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## Study the microbial diversity in the human palm's skin

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### Abstract

In the present study estimation of microbial diversity in skin normal microflora is done. Total 8 isolates were purified from 2 samples (OW1, OW2, OW3 & OW4) (FW1, FW2, FW3 & FW4) on the basis of colony morphology. In office worker the shape of colony were observed Spindle (OW1 & OW3) and two isolates (OW2 & OW4) were irregular. In office worker the colony pigmentation two isolates (OW2, OW3) were whitish and (OW1) are yellow and OW4 are creamy yellow in colony pigmentation. In colony elevation two isolates (OW1, OW3) were flat and (OW2) isolate were raised (OW4) were filamentous in colony elevation. In colony margin two isolates (OW1, OW4) are entire and (OW2) isolate were crenate, and (OW3) were observed lobate in colony margin. In Field worker the shape of colony were observed three isolates are irregular (FW1, FW2 & FW3) and one isolate (FW4) were rhizoidal in colony shape. In Field worker the colony pigmentation were observed two isolates (FW2, FW4) are creamy and One isolate (FW1) are whitish and (FW3) are yellowish in colony pigmentation. In colony elevation (FW1) isolate were flat, (FW2) isolate were raised, (FW2) isolate were convex & (FW4) were filamentous in colony elevation. In colony margin two isolates (FW1, FW2) are entire and (FW4) isolate were filamentous, and (FW3) isolate lobate were observed in colony margin. Total 8 isolates were purified from 2 samples namely, office work, (OW1, OW2, OW3 & OW4) field work, (FW1, FW2, FW3 & FW4) From office worker palm's skin (OW1, OW3) isolates were negative & (OW2, OW4) were positive for gram staining and in field worker palm's skin (FW1, FW3) isolates were negative & (FW2, FW4) were positive for gram staining. Total 8 isolates were purified from 2 samples namely, office work, (OW1, OW2, OW3 & OW4) field work, (FW1, FW2, FW3 & FW4), From office worker palm's skin (OW1, OW2, OW3 & OW4) all the 4 isolates were positive for catalase test. From field worker palm's skin (FW1, FW2, FW3 & FW4) all the 4 isolates were positive for catalase test.

**Keywords:** Human, normal micro flora, diversity, biochemical attributes, preservation

### Introduction

Skin microflora, is called skin microbiota, (Society of microorganism) that resides on the derma typically human skin. These are the bacteria of which are around 1,000 species on human skin from nineteen phylum. These are found in the epidermis and the upper parts of hair follicles. Skin microflora is usually Incapable of causing disease, and that are not dangerous to their host or offer a benefit. Resident microbes is a reason for skin diseases and enter in the blood system, which produce life approaching diseases like skin cancer, especially in immunocompetent human. The derma is a setting for controlled bacterial extension. Derma hold up the extension of commensal bacteria, which safe from the host into dangerous bacteria. Surroundings and local factors, host immunity being attached and lethal are complex relate to dermal contamination. In order for bacteria to be harmful, they must be allowed to stick fast, extension on, and occupy the host. Bacteria take over the many virulence genes that permit for increased in these rich ideal position. The derma is a fence that invade the hike of dangerous bacteria. The cutaneous microbicidal protection tool include the mechanical unbending of the horny layer and its low moist content, horny layer lipids, produce of lysozyme, acidity (pH 5), and defensins (Harder *et al*, 1997) <sup>[45]</sup>. Especially, most areas of derma are dried, create an unfavorable climate for bacterial replication. Derma is cool than normal human body temperature and slightly acidic in nature most of the bacteria are grow best at a neutral pH and at 37 °C temperature. If an organisms can evade cutaneous host defenses, the next line of the protection involves the immune system, or skin-associated lymphoid tissue (SALT).

### Materials and Methods

The present investigation were conducted in the laboratory Division of Microbiology at Career Point University Hamirpur, Himachal Pradesh during the year 2021-2022.

## Isolation of normal microbial flora of skin

### Collection of sample of a palm's skin

The Palm's Skin used for isolation were collected from location of Lower West Central Outer Himalayan zone (Career Point University Campus Tikker-Kharwarian) from Hamirpur district of Himachal Pradesh, India. Sampling was done in the month of July of the year 2022. Sample were stored in a sterile container.

### Isolation of Palm's skin

Isolates were separate from the Palm's Skin sample by stepwise dilution and spread plate method using Nutrient Agar medium at 28 °C.

### Morphological identification of normal microbial flora of skin

The different isolates were identified on the basis of morphological characteristics according to the Bergey's manual of determinative bacteriology (Geetha *et al.*, 2014)<sup>[47]</sup>. Purification was done by streak plate method (Singh & Lal, 2016)<sup>[48]</sup>.

### Morphological characterization

Morphological characteristics of isolates including colony morphology (Pigment, shape, margin and elevation), Gram reaction were investigated.

### Pigment production

The production of pigments was checked on Nutrient agar at 28-30 °C after 24 to 48 hours.

## Purification of selected isolates for Normal Microbial flora of Skin

### Purification

The isolated colonies Palm's skin were randomly selected and purified by streaking. Streak plate is a technique used for the isolation into a pure culture of the organisms, from a mixed population. The inoculum was streaked on Nutrient agar plates and incubated at 37 °C for 24 hours (microbeonline.com).

## Biochemical characterization of selected isolates

### Microscopic Observation-(Gram's Staining)

Four different reagents in the order of Crystal violet, Gram's iodine, Alcohol/ Acetone destaining reagents and Safranin were used for gram staining.

### Biochemical Characterization (Catalase) (Jani, S.A. *et al*, 2014)<sup>[46]</sup>.

A drop of 3% hydrogen peroxide was added to 48 h old bacterial colony on a clean glass slide and mixed. The effervescence indicates catalase activity.

## Results and Discussion

### Isolation of Normal Microbial flora of Skin

#### Collection of Palm's skin

The Palm's Skin samples used for isolation were collected from location of Lower West Central Outer Himalayan zone (Career Point University Campus Tikker-Kharwarian) from Hamirpur district of Himachal Pradesh, India. Sampling was done in the month of July of the year 2022. Sample was stored in a sterile container.



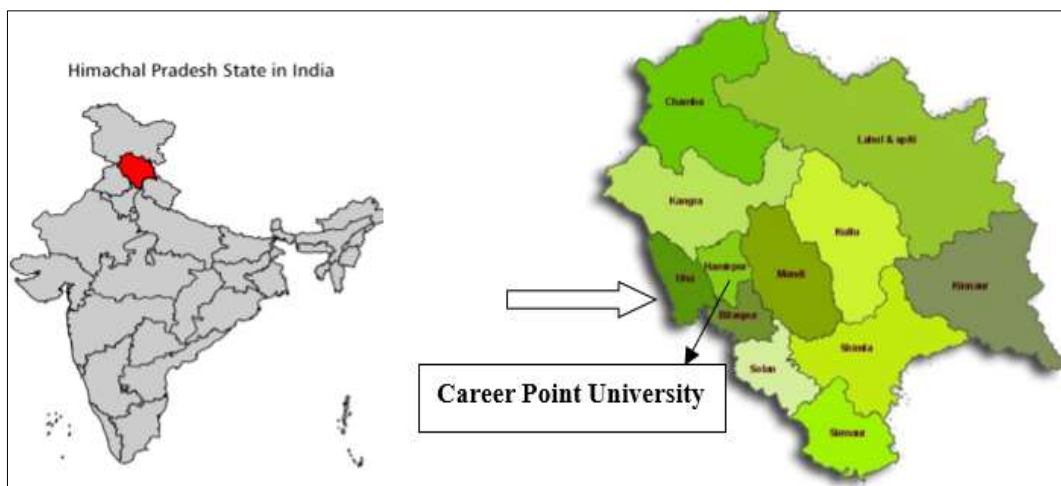
Name: Rattan Chand, Age: 72, Designation: Office boy, Sex: Male

**Plate 1:** Collection of palm's sample of office worker



Name: Bishan Dass, Age: 53, Designation: Gardner, Sex: Male

**Plate 2:** Collection of palm's sample of field worker human



**Fig 1:** Site of Sampling

**Isolation of Palm’s skin**

Isolates were isolated from the The Palm’s Skin by serial dilution and spread plate method using Nutrient Agar medium

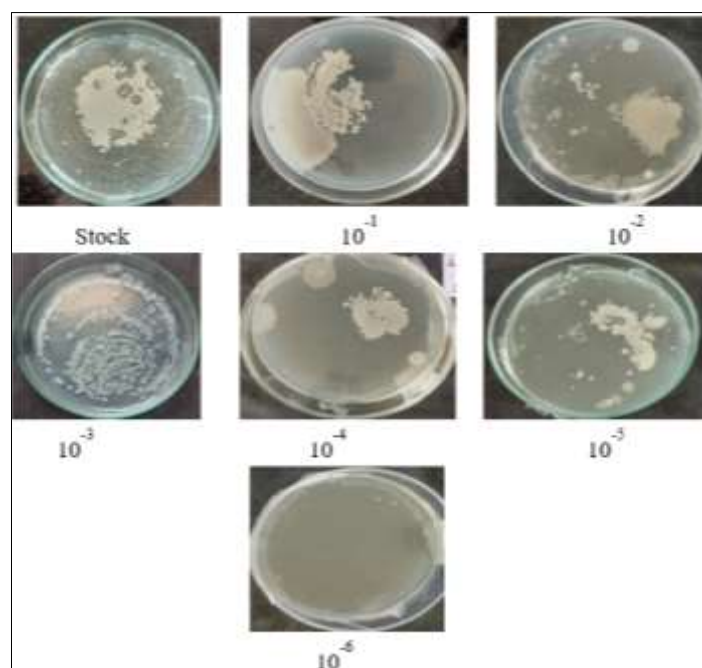
at 28 °C. at (Career Point University Campus Tikker-Kharwarian) from Hamirpur district of Himachal Pradesh, India.

**Table 1:** Microbial diversity from office worker palm’s skin sample

S.N.	Dilution factor	Colonies count	Cfu/ml
1	Stock	TMTC	-----
2	10 <sup>-1</sup>	250	2.50×10 <sup>-2</sup>
3	10 <sup>-2</sup>	220	2.20×10 <sup>-3</sup>
4	10 <sup>-3</sup>	190	1.90×10 <sup>-4</sup>
5	10 <sup>-4</sup>	140	1.40×10 <sup>-5</sup>
6	10 <sup>-5</sup>	80	8.0×10 <sup>-6</sup>
7	10 <sup>-6</sup>	20	2.0×10 <sup>-7</sup>

For the sample collection of Palm’s skin normal microflora one office Worker was selected. In palm’s skin sample of office worker 250 colonies were found in dilution factor 10<sup>-1</sup>

followed by 140 in 10<sup>-4</sup> and 20 colonies in 10<sup>-6</sup> dilution factor.



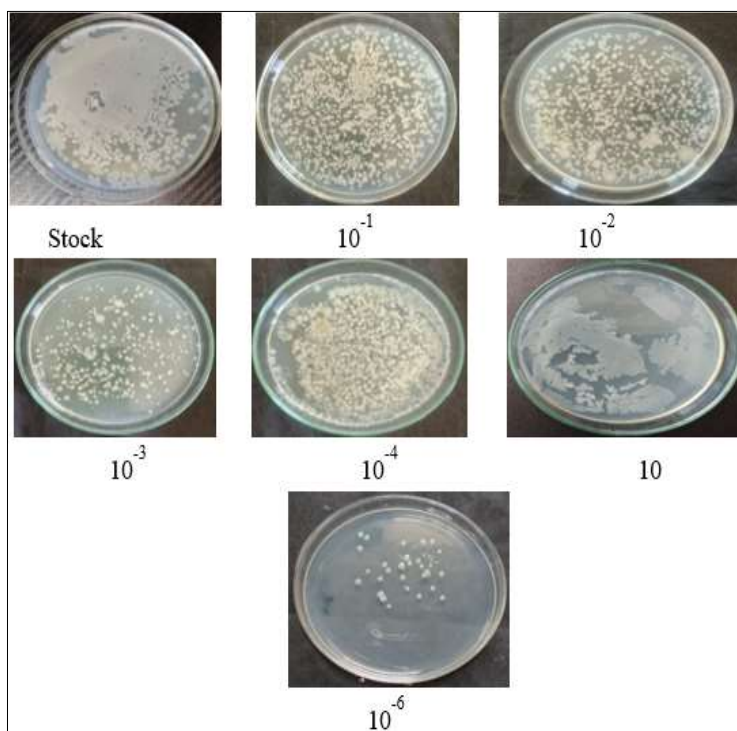
**Plate 3:** Microbial diversity in office worker palm’s skin sample

**Table 2:** Microbial diversity from field worker palm’s skin sample

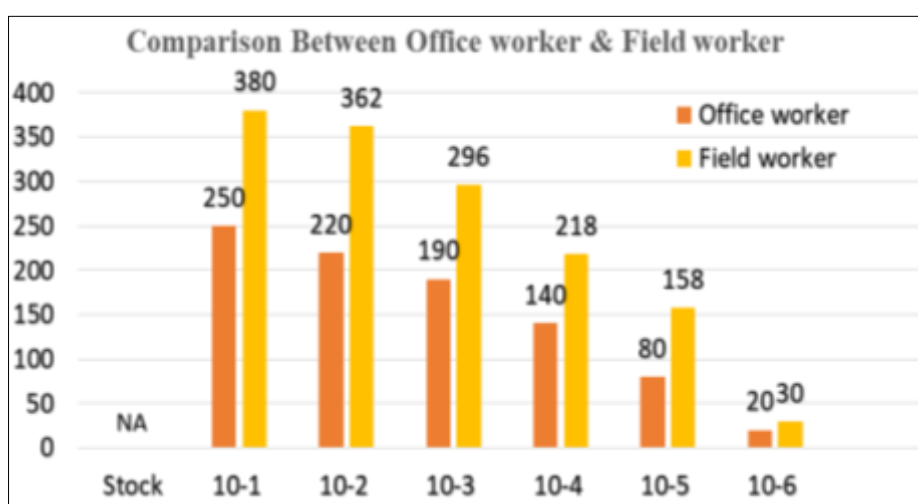
S.N.	Dilution factor	Colonies count	Cfu/ml
1	Stock	TMTC	-----
2	10 <sup>-1</sup>	380	3.80×10 <sup>-2</sup>
3	10 <sup>-2</sup>	362	3.62×10 <sup>-3</sup>
4	10 <sup>-3</sup>	296	2.96×10 <sup>-4</sup>
5	10 <sup>-4</sup>	218	2.18×10 <sup>-5</sup>
6	10 <sup>-5</sup>	158	1.58×10 <sup>-6</sup>
7	10 <sup>-6</sup>	30	3.0×10 <sup>-7</sup>

For the sample collection of Palm’s skin normal microflora one field Worker was selected. In the field worker palm’s skin

sample 380 colonies were found in dilution factor 10<sup>-1</sup> followed by 218 in 10<sup>-4</sup> and 30 colonies in 10<sup>-6</sup> dilution factor.



**Plate 4:** Microbial diversity in field worker palm’s skin sample



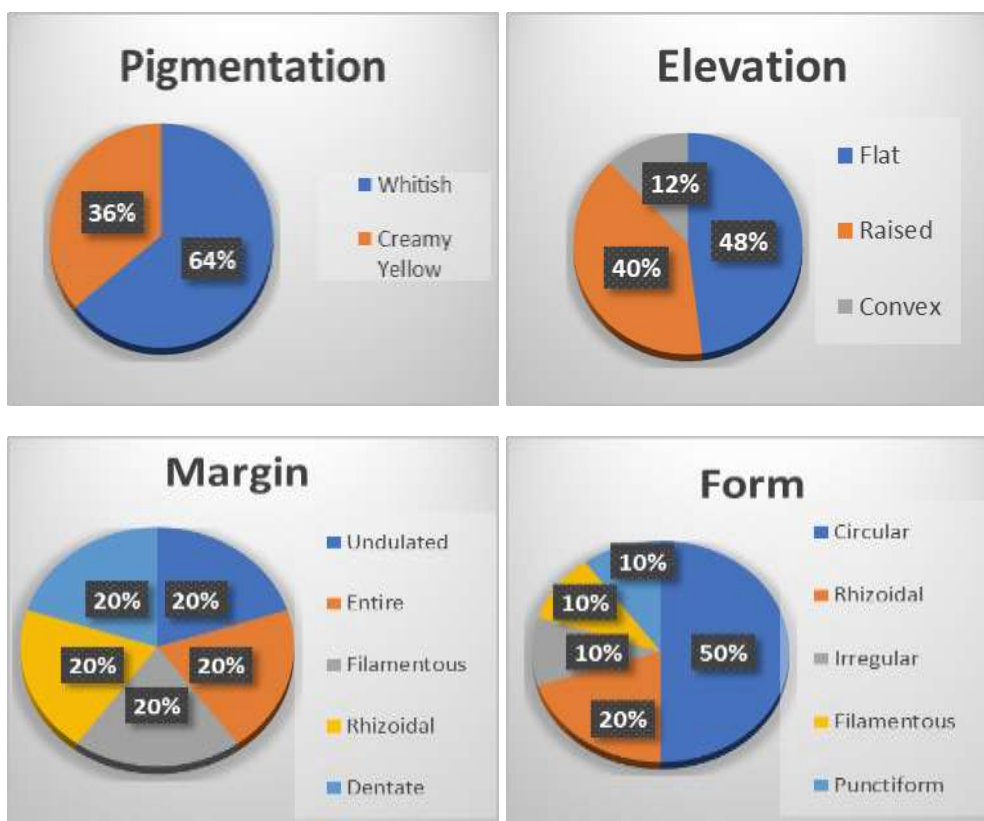
**Fig 2:** Morphological identification of Normal Microbial flora of Skin

In morphological identification of office worker palm’s skin sample based on their colony pigmentation which is 36% in creamy yellow & 64% in whitish, colony elevation which is 12% in convex, 48% in raised and 40% in flat, colony margin

which is 20% in each (i.e.) undulated, filamentous, rhizoidal & dentate and the colony form which is 10% in punctiform, 10% in irregular, 10% in filamentous 20% in rhizoidal and rest 50% are circular in form.

**Table 3:** Morphological identification of office worker Palm’s skin sample

S.N.	Dilution factor	Colony Pigmentation	Colony Elevation	Colony Margin	Colony Form
1	Stock	Creamy Yellowish, Whitish	Flat, Raised	Undulated	Circular, Rhizoidal
2	10 <sup>-1</sup>	Creamy Yellow	Flat	Entire, Undulated	Circular
3	10 <sup>-2</sup>	Whitish	Flat, Convex	Filamentous, Dentate	Rhizoidal, Irregular
4	10 <sup>-3</sup>	Whitish	Raised	Filamentous	Circular, Punctiform
5	10 <sup>-4</sup>	Creamy Yellow	Flat, Raised	Rhizoidal, Dentate	Circular, Rhizoidal
6	10 <sup>-5</sup>	Creamy Yellow	Raised, Convex	Rhizoidal	Filamentous, Circular
7	10 <sup>-6</sup>	Whitish	Flat	Entire	Punctiform



**Fig 3:** Morphological identification of office worker Palm’s skin sample

**Table 5:** Morphological identification of field worker Palm’s skin sample

S.N.	Dilution factor	Colony Pigmentation	Colony Elevation	Colony Margin	Colony Form
1	Stock	Yellowish, Whitish	Flat, Raised, Umbonate	Entire, Undulated	Circular, Irregular
2	10 <sup>-1</sup>	Yellow	Flat, Convex	Entire, Lobate	Circular, Punctiform
3	10 <sup>-2</sup>	Creamy Whitish, Light Yellow	Flat, Raised	Entire, Undulated	Circular, Irregular
4	10 <sup>-3</sup>	Yellowish	Flat, Raised	Filamentous, Entire	Rhizoidal, Irregular
5	10 <sup>-4</sup>	Yellowish, Creamy White	Crateriform	Entire, Crenate	Circular, Irregular, Filamentous
6	10 <sup>-5</sup>	Whitish	Flat, Convex	Rhizoidal, Lobate	Rhizoidal, Irregular
7	10 <sup>-6</sup>	Whitish	Flat	Entire	Circular, Punctiform

In morphological identification of field worker palm’s skin sample based on their colony pigmentation which is 35% in Whitish, 25% in creamy whitish, 25% in yellow & 15% in yellowish white, Colony elevation which is 10% in flat, 40% in raised and 50% in convex colony margin which is 15% in rhizoidal 20% in undulated 20% in entire 20% in dentate & 25% in filamentous and the colony form which is 10% in irregular 20% in rhizoidal 15% in filamentous 15% in punctiform and rest 40% are circular in form.

4.3 Purification of selected isolates for Normal Microbial flora of Skin & Throat sample the isolated colonies from Palm’s skin were randomly selected and purified by streaking. Streak plate is a technique used for the isolation into a pure culture of the organisms, from a mixed population. The inoculum was streaked on Nutrient agar plates and incubated at 37 °C for 24 hours. Total 8 isolates was purified from 2 samples namely, office work, (OW1, OW2, OW3 & OW4) field work, (FW1, FW2, FW3 & FW4).

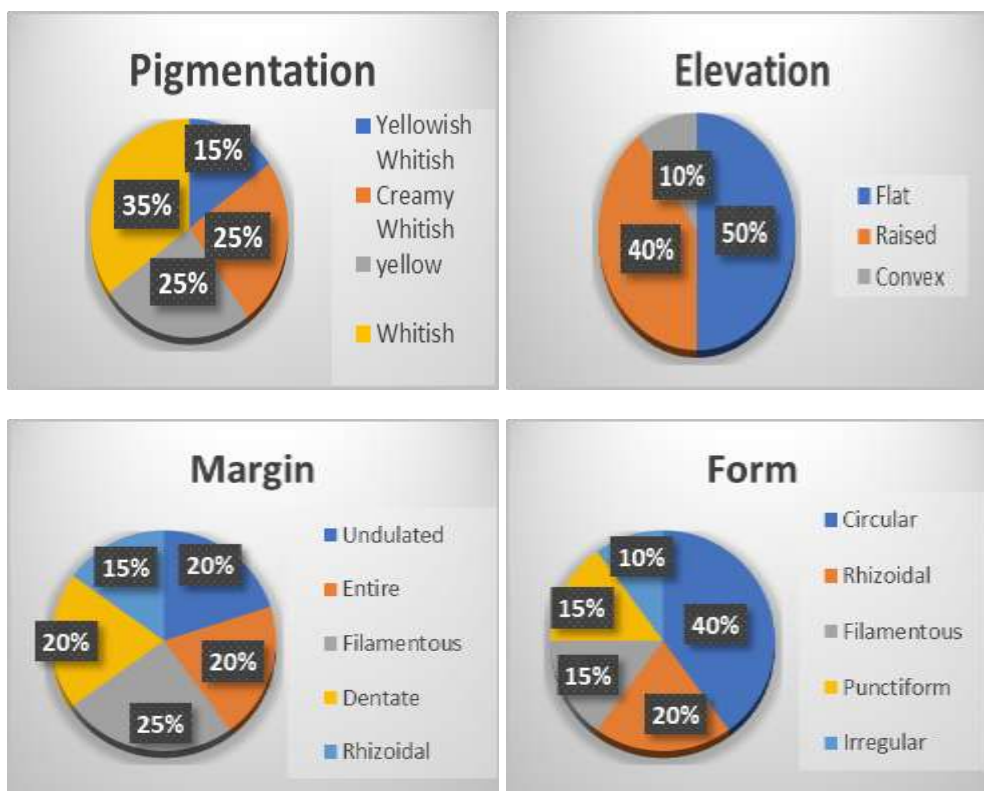


Fig 4: Morphological identification of field worker Palm's skin sample

Table 7: Purified isolate from office worker Palm's skin sample

S.N.	Dilution factor	Colony Pigmentation	Colony Elevation	Colony Margin	Colony Form
1	OW1	Yellow	Flat	Entire	Spindle
2	OW2	Whitish	Raised	Crenate	Irregular
3	OW3	Whitish	Flat	Lobate	Spindle
4	OW4	Creamy yellow	Filamentous	Entire	Rhizoidal

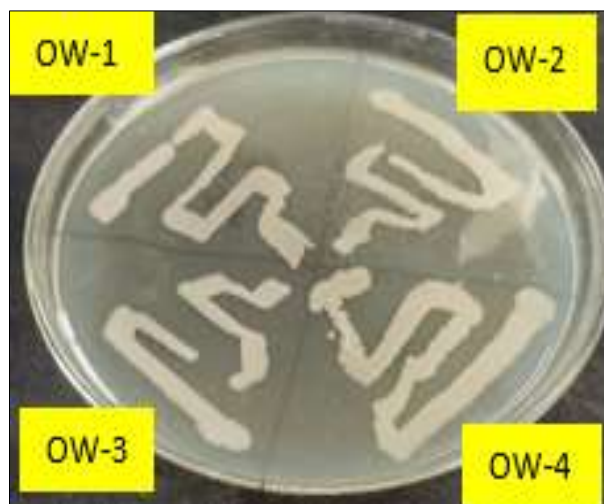
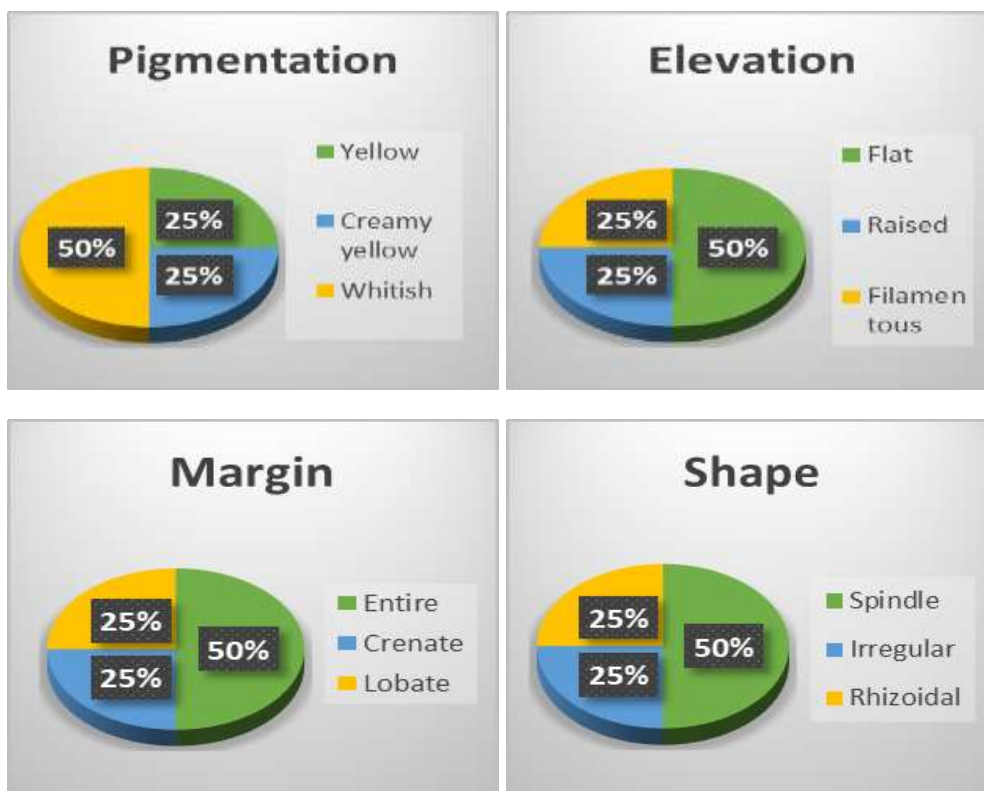


Plate 7: Purification of selected isolates from the office worker palm's skin

In Office worker the colony pigmentation were observed two isolates (OW2, OW3) are Whitish and one isolate (OW1) are Yellow and OW4 are Creamy Yellow in colony pigmentation. In colony elevation two isolates (OW1, OW3) were Flat and

(OW2) isolate were Raised (OW4) were filamentous in colony elevation. In colony margin two isolates (OW1, OW4) are entire and (OW2) isolate were Crenate, And (OW3) were observed Lobate In colony margin.



**Fig 6:** Purification of selected isolates from the office worker palm’s skin

**Table 8:** Purified isolate from Field worker Palm’s skin sample

S.N.	Dilution factor	Colony Pigmentation	Colony Elevation	Colony Margin	Colony Form
1	FW1	Whitish	Flat	Entire	Irregular
2	FW2	Creamy	Raised	Entire	Irregular
3	FW3	Yellowish	Convex	Lobate	Irregular
4	FW4	Creamy	Filamentous	Filamentous	Rhizoidal



**Plate 8:** Purification of selected isolates from the Field worker palm’s skin

In Field worker the shape of colony were observed three isolates are Irregular (FW1, FW2 & FW3) and one isolate (FW4) were Rhizoidal in colony shape. In Field worker the colony pigmentation were observed two isolates (FW2, FW4) are Creamy and one isolate (FW1) are Whitish and FW3 are Yellowish in colony pigmentation. In colony elevation (FW1) isolate were Flat, (FW2) isolate were Raised, (FW2) isolate were Convex & (FW4) were filamentous in colony elevation. In colony margin two isolates (FW1, FW2) are entire and

(FW4) isolate were Filamentous, and (FW3) isolate were observed in colony margin

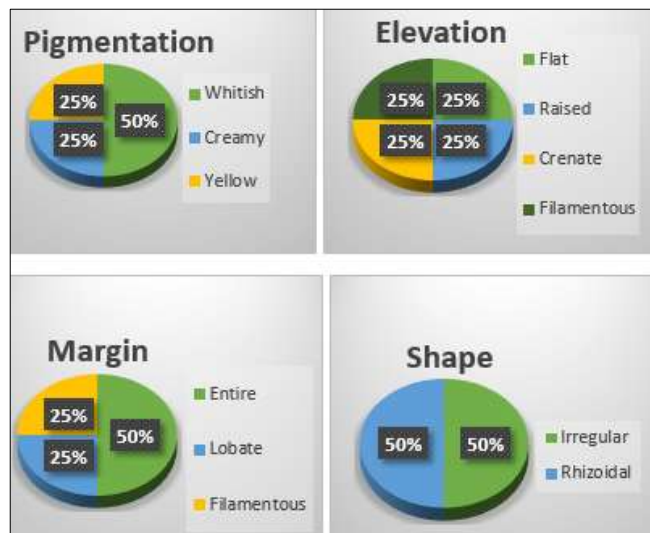
**Biochemical characterization of selected isolates**

**Microscopic Observation-(Gram’s Staining)**

Total 8 isolates were purified from 2 samples namely, office work, (OW1, OW2, OW3 & OW4) and field work, (FW1, FW2, FW3 & FW4).

**Biochemical Characterization (Catalase) (Jani, S.A. et al, 2014)**

A drop of 3% hydrogen peroxide was added to 48 h old bacterial colony on slide and mixed. This work shows catalase activity. Total 8 isolates were purified from 2 samples namely, office work, (OW1, OW2, OW3 & OW4) field work, (FW1, FW2, FW3 & FW4)

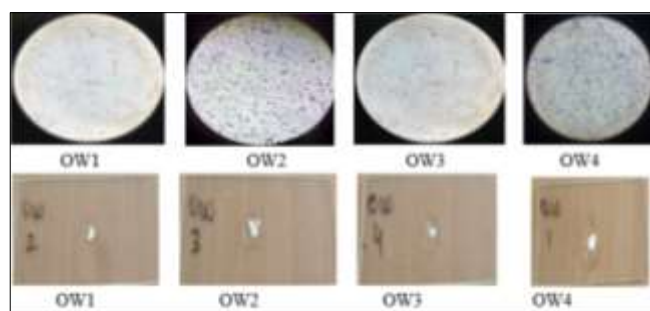


**Fig 7:** Purification of selected isolates from the Field worker palm's skin

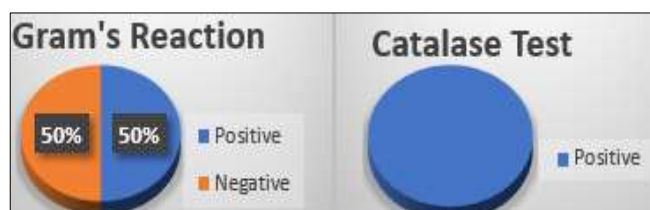
**Table 10:** Gram reaction and catalase test of selected isolates from office worker palm's skin sample

S.N.	Dilution factor	Catalase Test	Gram's Reaction
1	OW1	Positive	Negative
2	OW2	Positive	Positive
3	OW3	Positive	Negative
4	OW4	Positive	Positive

From office worker palm's skin (OW1, OW3) isolates were negative & (OW2, OW4) were positive for gram staining & all the 4 isolates were positive for catalase test



**Plate 10:** Gram reaction and catalase test of selected isolates from office worker palm's skin

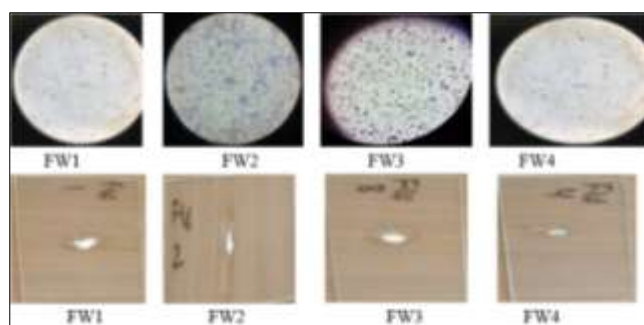


**Fig 9:** Gram reaction and catalase test of selected isolates from office worker palm's skin

**Table 11:** Gram reaction and catalase test of selected isolates from field worker palm's skin sample

S.N.	Dilution factor	Catalase Test	Gram's Reaction
1	FW1	Positive	Negative
2	FW2	Positive	Positive
3	FW3	Positive	Negative
4	FW4	Positive	Positive

In field worker palm's skin (FW1, FW3) isolates were negative & (FW2, FW4) were positive for gram staining & all the 4 isolates were positive for catalase test.



**Plate 11:** Gram reaction and catalase test of selected isolates from Field worker palm's skin



**Fig 10:** Gram reaction and catalase test of selected isolates from Field worker palm's skin

**Conclusion**

- In palm's skin sample of office worker 250 colonies were found in dilution factor 10-1 followed by 140 in 10-4 and 20 colonies in 10-6 dilution factor. In the field worker palm's skin sample 380 colonies were found in dilution factor 10-1 followed by 218 in 10-4 and 30 colonies in 10-6 dilution factor.
- Total 8 isolates were purified from 2 samples namely, office work, (OW1, OW2, OW3 & OW4) field work, (FW1, FW2, FW3 & FW4) on the basis of colony morphology.
- In Office worker the shape of colony were observed two isolates are Spindle (OW1 & OW3) and two isolates (OW2 & OW4) were Irregular in colony shape.
- In Office worker the colony pigmentation was observed two isolates (OW2, OW3) are Whitish and one isolate (OW1) are Yellow and (OW4) are Creamy Yellow in colony pigmentation.
- In colony elevation two isolates (OW1, OW3) were Flat and (OW2) isolate were Raised (OW4) were filamentous in colony elevation.
- In colony margin two isolates (OW1, OW4) are entire and (OW2) isolate were Crenate, And (OW3) were observed Lobate in colony margin.
- In Field worker the shape of colony were observed three isolates are Irregular (FW1, FW2 & FW3) and one isolate (FW4) were Rhizoidal in colony shape.



- In Field worker the colony pigmentation was observed two isolates (FW2, FW4) are Creamy and one isolate (FW1) are Whitish and (FW3) are Yellowish in colony pigmentation.
- In colony elevation (FW1) isolate were Flat, (FW2) isolate were Raised, (FW2) isolate were Convex & (FW4) were filamentous in colony elevation.
- In colony margin two isolates (FW1, FW2) are entire and (FW4) isolate were Filamentous, And (FW3) isolate lobate were observed in colony margin.
- Total 8 isolates were purified from 2 samples namely, office work, (OW1, OW2, OW3 & OW4) field work, (FW1, FW2, FW3 & FW4)
- From office worker palm's skin all the 4 isolates were positive for catalase test and in field worker palm's skin also all the 4 isolates were positive for catalase test
- From office worker palm's skin (OW1, OW3) isolates were negative & (OW2, OW4) were positive for gram staining and in field worker palm's skin FW1, FW3 isolates were negative & (FW2, FW4) were positive for gram staining
- From the current study it is concluded that the microbial load were found more in the field worker as compare to office worker palm's skin sample.

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