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Research Article

Isolation and Detection of Bacterial Strains from Cosmetics Products available in Pakistan

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Abstract: Cosmetics products are the most essential and frequently used components in our daily life. Besides improving human health, they provide healthy lifestyles and boost our self-esteem. Globally cosmetics market is projected to be 287 billion USD in 2021 to 415 billion USD in 2022. This research study aims at the isolation, identification, and characterization of bacterial strains isolated from cosmetics. Six bacterial colonies were isolated by inoculating different cosmetics products on tryptic soya agar media. All the strains showed optimum growth at 37 °C. All strains were assessed through biochemical tests by using different media such as MacConkey agar, SIM, and Simmons citrate agar and were further proceeded for nucleotide sequencing through Sanger sequencing. Different bacterial strains were revealed in cosmetics products including *Sphingomonas paucimobilis, Cytobacillus oceanisediminis, Robertmurraya andreesnii, Cytobacillus firmus, Falsibacillus pallidus*, and *Acinetobacter junii.* Most of these strains were found to be pathogenic however, *Sphingomonas* has the potential for bioremediation and can be utilized for degrading toxic compounds to make the environment better. Similarly, *Cytobacillus* is found to be involved in biomineralization and also aids in fermentation. Our results have shown that there is a dire need to assure strict safety regulations regarding cosmetics. Improper manufacturing practices can lead to the contamination of cosmetics which could lead to severe consequences of deteriorating the quality of health. Further studies are needed to explore the potential of these isolates so that they can be utilized to improve our health as well as the environment.

Keywords: Cosmetics, Contamination, Molecular Identification, Biochemical Tests.

1. INTRODUCTION

Personal care products are frequently utilized by people in daily life and getting popular across the globe due to extensive use. They are purchased without any hurdle as they do not lie under the same regulation as those of medicines. The word "Cosmetic" is derived from the Greek word "*Kosmetike tekhne"* meaning "Technique of dressing and decoration". Cosmetics are defined as the substances which are applied to the external surface of the human body to alter the appearance to look attractive, improve the texture of the skin, keep the body clean, and smell good, and for skin protection. There are seven main categories of cosmetics which include oral care products, skin care products, body care products, products of hair care, sun care, fragrance products i.e., perfumes, and decorative cosmetic products [1].

Cosmetics are comprised of a combination of chemical ingredients derived from natural as well as chemical sources. The vital ingredients of cosmetics include water, emulsifiers, preservatives, thickeners, pigments, glitters, fragrances, etc. However, these ingredients act as a medium for the transport of pathogens in the daily lives of people because mostly these components encourage the growth of microbes. Microorganisms can survive at a suitable temperature, pH, moisture, and metabolites [2]. Almost all cosmetics products fulfill these requirements and harbor the growth of microbes. Most cosmetics contain growth stimulators, organic as well as inorganic components, and are stored in

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a humid atmosphere which stimulates the growth of microorganisms. The microbial contamination of these personal care products deteriorates their quality ultimately affecting human health and resulting in severe consequences [3]

Cosmetics products become prone to contamination either during manufacturing in a container or during use by the consumer. In the first case, the manufacturer of the product must adopt all the safety protocols to avoid contamination so that the best quality of the product must be ensured. While in the second scenario, the user must keep the products in a safe place to avoid health issues [4]. The most important concern about cosmetics is that they are not labeled with their manufacturing and expiring date due to which chemicals used for preservation degrade at their specific time. Ultimately, contamination will occur and people keep on using such contaminated products without being in their knowledge. The microbial contamination of cosmetics results in the production of such toxins which cause severe irritation and allergy on the skin [5].

Microbial contamination of cosmetics products is a global health issue and causes nuisance among consumers, manufacturing industries, and clinicians. The cosmetics become prone to contamination due to impurity of raw material, due to use in a contaminated atmosphere, or poor personal hygiene [6]. Microbial contamination of cosmetics products is a global health issue and causes nuisance among consumers, manufacturing industries, and clinicians. The cosmetics become prone to contamination due to impurity of raw material, contaminated atmospheres, or poor personal hygiene [7]. The microbes not only modify the physical features of products like color, viscosity, flavor, and scent but also degrade the crucial components of products which results in severe consequences. The microbial interference may produce certain toxic compounds and metabolites which cause a severe allergic reaction to the skin [8].

The risks associated with contaminated products can have a significant impact on human health ranging from mild to severe diseases [9]. Pathogenic microbes have been isolated from cosmetics products which include *Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter,* and *Klebsiella pneumonia*, etc. which cause a range of diseases from severe skin allergies to respiratory infections along with bacteremia and urinary tract infections as well [10].

An accurate and early-stage diagnosis of a disease is crucial to avoid long-lasting effects and complications. The precise diagnosis of infection improves the effectiveness of the treatment required to alleviate that infection and prevents unnecessary practices and medication. The precise diagnosis of a disease prevents the outbreak of that disease and minimizes the development of antibiotic resistance [11]. Conventionally, bacterial infections are diagnosed with the help of culture methods, however, due to certain limitations like some bacteria being difficult to grow and their growth requirements being different and time-consuming procedures do not make it an ideal method for bacterial diagnostics. Over several decades, nucleic acid testing has revolutionized the diagnosis of disease and it is faster, more accurate, and more sensitive than the traditional culture methods [12]. The present study aimed to isolate and identify bacterial strains from cosmetics products available in Pakistan via biochemical testing and molecular characterization.

2. MATERIALS AND METHODS

Six branded products of cosmetics (lip-gloss, foundation, sunscreen, and eyeshadows) available in Pakistan were purchased. These were used because of their causal usage or daily usage. Different biochemical tests were performed which included SIM (Sulfur, Indole, Motility), Simmons Citrate Agar, and MacConkey Agar test. The molecular characterization was performed for the confirmation of isolated bacterial strains at the Molecular Systematic and Applied Ethnobotany Lab (MoSAEL), Department of Biotechnology, Quaid-i-Azam University, Islamabad.

2.1 Isolation of Bacterial Strains

The isolation of bacterial strains was done by providing them with suitable nutrients, temperature, and environment. Thus, different bacterial strains were obtained from different products of cosmetics. Different types of media were prepared including

TSA ((Tryptic soya agar), MacConkey Agar, SIM (Sulfur, indole, motility), Simmons citrate agar, and Urea base agar.

The serial dilution was performed for each sample. Each product of cosmetics was dissolved in autoclaved distilled water and DMSO (dimethyl sulfoxide) and then placed on the TSA media plates for the emergence of bacterial colonies. While the direct cosmetic samples (without dilution) were also taken with the help of sterilized inoculation loop and placed on TSA media at 37 °C. The emergence of bacterial colonies was sub-cultured and maintained. Morphologically different and pure colonies were selected for investigation.

2.2 Molecular Identification of isolated bacterial strains

The molecular characterization was done in the following steps as depicted below diagrammatically in Figure 1. To extract DNA from bacterial colonies, the simple plain boiling method was used for a higher yield of DNA. For this purpose, a single colony from overnight grown bacteria was picked with a micro-pipette and dissolved in 1ml distilled water in an Eppendorf tube. Then each Eppendorf tube was kept at 95 °C in a water bath for 10 minutes.

After boiling in the water bath, each tube was centrifuged at 1000 rpm for 5 minutes. The supernatant having the bacterial DNA was separated and stored at -20 °C and the pellet was discarded.

2.2.1 Polymerase Chain Reaction

All the components of the reaction mixture were added in an optimized quantity making the total volume of 20 µl in the PCR tube. The PCR reaction mixture was comprised of Green Master Mix (Thermo Scientific). Primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3) and reverse primer 1492R (5'-GGTTACCTTTTTTACGACTT-3') of 16S rRNA gene were added. Also, the template DNA and nuclease-free water were added to make the total volume of 20 µl.

2.2.2 Gel Electrophoresis and Purification of RCR Products

PCR amplified products were loaded on 1.5 % agarose gel, prepared by 1X TAE buffer along with 1kb ladder, and run for 30 min at 90 V. After that gel was visualized on UV. The required amplified bands were cut and subjected to Thermo Scientific GeneJET Gel Purification Kit for the purification of PCR products.

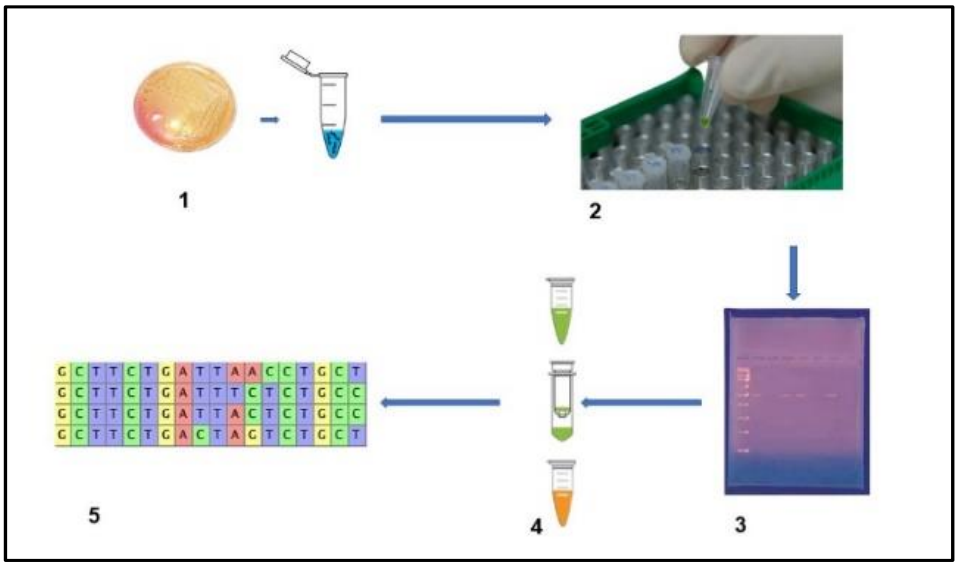

Fig. 1. The stepwise procedure of molecular characterization technique; 1. DNA extraction of pure bacterial isolates using the plain boiling method. 2. Polymerase chain reaction (PCR) using universal primers for the amplification of the 16S rRNA gene. 3. Agarose gel electrophoresis for the confirmation of amplified product. 4. Purification of amplified product from agarose gel electrophoresis. 5. Sanger sequencing and post-sequence analysis using bioinformatics tools.

According to the given protocol: the gel slice was first cut then binding buffer was added in the ratio of 1:1 and placed on the hot plate at the temperature of 65 °C for 10 minutes to melt the gel completely. Inverted mixing was done to homogenize the mixture. The mixture was vortexed briefly. The mixture was then transferred to the GeneJET purification column and centrifuged for 30-60 seconds. Flow through was discarded. Wash Buffer (700µl) was added to the GeneJET purification column and then centrifuged for 30-60 seconds. The flow-through was discarded and the purification column was placed back into the collection tube. The empty GeneJET purification column was again centrifuged for an additional 1 minute to completely remove the residue material. The GeneJET purification column was transferred to a sterile microcentrifuge tube with a 1.5 ml capacity. Elution buffer (30 µl) was added to the center of the GeneJET purification column membrane and centrifuged for 1 minute. The GeneJET purification column was then discarded and purified DNA was stored.

2.2.3 Sequencing of 16S rRNA Gene

After the elution of PCR products, they were processed for sequencing to Macrogen. The sequencing was carried out using forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3) by the Sanger Sequencing Method.

2.3 Phylogenetic Analysis

After 16S rRNA gene sequencing, the sequences were compared to the reference sequence retrieved from the NCBI (National Center for Biotechnology Information) for phylogenetic analysis. BLAST tool was used for the comparison of the similarity index. The most similar sequences having the highest similarity index were selected and aligned using the software MEGA X. The phylogenetic tree was constructed using the same software by using the maximum likelihood method at a 1000 bootstrap value. The maximum likelihood method predicted the evolutionary relationship of the strains to the closely related strains.

3. RESULTS

In this study, we revealed the diversity of bacteria isolated from six cosmetic products of various brands. A total of n=6 bacterial colonies were obtained through culturing on TSA media after 24 hours of incubation at 37 °C as shown in Figure 2 which were identified based on biochemical as well as molecular characterization. The tested products were taken from famous brands. The names of brands are not mentioned in the study due to commercialization issues.

3.1 Biochemical Tests

Various biochemical tests were performed to identify bacterial isolates which included SIM (Sulfur, Indole, and Motility), MacConkey Agar, and Simmons citrate Agar.

3.1.1 Sulfur test

A sulfur test was done to identify isolates that were gram-negative enteric bacillus based on the production of hydrogen sulfide gas which caused the formation of black precipitates. Four isolates showed positive Sulfur test as represented in (Figure 3A) while all other isolates were negative.

3.1.1.1 Indole test:

This test aimed at the differentiation of gram-negative bacteria based on the production of hydrolysis of tryptophan. Indole-positive bacteria produced pink to red color rings on the top surface of the media as shown in (Figure 3B) while no color change was observed for indole-negative bacteria. Five bacteria gave indole positive tests while the rest of them gave negative tests.

3.1.2 Motility test

The motility test identified the strains which were motile and could move from those which were non-motile. The motile isolates produced turbidity and cloudiness in the medium while non-motile isolated grew along the stab line only and did not produce any turbidity as represented in (Figure 3C).

3.1.3 Simmons Citrate Test

Citrate-positive bacteria changed the color of the media from green to bright blue while citrate-negative bacteria did not change the media color.

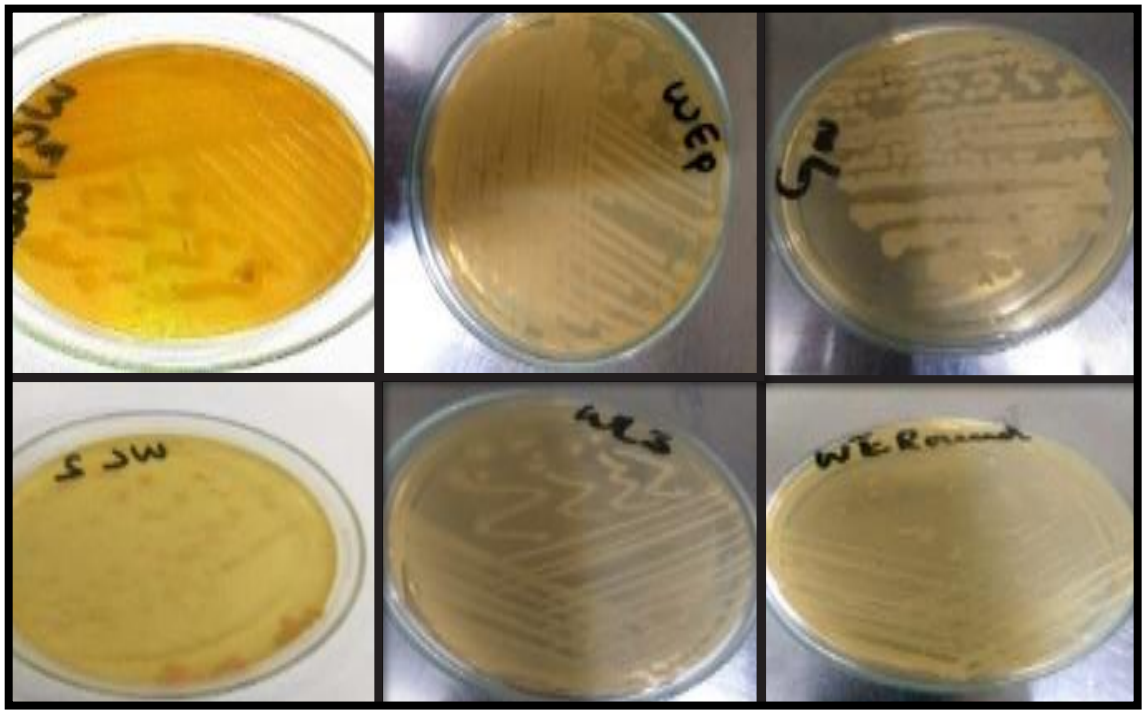

Fig. 2. Represents the bacterial colonies obtained after inoculation were mostly yellow, brown, and white in color and rest were transparent.

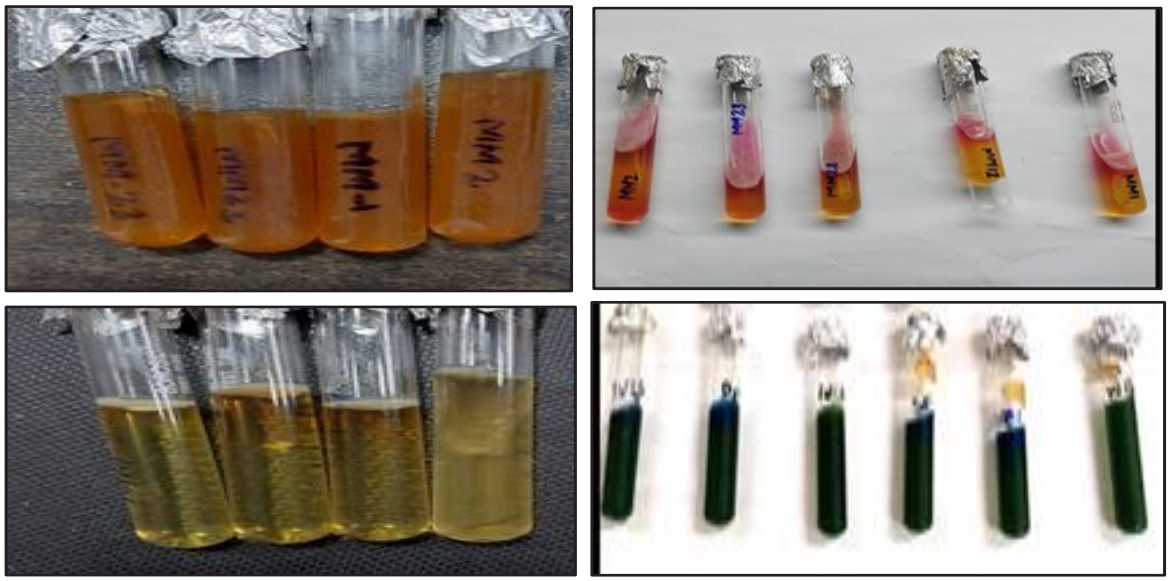

Fig. 3. SIM test and Simmons Citrate Test Results. A) Sulfur test: *A. junii, S. paucimobilis, C. firmus, R. andreesnii* gave sulfur positive test. B) Indole test: *C. oceanisediminis, S. paucimobilis, F. pallidus, R. andreesnii, C. firmus* gave indole positive test. C) Motility test: *C. firmus, A. junii, R. andreesnii* and *S. paucimobilis* were motile strains. D) Simmons Citrate Test: *C. oceanisediminis, F. pallidus, C. firmus, A. junii, R. andreesnii* and *S. paucimobili*s gave citrate positive tests.

Four isolates were found to utilize citrate as an energy source while the rest of the isolates were unable to do so as shown in Figure 3D.

3.1.4 MacConkey Agar Test

MacConkey agar test was used for the differentiation of gram-negative bacteria based on their ability to ferment lactose. Gram-positive bacteria did not grow on MacConkey agar Figure 4.

3.2 Molecular Identification

After the completion of PCR, the PCR products were visualized on 1.5 % agarose Gel as shown in (Figure 5).

3.2.1 Sequencing Analysis

After sequencing, the BLAST was used for comparing the sequences with reference sequences. The most similar sequences were retrieved. Sequencing results indicated one strain of *C. oceanisediminis* and another identified as *Sphingomonas paucimobilis*. There were also other strains including *C. firmus, R. andreesnii, C. pallidus*, and *A. junii*.

3.2.2 Construction of Phylogenetic Tree

Mega X was used to analyze the sequence of nucleotides of the bacteria isolated from cosmetics products in comparison with the reference sequence of nucleotides of bacteria from all over the world. The maximum likelihood method was used to evaluate the evolutionary history of strain. Phylogenetic analysis showed that isolate QAUT7F strain showed the most similarity to the *Cytobacillus firmus* strains found in Japan, QAU103 strain showed great similarity to *Sphingomonas paucimobilis* strains found in the USA while QAUT12R was found to be greatly related to the *Robertmurraya andreesnii* isolated from USA, Germany, and Belgium as shown in Figure 6. QAUT10F and QAUT11F show similarity to the *A. junii* strain and *F. pallidus* strain respectively, both the isolates have shown an evolutionary relationship with China (Figure 7). Figure 8 shows that QAU 112 isolate was significantly similar to the *C. oceanisediminis* strain of China.

4. DISCUSSION

Cosmetic products are inhabitable companions of everyone in daily life which enhances the elegance of the personality. The use of cosmetics has become indispensable as they are not merely used to improve appearance but also to keep the body in a healthy state. Cosmetics also act as an important vehicle for the transmission of pathogens to humans due to which concern about their use and safety is rising with time. They harbor a wide variety of microbes due to contamination from various sources which cause mild to severe allergic reactions leading to complicated life-threatening infections [13].

Current research studies have revealed bacterial diversity in cosmetics in terms of pathogenic as well as beneficial impacts. *Sphingomonas paucimobilis* is one of the bacteria isolated from cosmetics in my research work. It is a gram-negative, aerobic, and opportunistic pathogen and causes infections in immunocompromised individuals [14]. It causes soft tissue infection characterized by fluid and puss exudates from deep tissues. It is also reported to cause symptoms of pneumonia resulting in severe

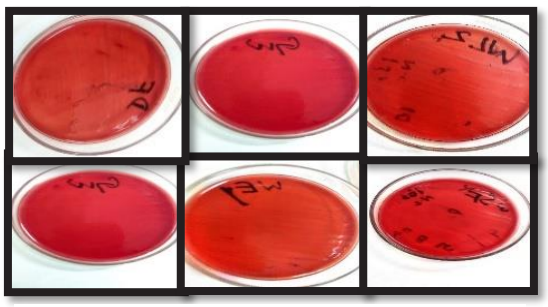

Fig. 4. Results of MacConkey Agar test which showed that almost all bacteria grew transparent as non-lactose fermenting while two strains were pink and lactose fermenting.

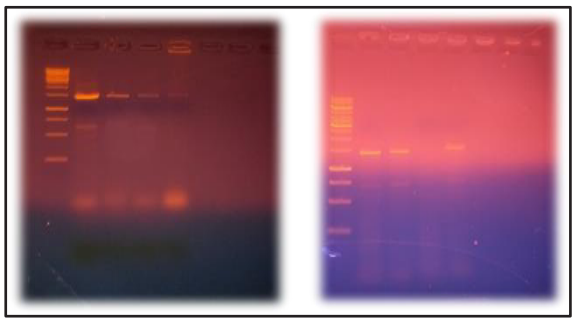

Fig. 5. Represents the PCR products of 1250bp compared with ladder of 1kb size.

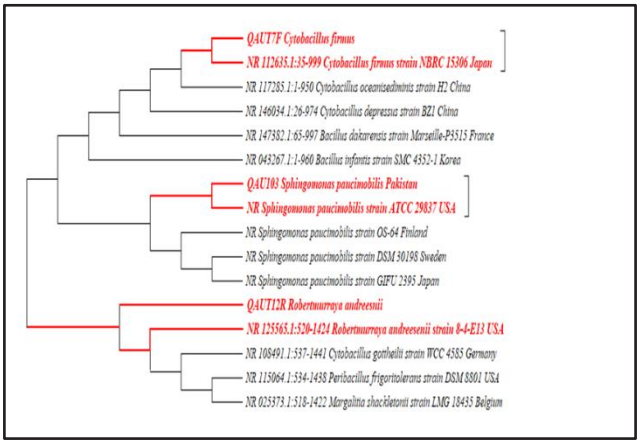

Fig. 6. Representation of phylogenetic tree of isolates constructed through maximum likelihood method (from n=14). The reference sequences were obtained from GenBank by using BLAST. QAUT7F which was identified as *C. firmus*, QAU103 was identified as *S. paucimobilis,* QAU12R identified as *R. andreesnii.*

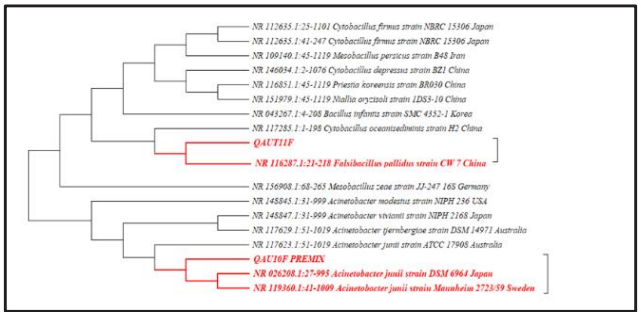

Fig. 7. Representation of phylogenetic tree of isolates constructed through maximum likelihood method (from n=16). The reference sequences were obtained from GenBank by using BLAST. QAU10F PREMIX and QAUT11F were identified as *A. junii* and *F. pallidus* respectively.

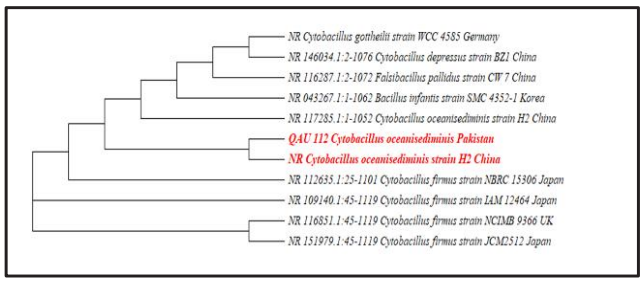

Fig. 8. Representation of Phylogenetic tree of isolate QAU112 (identified as *C. oceanisedminis*) constructed through the maximum likelihood method (from n=10). The reference sequences were obtained from GenBank by using BLAST.

difficulty in breathing [15]. It is not just significant in clinical terms but it has significant importance in the environment as it is the fundamental agent used in bioremediation. This bacterium can degrade aromatic compounds and it is an effective and eco-friendly tool for the degradation of pollutants and carcinogenic compounds from the soil. These bacteria can be used for *in-situ* bioremediation after enhancing the production of biomass in bioreactors. Further work and research are needed to explore and uncover the capacity of this bacteria to perform bioremediation and make our environment free of harmful substances. Recombinant DNA technology can be used to identify the genes responsible for bioremediation and recombinant strains can be developed to remediate the environment [14].

Another bacterial strain isolated from cosmetics in my research work is *C. oceanisediminis*. It is a gram-positive, rod-shaped, and aerobic bacteria found in marine sediments. It is not known whether it is pathogenic or not pathogenic. It helps in fermentation through the production of acetate, lactate, and ethanol [16]. They play an important role in biomineralization. It is a novel strain reported to be isolated from marine systems. Further research is needed to explore its potential and its effects on humans and animals [17].

A. junii has been isolated from cosmetic products. It is a gram-negative bacteria and coccobacillus in morphology. It is a well-known infectious agent and can colonize the skin, gastrointestinal tract, and respiratory pathways. It has been reported to cause a severe form of pneumonia associated with ventilators [18]. It is a notorious pathogen and also a causative agent of blood infection as well as urinary tract infection. It is one of the pathogens involved in eye infections and damages the eye epithelium which leads to corneal ulcers. A corneal ulcer is one of the most common diseases caused due to use of contaminated mascara and eyeliner [19].

R. andreesnii strain is another bacterium isolated in my research work. It is a gram-positive, motile, and rod-shaped bacterium. It is an opportunistic bacterium and causes gastrointestinal infections. It is reported to cause endocarditis, blood infection, pneumonia, and skin infection. It causes morbidity in immunocompromised patients and people having diabetes or serious injuries [20]. *C. firmus* has been isolated from one of the cosmetics samples. It is a gram-positive, aerobic bacterium. It is not known to have pathogenic effects on humans and does not cause any significant diseases in humans [21]. It has significant importance in promoting plant growth by interfering with and inhibiting nematode and cysts growth [22]. It is also used for the preparation of animal feed as probiotic supplements which have played a significant role in improving animal health [23].

5. CONCLUSION

Different bacteria have been isolated from cosmetics products which include *S. paucimobilis, C. oceanisediminis, F. pallidus, A. junii*, *C. firmus*, and *R. andreesnii*. Some of these bacteria are pathogenic and can cause numerous kinds of infectious diseases. Whereas beneficial bacterial components have been derived from these bacteria which have the potential to give maximum benefits to us and the environment as well. Cosmetics companies should develop a monitoring system for checking the quality of products and to assure product safety. A sterile environment should be used to manufacture products. Personnel should implement standard hygiene practices. The raw material used for products should be labeled and verified by the quality assurance officer. The closed system should be preferred for manufacturing products. The finished cosmetics products should be approved by quality control management before releasing in the market. Consumers should also follow the safety protocols during the use of products as well. They should check the manufacturing and expiry date before buying products and high-quality brands should be used instead of relying on cheap and lower-quality products. Bacterial count should be zero in these products because at a certain stage, they may cause serious harm to human health. However, some strains are still unknown whether they are pathogenic or not and there is a dire need to explore and check the metabolic profile of these bacteria so that they can be employed for therapeutic purposes.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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