

## SCIENTIFIC STUDY ON HOMO SAPIEN SKIN DE-PIGMENTARY DISORDER IN THE LIGHT OF HOMO SAPIEN GUT MICROBIOME ECOSYSTEM

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Article Received on 17/01/2023

Article Revised on 07/02/2023

Article Accepted on 27/02/2023

### ABSTRACT

**Introduction:** Homo sapien skin depigmentary disorder among Asians, Africans, Arabians, etc at the tropics has not been seen in the light of the Homo sapien gut microbiome. So this study. **Methods and material:** Four volunteers: Ind223265A, Ind223265B, Ind223265C, and Ind223265D, with skin dark, skin lightly dark, skin wheatish, and skin white respectively, participated in this study. RNA Sequencing and 16S Metagenomic Analyzer have been applied in the analysis of sterile fecal swabs of these volunteers. **Result:** The bacterial composition and percentage of vitamin K biosynthesizing bacteria differed in the gut microbiomes of all the volunteers. In Ind 223265A, the strong vitamin K biosynthesizing bacteria: *Clostridium* (10%) and *Flavobacterium* (17%) have been identified in its gut microbiome whereas in Ind 223265B, *Prevotella* (73%) with the ability to biosynthesize vitamin K has been found. In Ind 223265C, moderate vitamin K biosynthesizing bacteria have been seen in his gut microbiome, while in Ind 223265D, the gut microbiome registered the absence of strong and moderate vitamin K biosynthesizing bacteria and displayed poor vitamin K biosynthesizing bacteria. **Conclusion:** The vitamin K is the main complementary factor for the determination of Homo sapien skin pigmentary order. This study endorses the Sawhney theory of vitamin K deficiency in humans at tropics with the skin depigmentary disorder (HES Leucoderma). Probiotics of strong or moderate vitamin K biosynthesizing bacteria appear to be the only option to treat skin depigmentation disorder at the tropics.

**KEYWORDS:** HES, leucoderma, microbiome, 16s metagenomics, vitamin K, RNA sequencing.

### INTRODUCTION

In humans, the gut microbiome has been found having the large number of bacteria and the greatest number of species compared to other areas of the body.<sup>[1]</sup> In human, after one of two years after birth, the gut microbiome is established, providing a barrier to pathogenic organisms<sup>[2,3]</sup>

The relationship between some gut microbiome and humans is not merely commensal (non-harmful existence), but rather a mutualistic relationship.<sup>[4,5]</sup> The gut microbiome, housing over 99% anaerobic bacteria, are said to be the second heart of the Homo sapien body and performs positively in the fermentation of dietary fiber into short-chain fatty acids (SCFAs) such as acetic acid, and butyric acid which are absorbed by the host,<sup>[1,5]</sup> the synthesis of vitamin B (B1, B2----- B12) and vitamin K.<sup>[2,4]</sup> to meet the biodeMANDS of the body and metabolization of bile acids, sterol, and xenobiotics.<sup>[1,3]</sup> etc. The dysregulation of gut microbiome has been correlated with the host of inflammatory and autoimmune conditions.<sup>[1,6]</sup> The disruption in gut

microbiome would depress the human body of many bacteria-driven benefits.<sup>[4]</sup> With the change in diet and health, the composition of human gut microbiomes changes,<sup>[1,6]</sup> Wang et.al.<sup>[7]</sup> reported a systematic review, examining the preclinical and small human trials with certain commercially available strains of probiotic bacteria and identified those that had the most potential to be useful for central nervous system disorder.

The microbial composition of the gut microbiome varies across the digestive tract. In the stomach and small intestine, the presence of relatively few species of bacteria has been generally found.<sup>[6,8]</sup> In contrast, the highest microbial density in any habitat upon earth has been recorded.<sup>[9]</sup> with upto 10<sup>12</sup> cells per gram of intestinal content. These bacteria represent between 300 and 1000 different species.<sup>[6,8]</sup> However, 99% of bacteria come from 30 or 40 species. As a result of their abundance in the intestine, the bacteria also make up to 60% of dry mass of feces. The gut microbiome contains fungi, protists, archaea and viruses but less is known about their activities.<sup>[10]</sup>

Over 99% of the gut bacteria are anaerobes. It has been reported that these gut flora have around a hundred times as many genes in total as there are in human genome.<sup>[11]</sup> Overgrowth of bacteria in the small intestine can lead to intestinal failure. In addition the large intestine contains the largest bacterial ecosystem in the human body.<sup>[4]</sup> About 99% of the large intestine and feces flora are made up of obligate anaerobic such as Bifidobacterium.<sup>[12]</sup> The factors, that disrupt the microorganism population of the large intestine include antibiotics, stress and parasites.<sup>[4]</sup>

The human virome is mostly bacteriophages.<sup>[13]</sup>

Caesarean section, antibiotics and formula feeding may alter the gut microbiome composition.<sup>[14]</sup> The children treated with antibiotics have less stable, and less diverse floral communities.<sup>[15]</sup> Caesarean sections have been shown to be disruptive to mother offspring transmission of bacteria, which impacts overall health of the offspring by raising risks of disease, such as celiacs, asthma, and type, diabetes. This further evidences the importance of a healthy gut microbiomes.

The restoration are being explored, typically involving exposing the infant to maternal vaginal contents and oral probiotics.<sup>[15]</sup>

Sawhney<sup>[16]</sup> concluded on vitamin K deficiency as the precipitating factor for Homo sapien skin depigmentary disorder, which happens to Homo sapiens at the tropics. Under all circumstances bacterially enough vitamin K is biosynthesized in the body to meet the body's bio-demands excluding that of skin organ where melanolipoprotein pigment is biosynthesized.

Homo sapien skin de-pigmentary disorder (HES, leucoderma) among the human black race at the tropics has been the subject of attention to the scientific world. All attempts to understand its aetiology and treatment extended have failed. This skin condition has been registered as refractory. In the Hindu sacred book, Atharveda, the Homo sapien skin depigmentary disorder had been misinterpreted passing on misconceptions from generation to generation, which had/has delayed its resolution. Sawhney visited this subject again recently putting to rest all the age old misconceptions thereon and generating positive awareness of this skin condition in India and other countries at the tropics. In 1994 Sawhney<sup>[17]</sup> found melanin as a misnomer and coined it as melano- lipoprotein. In 2012 the same author<sup>[18]</sup> coined this skin condition scientifically as HES (hepto epidermal syndrome), disregarding earlier known nomenclature as leucoderma etc. and published the data in Nature Proceedings. In 2020 Sawhney<sup>[16]</sup> published data in wjpls on cracking Homo sapien skin pigmentary disorder, Homo sapien skin depigmentary disorder, and its attended skin cancer and their rehabilitation with Napthoquenone Theory. In 2022 the research paper

published by Sawhney<sup>[19]</sup> in wjpls helped conclude the nongenetic nature of Homo sapien depigmentary disorder.

Sawhney concluded on the vitamin K deficiency and the etiology of homo sapien depigmentary disorder, but could not assess the Homo sapien gut microbiome and its role in the biosynthesis of vitamin K, meeting out total organal bio-demands of Homo sapien body of vitamin K including that of skin organ. So this study.

## MATERIALS AND METHODS

This study has been done analytically. Four Homo sapien volunteers participated in this study, having different skin tones (skin dark, skin lightly dark, skin Wheatish, and skin white, hereafter to be read as Ind 223265A, Ind 223265B, Ind 223265C, Ind 223265D. Volunteers helped in the collection of the individual sterile fecal swab. Analysis of the 4 sterile fecal swabs was carried out using the RNA sequencing method and the 16S Metagenomics analyzer.

## RESULTS

The fecal samples of four volunteers (Ind 223265A, Ind 223265B, Ind 223265C, and Ind 223265D) analyzed, applying the RNA sequencing method and 16S Metagenomics Analyzer, have been found in fine state (54%, 52%, 55%, and 54.5% respectively) with details in Metagenomics Report 1. The Phylum Level/ Classes Level / Order Level / Families Level/ Genus Level- Taxonomy plot analysis are given in Metagenomics Reports 2-6.

The Dendrogram was constructed based on the distance measure of the Bray-Curtis index with a ward clustering algorithm (Metagenomics Report 7). The top 10 enriched genera, for Ind 223265A, Ind 223265B, Ind 223265C, and Ind 223265D are displayed in Metagenomics Reports; 8-11. Comparative analysis of these fecal samples: (Ind 223265A, Ind 223265B, Ind 223265C, and Ind 223265D) had been shown through Dendrogram construction (Metagenomics Report 12).

Comparative study data on the presence of vitamin K biosynthesizing bacteria in the gut microbiome of individual: Ind 223265A, Ind 223265B, Ind 223265C and Ind 223265D candidates has been given in Table 1.

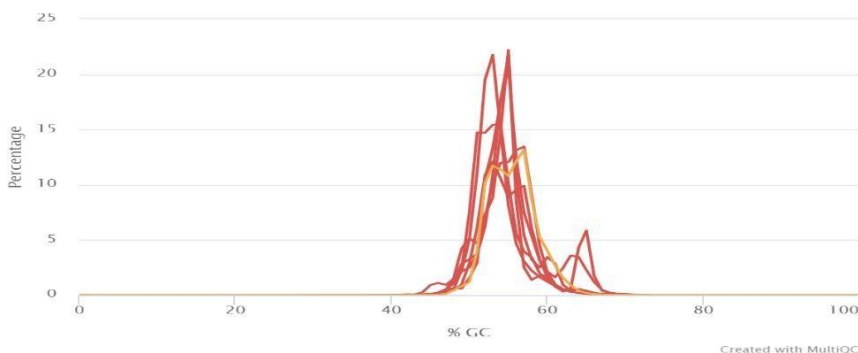
**Table 1: Comparative Study of Top 10 Vitamin K Biosynthesizing Bacteria in a Sterile Fecal Swab of Ind2223265A, Ind2223265B, Ind2223265C, and Ind2223265D Volunteers.**

Ind2223265A	Ind2223265B	Ind2223265C	Ind2223265D
<i>Serratia Lactobacillus</i>	<i>Serratia Lactobacillus</i>	<i>Serratia Lactobacillus</i>	<i>Serratia Lactobacillus</i>
		28%	56%
<i>Bifidobacterium</i>	<i>Bifidobacterium</i>	<i>Bifidobacterium</i>	<i>Bifidobacterium</i>
	1%	8%	14%
<i>Streptococcus</i>	<i>Streptococcus</i>	<i>Streptococcus</i>	<i>Streptococcus</i>
		19%	8%
<i>Bacillus</i>	<i>Bacillus</i>	<i>Bacillus</i>	<i>Bacillus</i>
<i>E.coli</i>	<i>E.coli</i>	<i>E.coli</i>	<i>E.coli</i>
<i>Flavobacterium</i>	<i>Flavobacterium</i>	<i>Flavobacterium</i>	<i>Flavobacterium</i>
17%			1%
<i>Enterococcus</i>	<i>Enterococcus</i>	<i>Enterococcus</i> 1%	<i>Enterococcus</i>
<i>Prevotella</i>	<i>Prevotella</i>	<i>Prevotella</i>	<i>Prevotella</i>
	73%		
<i>Clostridium</i>	<i>Clostridium</i>	<i>Clostridium</i>	<i>Clostridium</i>

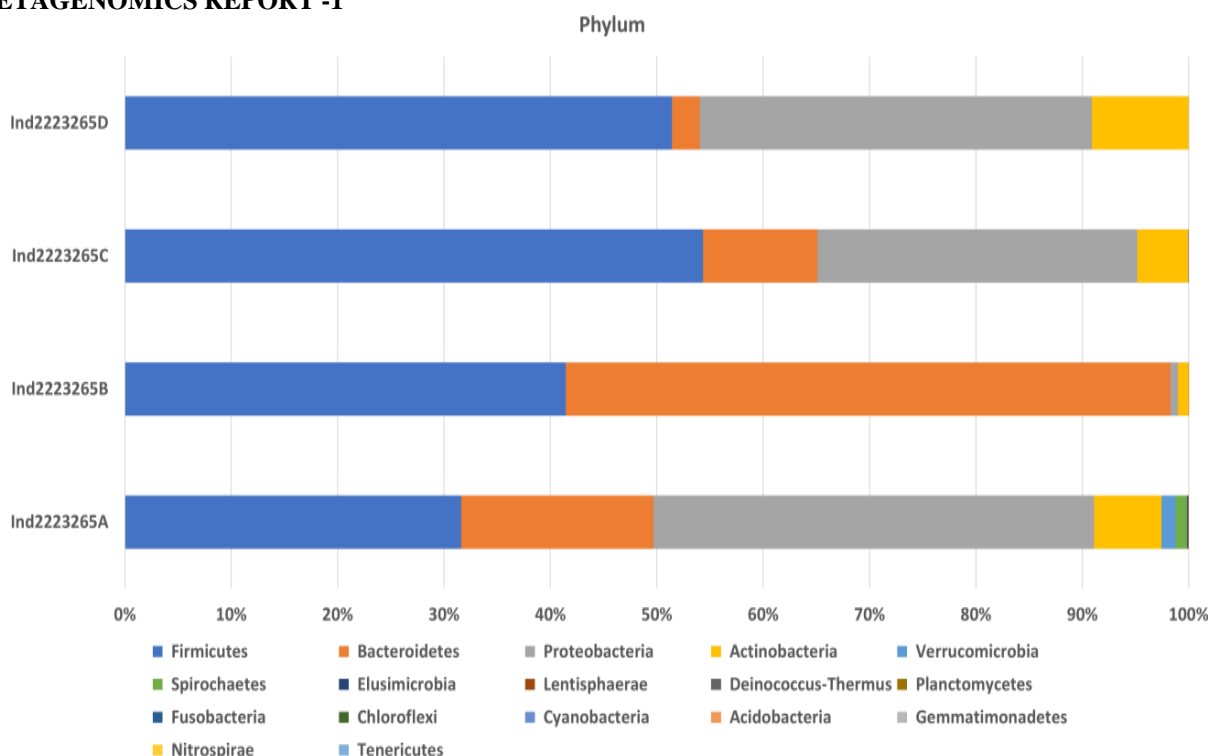
10%

The bacteria against which % has not been shown, display less than 1%

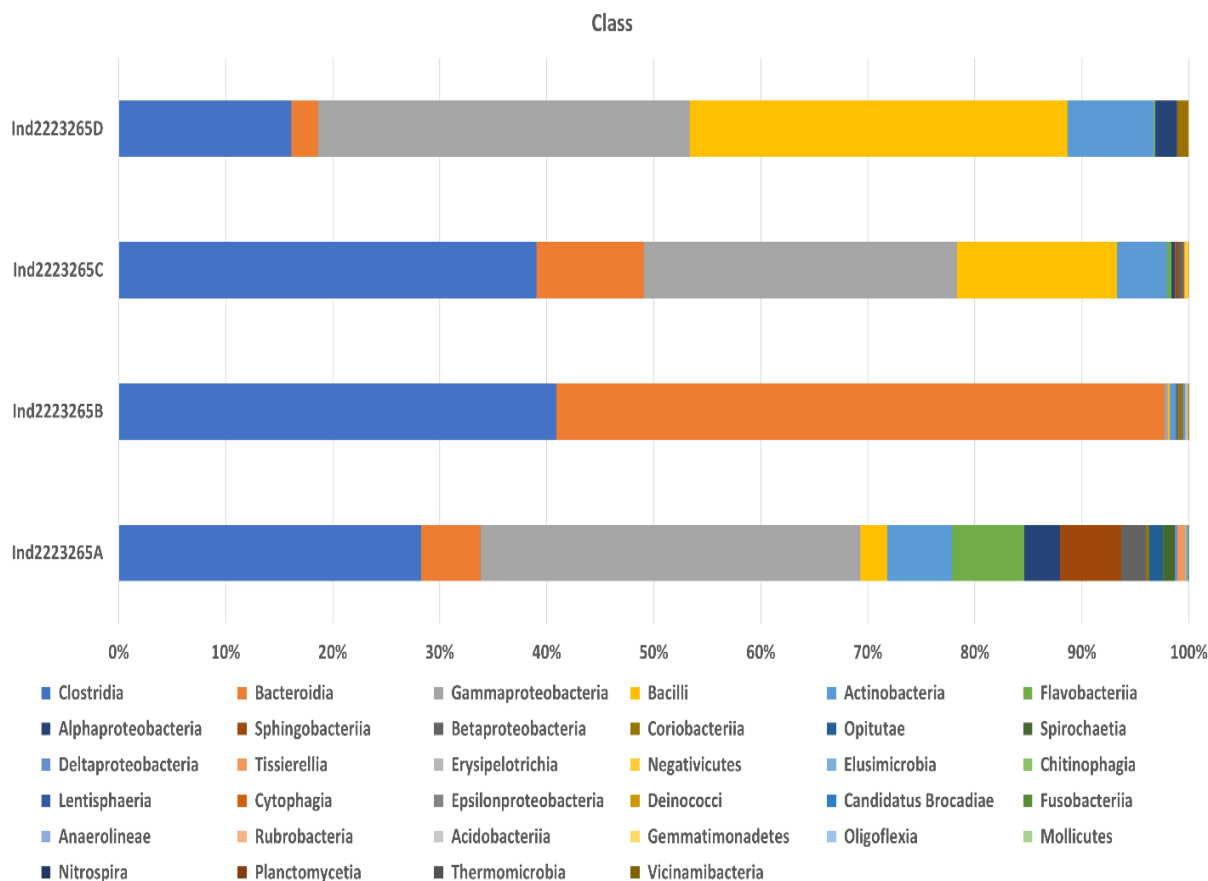
FastQC: Per Sequence GC Content



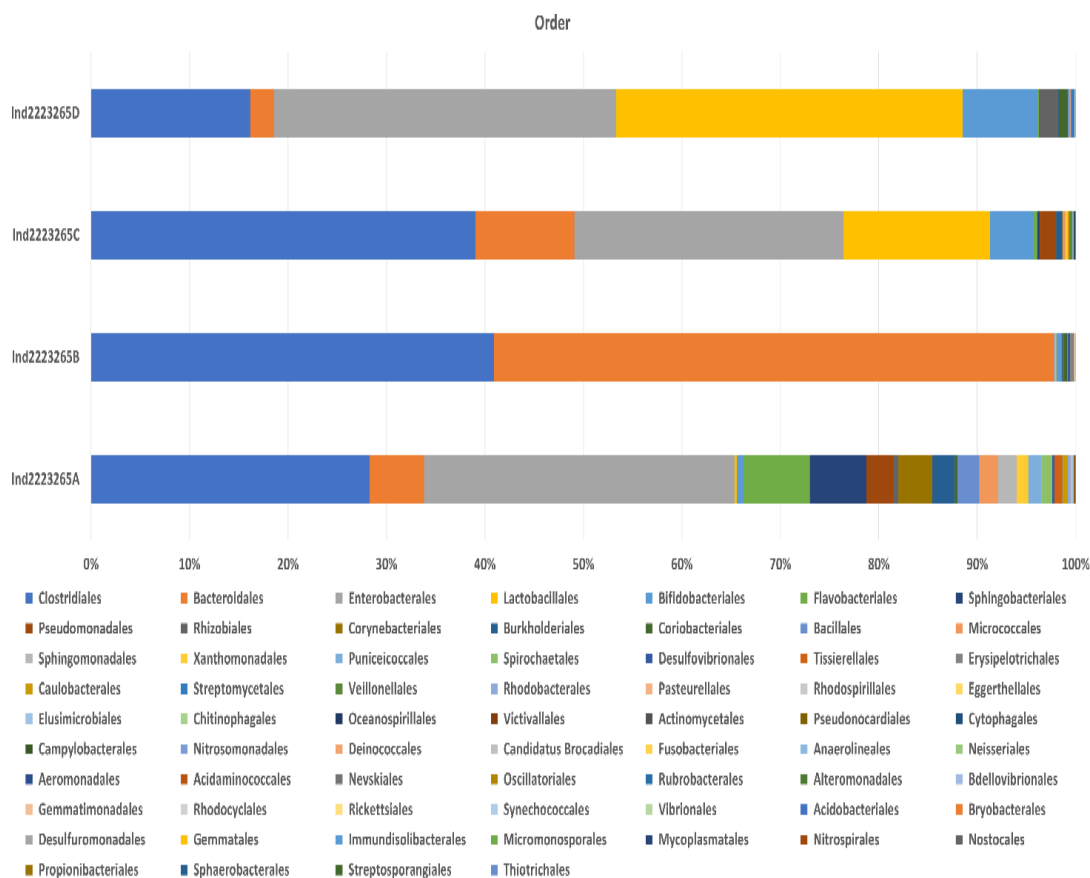
**METAGENOMICS REPORT -1**



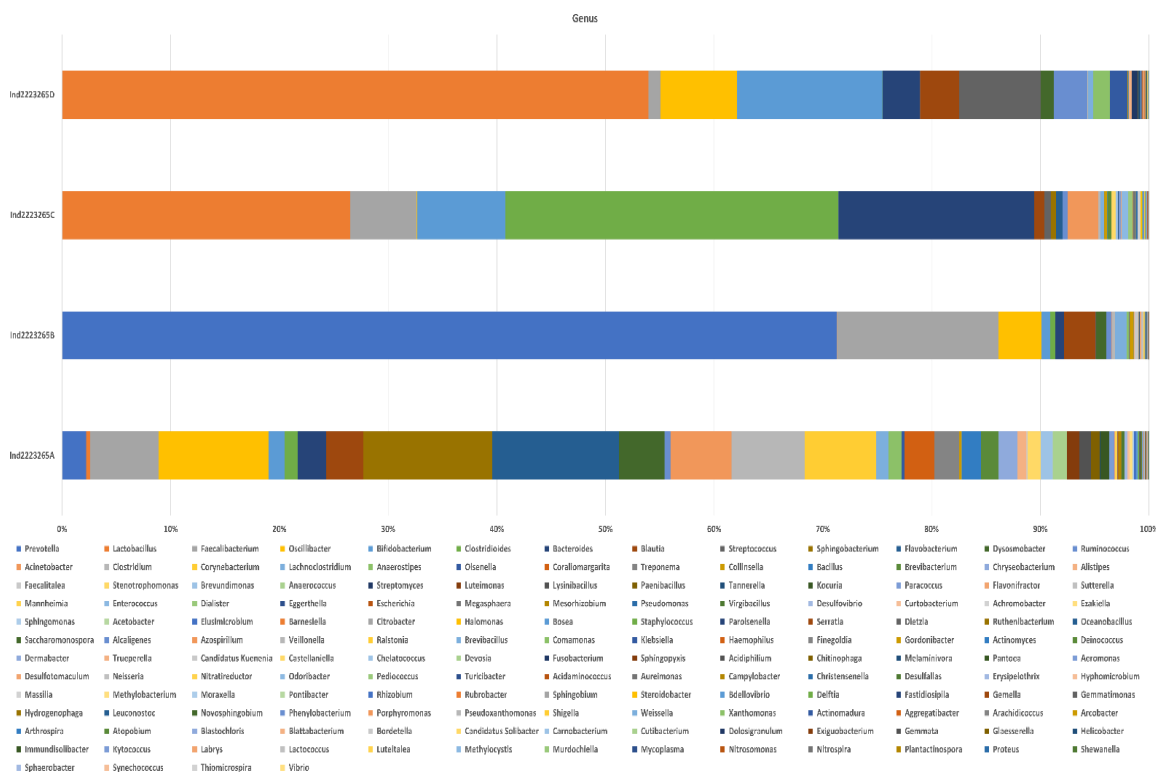
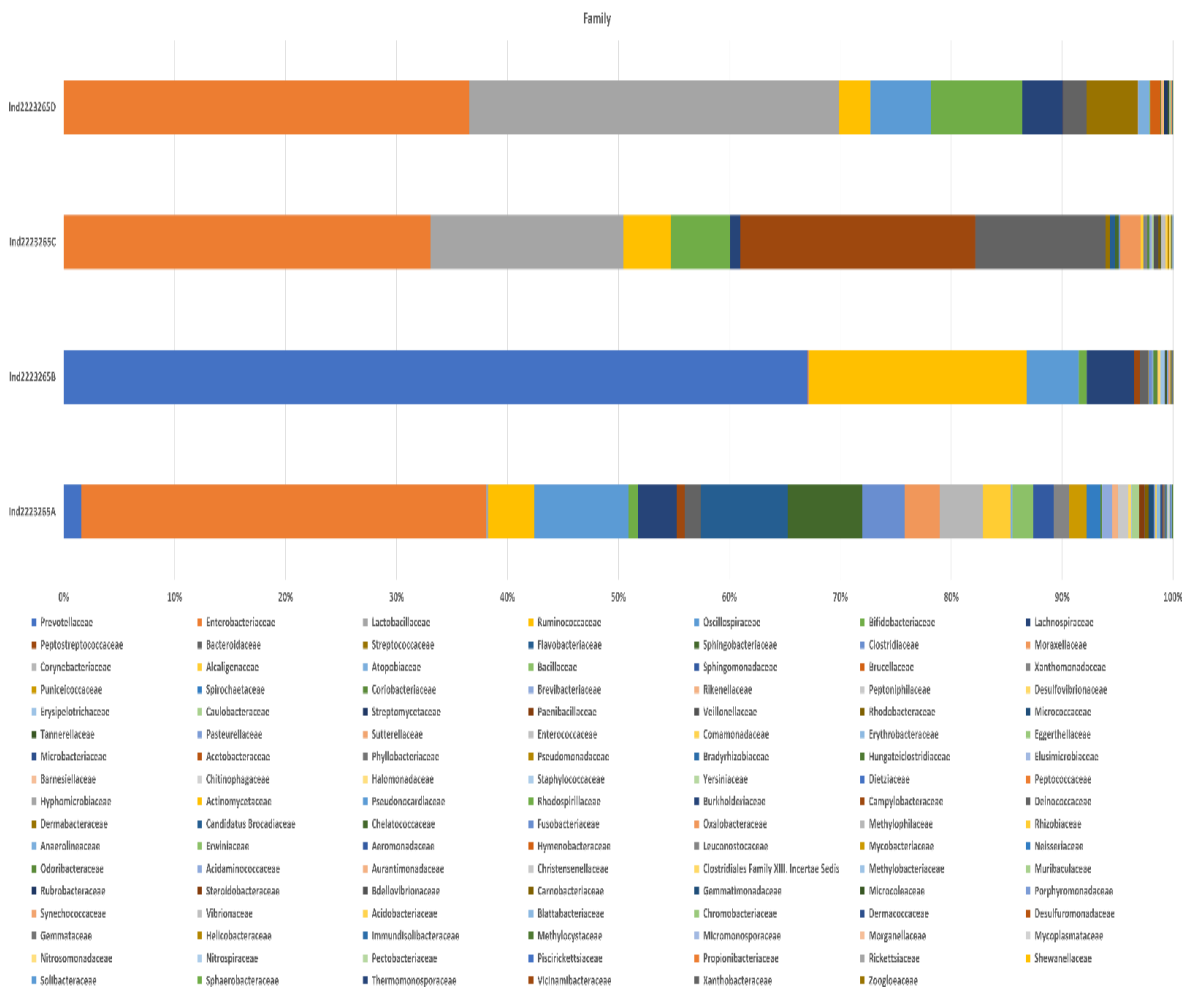
**Metagenomic Report-2: Phylum level- Taxonomy Plot Analysis.**

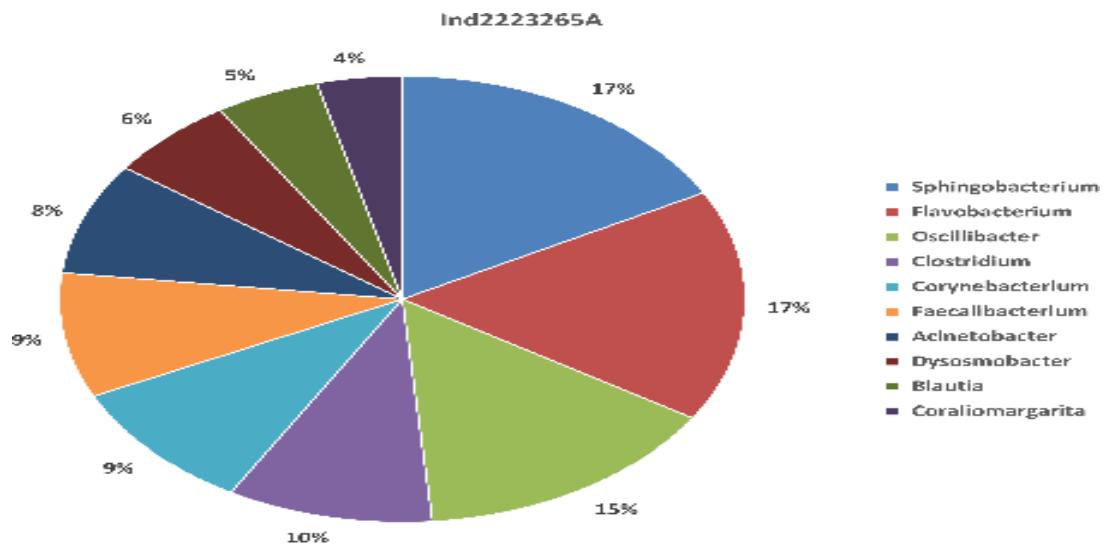
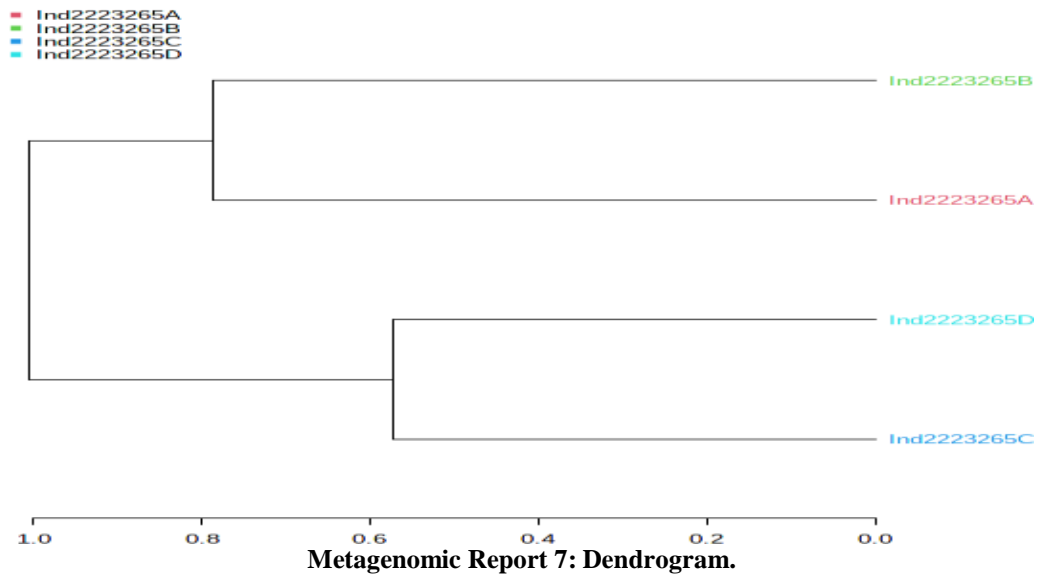


**Metagenomic Report -3: Classes level- Taxonomy Plot Analysis.**

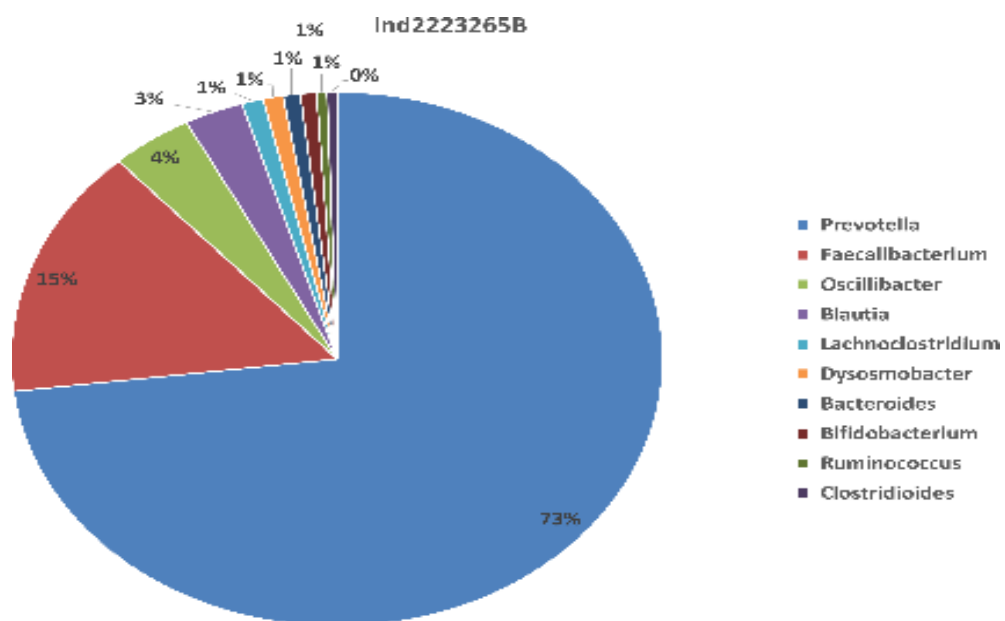


**Metagenomic Report-4: Order level- Taxonomy Plot Analysis.**

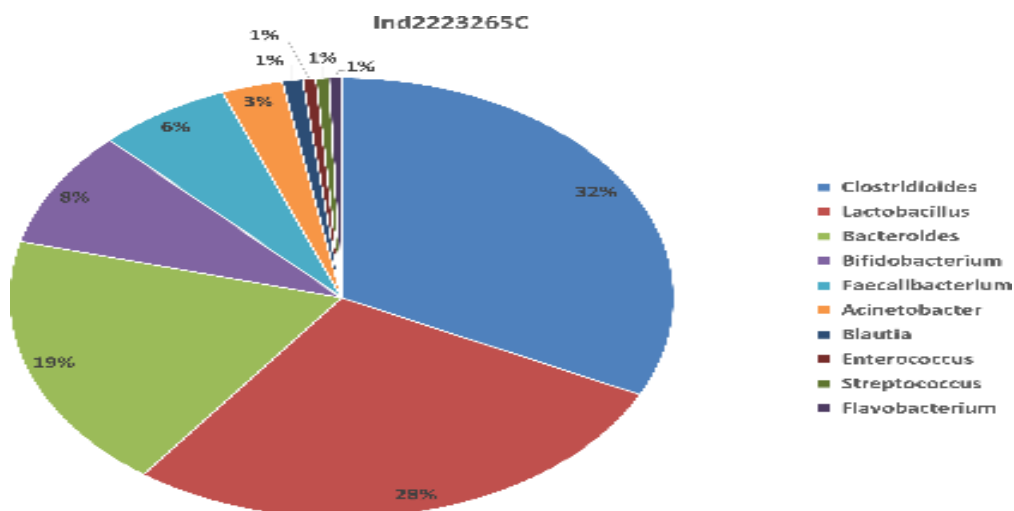




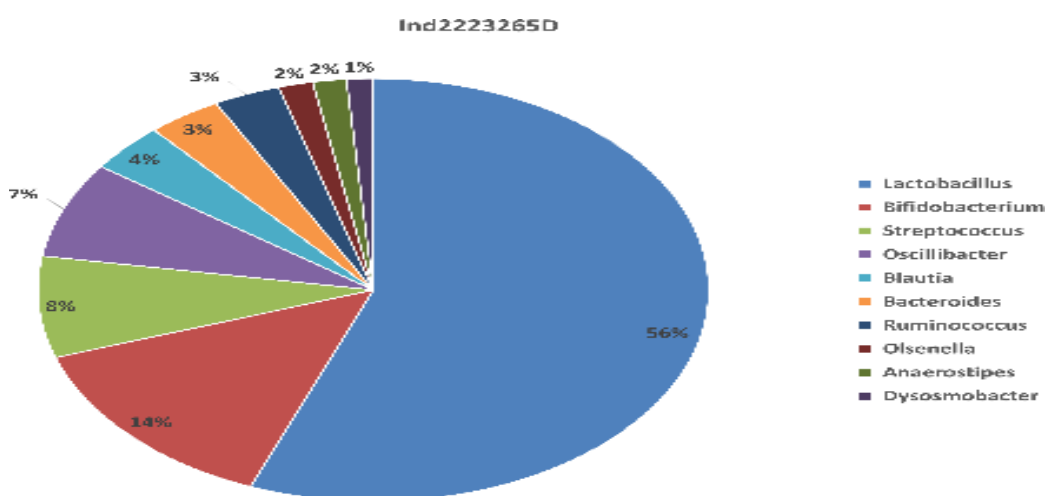
Metagenomic Report 8: Pie-chart on the bacterial population in a fecal swab of ind2223265A.



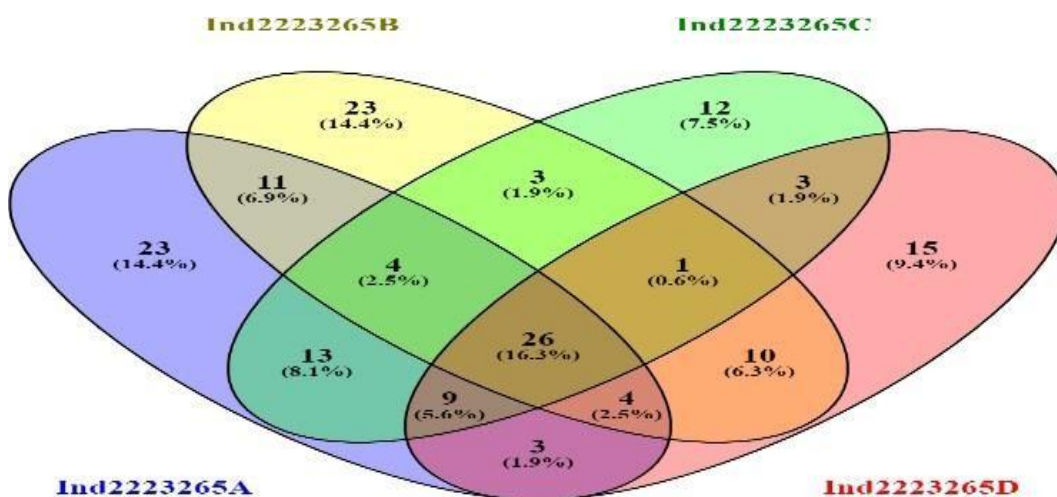
Metagenomic Report 9: Pie-chart on the bacterial population in a fecal swab of ind2223265B



Metagenomic Report10: Pie-chart on the bacterial population in a fecal swab of ind2223265C



Metagenomic Report 11: Pie chart on the bacterial population in a fecal swab of ind2223265D



Metagenomic Report12: Comparative analysis of ind2223265A, Ind 2223265B, Ind2223265B,Ind2223265C, Ind222326

**DISCUSSION**

The human gut microbiome consists of bacteria with defined duties under the principles of nature. This study is limited to vitamin K biosynthesizing bacteria and its role to meet the challenges of Homo sapien depigmentary

disorder. The defined and known biosynthesizing bacteria are; *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Enterococcus*, *E.coli*, *Bacteroides*, *Clostridium*, *Serratia*, *Prevotella*, and *Flavobacterium etc.* The vitamin K biosynthesizing bacteria in the gut



microbiome of individual fecal samples could be culled from the different Metagenomics Reports thereon. The Ind 2223265 A (skin dark), sample had 10 vitamin K biosynthesizing bacteria having less than 1% bacteria population with the exception of *Clostridium* (10%) and *Flavobacterium* (17%), whereas Ind 2223265 B (skin lightly dark) volunteer's gut microbiome had been found to contain vitamin K biosynthesizing bacteria, having for less than 1% numbers in gut microbiome, with the exception of *Bifidobacterium*, and *Prevotella* which had been found to have 1% and 73% bacteria respectively in gut microbiome. The other bacteria showing less than 1% bacteria were *Lactobacillus*, *Streptococcus*, *E.coli*, *Clostridium*, *Serratia*, and *Flavobacterium*. The complete absence of *Enterococcus* in the gut microbiome of Ind 2223265 B had been registered. The Ind 2223265 C volunteer with wheatish skin complexion displayed the presence of 10 gut microbiome, out of which 4 (*E.coli*, *Clostridium*, *Serratia*, and *Prevotella*) had been found having less than 1% numbers. The *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Enterococcus*, *Bacteroides*, and *Flavobacterium* had been found to have 28%, 1%, 8%, 1%, 19%, and 1% bacteria in the gut microbiome. In the Ind 2223265 D volunteer with skin white, only 3 bacteria: *Lactobacillus*, *Streptococcus*, and *Bacteroids* had been found to have vitamin K biosynthesizing capacity, having 56%, 8%, and 3%. The other bacteria, *Bifidobacterium*, *Enterococcus*, *E.coli*, *Clostridium*, *Prevotella*, and *Flavobacterium*, had far less than 1% of the population. Comparative list of vitamin K biosynthesizing bacteria in Ind 2223265 A, Ind 2223265 B, Ind 2223265 C and Ind 2223265D are shown in the Table 1.

Sawhney<sup>[16]</sup> concluded on vitamin K deficiency as the precipitating factor of Homo sapien skin depigmentary disorder (HES) in a research paper published in wjpls in 2022. Examination of the Table<sup>1</sup> showed that the best vitamin K biosynthesizing bacteria in Homo sapien's gut microbiome are *Clostridium* (10%), *Flavobacterium* (17%) (Ind 2223265 A with skin dark) and *Prevotella* 73% (Ind 2223265 B with skin lightly dark). The other bacteria varying in percentage in Ind 2223265 C and Ind 2223265 D cases appeared to be moderately contributing to vitamin K biosynthesis. The three bacteria: *Clostridium*, *Flavobacterium*, and *Prevotella* had been found absent in Ind 2223265D. In Ind 2223265C 1% *Flavobacterium* had been noticed. In conclusion, the vitamin K biosynthesizing bacteria assumed the following order. Ind 2223265A > Ind 2223265B > Ind 2223265C > Ind 2223265D. This means that vitamin K is biosynthesized in gut microbiome in all cases, though in varying volumes.

The Homo sapien body, under the laws of Nature, functions on the principles of priority. Vitamin K is one of the bioessentials required by the body very badly for body survival and functioning, without which the body could have collapsed irreversibly. The Ind 2223265 A gut microbiome especially with 10% *Clostridium* and

17% *Flavobacterium* biosynthesize vitamin K in line with the organal requirement including that of epidermal part of skin organ where relentless sun high-intensity UVB photoreacts with the epidermally defined threshold limits of vitamin K to trigger and migrate melanolipoprotein to the Homo sapien body's stratum granulosum (first layer of skin) whereas in Ind 2223265B the *Prevotella* (73%) appears to be main bacteria which biosynthesize enough vitamin K, meeting all the organal biodemands including that of skin organ and making it thus possible to pigment (light-dark) stratum granulosum. The Ind 2223265C with the absence of bacteria found in Ind 2223265A and Ind 2223265B could biosynthesize vitamin K, resulting in meeting organal biodemands including that of the skin organ, but causing less biosynthesis of melanolipoprotein and transfer to stratum granulosum. So Ind 2223265C assumed skin wheatish complexion. The Ind 2223265D showed the absence of the dominating vitamin K biosynthesizing bacteria as found in Ind 2223265A and Ind 2223265B, and had *Lactobacillus* (56%), *Streptococcus* (8%) and *Bacteriodes* (3%) as dominating bacteria in gut microbiome, which biosynthesize enough Vitamin K to meet out the biodemands of the whole body but that of skin organ, where biosynthesis of melanoprotein (pigment) occurs under the laws of nature. So Ind 2223265D assumed skin white (Homo sapien skin depigmentary disorder or HES as reported in Nature Proceeding by Sawhney). In line with Hindu mythology there are 8400000 ecosystems out of which the skin pigmentation of 8399999 are genetically defined and their offsprings are born with the defined skin pigmentation whereas the skin pigmentation of Homo sapien as concluded on by Sawhney, is biochemically defined. Homo sapien child is born with the constitutive light-toned coat, fully loaded epidermally with vitamin K threshold. Nature strategised exploiting a binary of epidermal vitamin K loaded epidermally at prenatal level in Homo sapien and relentless skin high intensity UVB, which has penetrating limit to epidermis, where the melanocytes and the cluster of skin layers (stratum basale, stratum spinosum and Stratum granulosum) are placed and established by nature. Both of which assume dormancy unless and until they are energized. The binary Vitamin K and the relentless sun high-intensity UVB photoreact as explained by Sawhney to produce OPE (offspring photon energy) with the inherent property to break the dormancy as said above, energizing melanocytes and three skin layers with the production of melanolipoprotein and its subsequent layer to layer migration to stratum granulosum, pigmenting it. As a corollary, the epidermally defined vitamin K is an essential bioessential to bring about skin pigmentary order in human, since relentless sun high-intensity UVB is constant at tropics. Nature conceptualized evolved and developed Homo sapiens species in Africa at tropics. Secondly, epidermal vitamin K deficiency causes Homo sapien skin depigmentary disorder among Homo sapiens at tropics.



Blood coagulation is essential in order to survive the Homo sapien body and its function. In absence of vitamin K, the coagulating factor, the Homo sapien shall collapse. The Homo sapien body works on the principle of priority. The vitamin K is produced bacterially only by Homo sapien gut microbiome. The body utilizes vitamin K first for blood coagulation and bone health etc, and then made available to the epidermis for manufacturing melanolipoprotein. In Ind2223265A, and Ind 2223265B, the high volume of vitamin K is biosynthesized by *Clostridium* (10%) and *Flavobacterium* (17%), and *Prevotella* (73%) respectively, meeting out biodeMANDs of the whole Homo sapien body including that of skin. So they have developed skin dark and skin lightly dark. In Ind 2223265C all the above, three bacteria are completely absent. The bacteria *Lactobacillus*: 26%, *Bifidobacterial* (8%), and *Bacteroids* 19% biosynthesize vitaminK in the gut moderately, satisfying the blood coagulation, etc, but the moderate supply of vitamin K to epidermis, causing to produce moderately melanolipoprotein, pigmenting stratum granulosum slightly. Thus Ind 2223265C developed a skin wheatish tone. In Ind2223265D, the presence of three vitamin K-producing bacteria: *Lactobacillus*; 56%, *Streptococcus*; 8% and *Bacteroids*; 3% which produce vitamin K sufficiently enough to satisfy blood coagulation conditions, etc, depriving off the epidermis of vitamin K supply. Thus Ind 2223265D assumed skin white (Homo sapien depigmentary disorder), To achieve Homo sapien pigmentary order over and above homo sapien depigmentary disorder as seen in Ind 2223265D, probiotics *Prevotella*, probiotics *Clostridium*, and probiotics *Flavobacterium*, which are far less than 1% in gut microbiome in this case, are the only option for high production of vitamin K bacterially. The study helped conclude on the absence of the strong vitamin K biosynthesizing bacteria in the candidate: Ind 2223265D (with skin depigmentary disorder) gut microbiomes, and agree to vitamin K deficiency as skin aetiology of skin depigmentary disorder (HES) in humans at tropics as said by Sawhney. The probiotics of strong vitamin K biosynthesizing bacteria; *Clostridium*, *Flavobacterium* and *Prevotella* etc are the only option to strength in Ind2223265D gut microbiome with these bacteria to effect the pigmentation of skin stratum granulosum. The Homo sapien gut microbiome registered decreasing number of strong vitamin K biosynthesizing bacteria with the reduction of the skin pigmentary loss of stratum granulosum as: Ind2223265A > Ind2223265B> Ind2223265C> Ind2223265D.

## CONCLUSION

The absence of strong vitamin K biosynthesizing bacteria such as *Clostridium*, *Flavobacterium* and *Prevotella* etc in the gut microbiome of the skin depigmentary disorder patient, confirms the theory of Sawhney on vitamin K deficiency as the precipitating factor for skin depigmentary disorder in humans at tropics. Only option to meet the vitamin K deficiency in humans with this skin condition is the probiotics of these three bacteria.

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