used to convert L-tyrosine into melanin pigment by acting as a precursor. Tyrosinase is a copper protein that is a member of the polyphenol oxidase family. Tyrosinase converts tyrosine to L-DOPA (3, 4-dihydroxyphenyl-Lalanine), which is then auto oxidized to indol-5, 6-quinone and subsequently transformed into dopachrome. Dark brown is produced when the latter spontaneously polymerizes into DOPA-melanin (MT Shaaban *et al*.,2013). Using DOPA, a variety of fungi may produce brown or black pigments from L-tyrosine. The best-characterized melanization mechanism from L-tyrosine is the DOPA-Melanin pathway, where tyrosinases or laccases hydroxylate tyrosine via DOPA to DOPA-Quinone, which subsequently auto-oxidizes and polymerizes. However, a process involving the buildup and auto-oxidation of tyrosine catabolism intermediates may also result in the production of brown pigments from L-tyrosine. For example, tyrosine is converted to p-hydroxyphenylpyruvate (HPP) and homogentisic acid (HGA) to create pyo-melanins. Alkapto-Melanin and Pyo-Melanin are simply two names for the same pigment. However, the pigment that people generate is known as Alkapto-Melanin, while the pigment that microorganisms produce is frequently referred

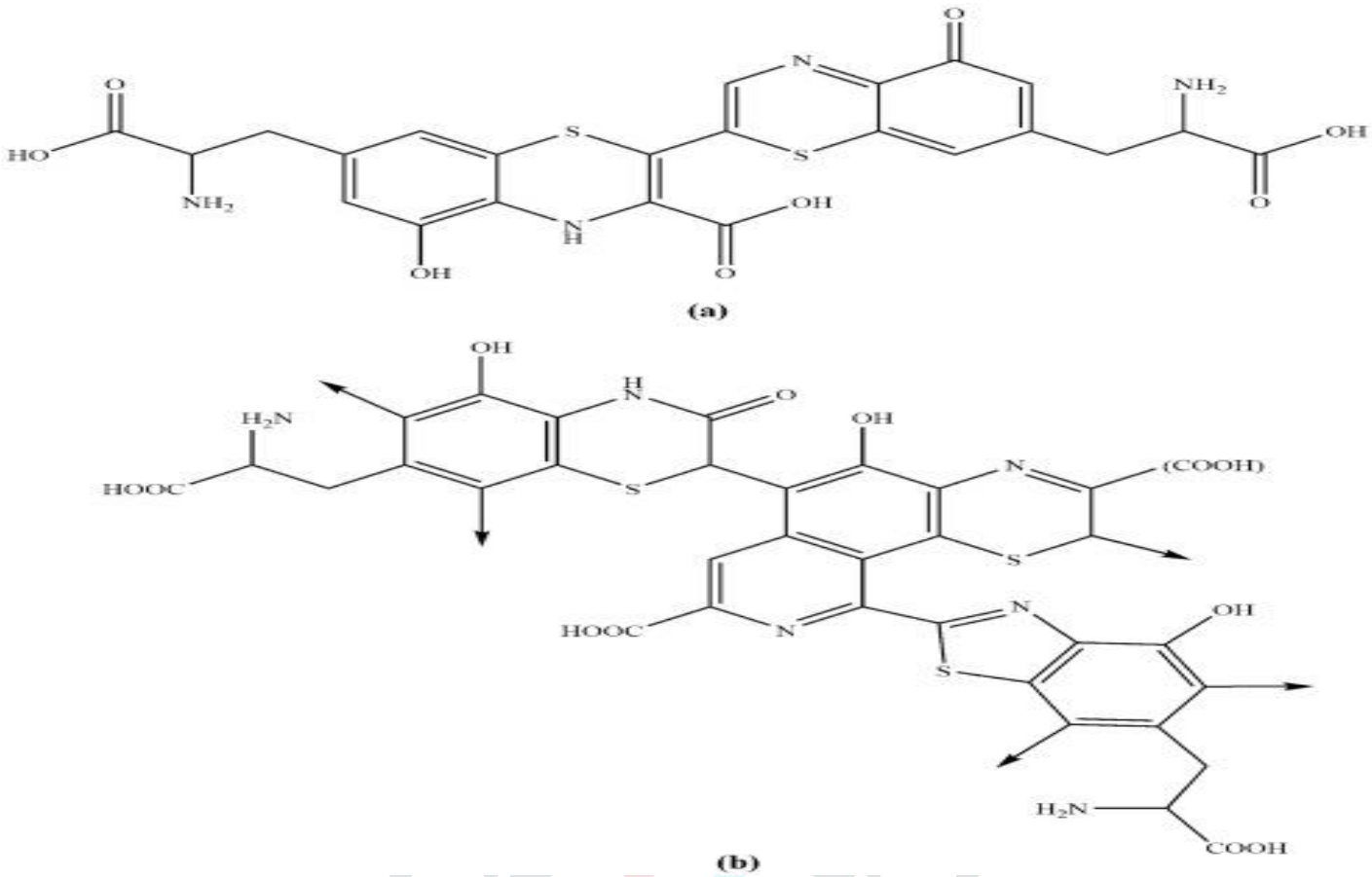
to as Pyo-Melanin. Yabuuchi and Ohyama were the first to use the word "pyo-melanin" to describe a brown substance that was soluble in water (S Singh *et al.*,2013).

2.2 PROPERTIES OF MELANIN: -

The combination of carotenoids, oxy-deoxy-hemoglobin, and most importantly different forms of melanin, as well as how melanin is distributed and packaged in melanosomes, are the primary factors that determine skin color. Dendritic melanocytes, which make up just 1% of epidermal cells, create melanosomes, which are particular ovoid organelles that are responsible for producing melanin. In order to shield DNA from ultraviolet light, melanin generated in melanosomes is transported by dendrites to neighboring keratinocytes and accumulates as supranuclear "caps" in the perinuclear region of keratinocytes and melanocytes (M Brener *et al.*,2008). The phototype determines the skin's melanin content. Endogenous melanin is insufficient for complete protection in the majority of morphologies, particularly during the summer. Therefore, commercial sunscreens ought to include photoprotective molecules (F Solano *et al.*,2020). Numerous physicochemical traits of animal-derived melanin are present in pigments made by several Streptomyces strains. It was shown that the melanins generated by Streptomyces strains were insoluble in acidic solutions with a pH below 2.0 but soluble in aqueous alkali (0.1 M NaOH) and DMSO. Some of these melanins, including the pigments made by S. roseochromogenes ATCC 13400 and S. nashvillensis DSM 40314, were either fully or gradually soluble in water (S Singh *et al.*,2013).

2.3 STRUCTURE OF MELANIN: -

Melanin is known to be a high molecular weight amorphous polymer. The precise structure of melanin is still unknown, despite the fact that it is known to be created via the polymerization of phenolic and indolic chemicals. These polymers create planar sheets that resemble graphite and then aggregate hierarchically to create a colloidal particle. These melanin particles can have widths of hundreds of nanometers, depending on where they come from. Although melanin's structure is resistant to acid hydrolysis, it can be broken down by oxidative substances like hydrogen peroxide or permanganate or by alkaline environments(RJB Cordero**,** A Casadevall *et al.*,2020). There are five primary types of melanin, a pigment that is widely distributed in many different organisms. These include eumelanin, which is found in both plants and animals, pheomelanin, which is found in both plants and animals, allomelanin, which is found in plants, neuromelanin, which is found in animals, and pyomelanin, which is found in fungi and bacteria (W song *et al.*,2023). Since melanin is found in all living things in all biological kingdoms, it is a significant natural biomaterial (W Cao *et al.*,2021). Electron microscopy has been used to examine melanocytes and melanin granules in hyperplastic human skin lesions as well as normal human and cat skin. In the epidermis, or on the epidermal side of the dermal membrane, melanocytes have always been found as free cells
(P Drochmans *et al.*,1960).



2.4 FUNCTION OF MELANIN: -

Human melanin's main job is to shield us from the sun's ultraviolet (UV) rays by acting as a sunscreen (WL Morison *et al.*,1985). Numerous pharmacological and biological characteristics are displayed by it. It is widely known that it is a molecule with unique qualities and functions that impact overall health, such as immunological and photoprotective effects. Only lately have its hepatic, gastrointestinal, immunomodulatory, radioprotective, antioxidant, anti-inflammatory, and hypoglycemic properties been identified and investigated (AS EI Obeid *et al.*,2017). One of the primary distinct functions of both normal and malignant melanocytes is the multistep, highly controlled pathway that produces melanin. Although melanin's primary purpose is to shield the skin from UV rays, the pigment can also control epidermal homeostasis, which can influence the behavior of melanoma (RM Slominski *et al.*,2015). Melanins are not essential for fungal growth but appear to be important for the virulence of several pathogens *(*R Romero *et al.*,2000).

3. ADVANTAGES: -

3.1 PHOTO PROTECTIVE ROLE OF MELANIN: -

One of the harshest things for human skin is direct sun exposure. UV light is one of the electromagnetic spectrum's components found in sun radiation. Human skin has a photoprotective pigment called melanin that protects against UVB, UVA, and blue visible light in addition to the stratospheric ozone layer blocking the most dangerous UVC rays (F Solano *et al.*,2020). UV light absorption energy suggests that melanin structural components are photoexcited, and the ensuing energy transfer can cause a number of consequences on nearby molecules. Melanin may turn into a photosensitizer rather than a photoprotector as a result of these activities (F Solano *et al.*,2016).

3.2 ROLE OF MELANIN IN ENVIRONMENTAL PROTECTION: -

Free-living microorganisms' capacity to produce melanin is probably linked to an advantage in environmental survival. Accordingly, melanin is constitutively synthesized by a variety of fungi, and soils can also melanized facultative melanotic microorganisms such as C. neoformans. Melanin prevents photo-induced damage by absorbing a wide spectrum of electromagnetic radiation, which gives them resistance to UV light. Actually, melanin has been utilized in photoprotective lotions for commercial purposes. When exposed to UV radiation, melanized C. neoformans cells were less vulnerable than non-melanized cells. Monilinia fructicola, Phaeococcomyces sp and S. schenckii have all been shown to be protected against UV, solar, or gamma radiation.

Environmental heavy metals that may be harmful to cells can be bound by melanins. Compared to non-melanized cells, melanized C. neoformans cells are more resilient to death from silver nitrate, a substance that is extremely poisonous to bacteria and fungi. Metal binding has not been shown to have a protective function in other microorganisms, despite the fact that other fungal melanin does (JD Nosanchuck *et al.*,2003).

4. CHALLENGES: -

Melasma is a chronic, acquired hyperpigmentation that primarily affects women. Although the exact cause of melasma is unknown, ultraviolet (UV) exposure is a major contributing factor. Melasma is difficult to treat because it is a chronic and recurring condition. The existing treatment frequently produces less than ideal results and unfavorable side effects. The treatment's three main tenets are UV protection, melanin synthesis inhibition, and melanin removal route expansion(SAA Suryan *Tari et al.*,2020). The primary defense against UV rays is provided by melanin. Albinos and other people with melanin synthesis deficiencies are therefore vulnerable to severe sun-induced skin damage and tumor formation, particularly if they reside in nations with long days. Dark skin, on the other hand, is less vulnerable to harm from prolonged exposure to sunlight. Their sole apparent drawback is that they have trouble synthesizing vitamin D when exposed to low light levels (O Braun-Falco *et al.*,2000). The primary pigment that gives human skin its color is eumelanin. In the basal layer of the epidermis, this black/brown pigment is located in membrane-bound organelles called melanosomes that are present in specialized cells called melanocytes. The phases involved in melanogenesis in the epidermis are highlighted in this overview, as are the problems in skin pigmentation that arise from the failure of some crucial steps in this process. Tyrosinase, a key enzyme in melanin formation, is found in melanosomes, which are formed via a sequence of events in the melanocyte. They are given to the nearby keratinocytes in the epidermis by the melanocyte dendrites. The melanosomes in keratinocytes can be found individually or in clusters, and they begin to break down as the keratinocytes ascend in the epidermis. This series of actions is crucial (MW Lambert *et al.*,2019).

5. APPLICATION OF MELANIN: -

Melanin in fungi and bacteria are typically noted for their significant contribution to the pathogenic organisms' pathogenicity. Melanin pigments may now be transformed into useful materials in a variety of sectors, including environmental remediation, biomedicine, materials science, green technology, and cosmetics, thanks to the development of new knowledge and technologies.

Melanin has also been used to create ecologically safe silver nanostructures in another kind of application. The food and health industries may find use for these melanin-mediated silver nanostructures, which exhibit broad-spectrum antibacterial activity against food pathogens. Melanin is used in dermal and cosmetic applications, such as hair coloring and sunscreen. In environmental applications, melanin can be used as a metal chelator. Melanin-based composites can remove up to 94% of lead (II) from water systems by combining fungal melanin with other polymers including polycaprolactone and polyurethane. Many research has reported on the prospective uses of melanin; however, they are still in the early stages of development and have not yet (AN Trana-LY *et al.*,2020).

Melanin can be utilized as an adsorbent in industry to reduce heavy metal pollution. Heavy metals in various chemical forms and states are highly migratory, enriched, latent, and bio toxic and accumulate when they enter the environment and human body, threatening ecological environment safety and human health. Food packaging can leverage melanin's potent antibacterial, anti-radiation, and antioxidant properties to prolong the shelf life of food items. Humoral immunity, cellular immunity, and both specific and non-specific (including carbon scavenging) immunity can all be markedly enhanced by melanin. A notable rise in IL-6 releases and expression in retinal epithelial cells suggests that calf RPE melanin may play a role in controlling the retinal immune response (L Guo *et al.*,2023).

6. MELANIN BASED PRODUCT: -

WHITENING PRODUCT: -

As previously stated, melasma is typified by an overabundance of melanin in the dermis and epidermis. Tyrosinase is a crucial enzyme in the synthesis of melanin, which is produced and stored in melanocytes through a sequence of oxidative reactions. From there, it is transferred to nearby keratinocytes through dendritic processes, resulting in the appearance of brown spots and patches on the skin's surface.

SUNSCREEN PRODUCT: -

Therefore, a sensible and efficient use of sunscreens can lower melanin production, minimize skin inflammation, preserve the skin barrier, and stop melasma from recurring. This is a crucial adjuvant therapy and the cornerstone of melasma treatment. However, some moisturizing components that are added to sunscreens can also aid in the restoration of the skin's protective layer.

MOISTURIZING PRODUCT: -

Effective moisturizing skin care solutions can improve the epidermis's moisture content, aid in restoring the skin's barrier function, lessen skin dryness and peeling, and smooth out the skin. The four primary categories of moisturizing skin care products are occlusives, humectants, emollients, and rejuvenators. Occlusives, including petrolatum and dimethicone, can create a hydrophobic coating on the skin's surface to prevent water loss.

CONCLUSION: -

The complex group of soil-dwelling microbes known as actinomycetes is well-known for producing a variety of bioactive substances, including melanin. One important biochemical characteristic of actinomycetes that helps with their survival and environmental adaptability is the formation of melanin, especially in species like Streptomyces and Nocardia.

Melanin protects bacteria from UV light, oxidative stress, and antibiotic agents, among other things. There are several possible uses for the research of melanin formation in actinomycetes, such as the creation of industrially useful natural pigments, novel medicinal agents, and biotechnological developments in agriculture and medicine. Furthermore, by comprehending the molecular processes underlying melanin manufacturing in actinomycetes, new approaches to regulating pigment production and investigating its functions in microbial ecology and human health may be developed.

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