Journal of Biotechnological Research

Vol. 1, No. 1, 22-36, 2016

Isolated and Identification of Some Isolated Bacterium from Human in Egypt

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ABSTRACT

Microbes are an often overlooked part of an ecosystem because of their small size and lack of morphological characters; however their presence is vital for every kind of known life. The human microflora is an essential and play very important role in health and disease. This study was done for isolates and identifies some samples from deferent section in the human body from Egypt. Twenty samples were collected as following: Five saliva samples of 3 children school volunteers aged (4-10 years) were collected from 3 schools in Alexandria Governorate, Egypt and 2 adult samples aged (22,30 years) were collected randomly. Five feces samples of infant babies aged (3-6 months).Ten teeth samples were collected from adult teeth roots. From our results 42 predominant colonies morphology isolated on MRS agar, BHI agar, MSA and SF agar from 12, 16 and 14 samples of saliva, teeth and faeces respectively. This found to be 24 cocci cells, 14 rods cells and 4 yeasts cells. These bacteria were identified as: 1 Staph.lentus; 5 Staph.xylosus; 1 B. brevis; 3 Lactobacillus acidophilus; 2 Lactobacillus plantarum; 8 Bacillus sp. and 18 Ec.faecalis

Keywords: Human microflora, *Lactobacillus acidophilus, Ec.faecalis, B. brevis.*

DOI: 10.20448/805.1.1.22.36

Citation | Fawzia M. A. El- Sakhawy; Ali Abd. El-L. Khattab; Hoda Mahrous; Atef M. Ibrahim (2016). Isolated and Identification of Some Isolated Bacterium from Human in Egypt. Journal of Biotechnological Research, 1(1): 22-36.

Funding : This study received no specific financial support.

Competing Interests: The authors declare that they have no competing interests.

History : **Received:** 9 June 2016/ **Revised:** 23 June 2016/ **Accepted:** 28 June 2016/ **Published:** 2 July 2016

Publisher: Online Science Publishing

1. INTRODUCTION

The number of microbial species that exists on the earth today is unknown, and classifying them becomes a massive project because of their small size, a traditional requirement for colony isolation, a requirement for a microbiological or biochemical biotype identification, and large diversity. Molecular techniques have yielded estimates that less than 10% of the organisms in an ecosystem have been identified using traditional methods [11]. In the 19th century the development of pure culture techniques that allowed scientists to isolate and characterize prokaryotic organisms were developed by Robert Koch who stipulated that pure cultures of the pathogenic organism must be isolated from its host and grown in pure culture [11].

The midgut, in contrast, showed quite a bit of microbial density. Approximately 10^8 colonies per midgut were counted when incubated aerobically and 3 x 10^8 colonies were isolated when incubated anaerobically. A dilution series isolated on medium containing carboxymethyl cellulose demonstrated that the midgut of both roach species contained 10^6 bacteria that grew under aerobic conditions and 107 that grew anaerobically. The most common microbes that were isolated from the midgut included anaerobes, *Enterobacter agglomerans* and *Klebsiella oxytoca*, and *Citrobacter freundii*. Electron microscopic examination of the midgut also showed that several types of insects harbor bacteria between the peritrophic membrane and midgut epithelium with occasional protozoa and spirochetes also present [2].

The characteristics of mouth are ecologically distinct from all other surfaces of the body and determine the types of microbes that are able to persist, so that not all of the microorganisms that enter the mouth are able to colonize. Moreover, distinct habitat exists even within the mouth, each of which will support the growth of a particular microbial community because of their precise biological features. Habitats with obviously different ecological conditions include mucosal surfaces (such as lips, cheeks, palate, and tongue) and teeth. The properties of mouth as a microbial habitat are dynamic and will change during the life of an individual [15]. The oral cavity is potentially susceptible to a variety of infections due to the presence of numerous proliferative microorganisms that cause fungal, viral and bacterial infections [17]. Dental caries can be characterized as a multifactorial infection directly related to three main factors: Microflora, substrate and host. Some carious processes may develop to the point where dental pulp is affected. Microbial involvement has been indicated as the main factor associated with the etiology of endodontic diseases [20].

The human GI microbiota is considered a tissue, not an organ, and is used in FMT to implant in a recipient's GI tract. To understand the utility of FMT, it is first necessary to appreciate the compositional complexity of the GI microbiota, along with its associated functional implications. There are about 10^{14} bacterial cells in our body and most of these bacterial cells reside in the GI tract. Only about 30 %of the GI microbiota is detectable by culture-based techniques [6].

The present study isolates and identifies some deferent bacteria from deferent section in the human body from Egypt.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Culture media

2.1.1.1. De Man, Rogosa, and Sharp (M.R.S.)

M.R.S. agar medium was prepared according to De Man, et al, [3] having following composition per liter:

Peptone	10 gm
Lab-lemco powder	8 gm
Dextrose	20 gm
Yeast extract	4 gm
Tween 80	1 ml
Dipotassium hydrogen phosphate	2 gm
Sodium acetate.3H2O	5 gm
Triammonium citrate	2 gm

Magnesium sulphate.7H2O	0.2 gm
Manganese sulphate.4H2O	0.05 gm
Distilled water to	1000 ml

pH 6.2

2.1.1.2. Violet Red Bile agar (V.R.B.A)

V.R.B.A. medium was prepared as described in Difco"s Manual, [4]. The composition of the medium is:

Yeast extract	3 gm
Peptone	7 gm
Bile salts No.3	1.5 gm
Lactose	10 gm
Sodium chloride	5 gm
Agar	15 gm
Neutral red	0.03 gm
Crystal violet	0.002 gm
Distilled water to	1000 ml

pH 7.4

2.1.1.3. Mannitol Salt Agar (M.S.A)

M.S.A medium was prepared according to Difco"s Manual, [4] having following composition per liter:

Beef extract	1 gm
Proteose peptone No.3	10 gm
Sodium chloride	75 gm
D-Mannitol	10 gm
Agar	15 gm
Phenol red	0.025 gm
Distilled water to	1000 ml

PH 7.4

2.1.1.4. Brain Heart Infusion Agar (B.H.I.A):

B.H.I.A was obtained from Difco"s Manual, [4]. Having the following composition:

Calf brain	200.0 gm
Beef heart	250.0 gm
Proteose peptone	10.0 gm
Dextrose	2.00 gm
Sodium chloride	5.00 gm
Disodium phosphate	2.50 gm

For propagation of phathogenic cocci and other fastidious organisms associated with blood culture work and allied pathological investigations

2.1.1.5. Plate Count Agar Media (P.C.A)

P.C.A media was obtained from Difco"s Manual, [4] as the following composition:

Beef extract	3 gm
Peptone	5 gm
Yeast extract	2.5 gm
Tryptone	5 gm
Dextrose	1 gm
Agar	15 gm

PH 7

2.1.1.6. Streptococcus Faecalis Agar Medium (S.F)

S.F agar media was obtained as described in Difco"s Manual, [4] as the following composition:

Tryptone	20 gm
Glucose	5 gm
Dipotassium phosphate	4 gm
Monopotassium phosphate	1.5 gm
Sodium azaid	0.5 gm
Sodium chloride	5 gm
Bromocresol purple	0.032 gm

PH 6.9

2.2. Methods

2.2.1. Collection of Samples

Twenty samples were collected as following:

- Five saliva samples of 3 children school volanteers aged (4-10 years) were collected from 3 schools in Alexandria Governorate,Egypt and 2 adult samples aged (22,30 years) were collected randomly.
- Five feces samples of infant babies aged (3-6 months).
- Ten teeth samples were collected from adult teeth roots.
- The mentiond samples were collected along one year (2011-2012). All samples were collected in wide mouth sterile (100 ml) containers aseptically, labeled and immediately transported to the laboratory in an ice-box where they were processed immediately for:

2.2.1.1. Isolation of Some Microbial Groups

A loop of each sample was streaked on MRS Agar, Brain heart infusion agar , S.F.A , M.S.A and V.R.B.A according to Difco's Manual for determinative microbiology, then cultured at aerobic and anaerobic conditions at 37ºC for 48hrs. Abredomenant colonies were purified and examined for some bacteriological tests included gram stain, motility, catalase test, salt tolerance, oxidase test.

2.2.1.2. Microbiological Identification Tests

Representative colonies isolated throughout the present investigation were purified, kept on slants and examined microscopically using Gram staining (Hucker's modification).

Isolates were also tested for their ability to produce catalase.

Accordingly, the isolates were grouped into:

1- Gram positive catalase negative coccoids in chains. No further attempts were, however, considered as belonging to either the Genus Streptococcus or the Genus *Leuconostoc*.
2- Gram positive catalase positive coccoids. To differentiate between members of the Genus Micrococcus and that of *Staphylococcus*, the test for the ability to produce acid from glucose under various cultural conditions was used.
3- Gram positive, catalase positive rods.
4- Gram positive, catalase negative rods. With isolates belonging to the above mentioned two groups no identification tests were performed.
5- Gram negative catalase positive rods.

Strains that were found able to ferment lactose with the production of acid and gas were subjected to the four conventional IMVIC tests.

2.2.1.2.1. Gram Staining

It is a complex staining method to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls, according to [1].

2.2.1.2.2. Catalase Test

To detect catalase activity, a drop of broth culture was transferred onto a clean slide, flooded with a drop of H_2O_2 solution 30% and observed for the production of effervescence [9].

2.2.1.2.3. Salt Tolerance

Salt tolerance test is particularly useful for presumptive identification of enterococci group D organisms, which have the specific ability to grow in the presence of 6.5% NaCl incorporated into a specific broth culture media.

Brain heart infusion and MRS supplemented with 4%, 4.5% and 6.5% and bromocresol purple as a pH indicator is used for this purpose according to the method in Biochemical tests for gram positive bacteria [23].

2.2.1.2.4. Gelatin Liquification Test

The gelatin stab method employs according to Biochemical identification tests. Nutrient gelatin deep tubes that contain 12% gelatin, heavy inoculums from a pure culture of the test organism is stabled into the media and incubated for at least 48 hrs, then placed into refrigerator for 30 min. If the organism has produced sufficient gelatinase, the tubes will remain liquid and do not solidify in the refrigerator.

2.2.1.2.5. Starch Hydrolysis Test

The starch agar is inoculated with the pure colony and incubated at an appropriate temperature then iodine solution is added to the surface of the agar media which turned to blue-black in the presence of starch. Absence of the blue-black color indicates that starch is no longer present (Biochemical tests).

2.2.1.2.6. Motility

To determine if an organism is motile or non motile by inoculating an 18 to 24 hr pure culture in the center of MRS agar or B.H.I.agar medium by needle to a depth of 0.05 inch, then incubated at 35º C for 24 to 48 or more according to (the biochemical tests).

2.2.1.3. IMVIC Tests

a- Indol Production Test

The test was made by including 10.0 ml of tryptone water (1% tryptone in distilled water) with the test organism. Tubes were incubated for 24 hr, at 37ºC, then 0.2 to 0.3 ml. of Kovac's reagent were added to 5.0 ml of the 24 hr. culture. The formation of a dark red colour on the surface indicated a positive test.

Kovac's reagent was prepared by dissolving 5.0 gm of p-dimethyl amino benzaldehyde in 75 ml .of amyl alcohol, followed by the addition of 25 ml .of concentrated hydrochloric acid.

b- Methyl Red Test

This test was carried out by inoculating the tested isolates into 10 ml. medium which was prepared to have the following composition:

Peptone	7.0 gm.
Dextrose	5.0 gm.
K₂HPO4	5.0 gm.
Distilled water	1000.0 ml.

Final pH 6.9

5 drops of methyl red solution were added to 5.0 ml of each culture. Positive reaction was indicated by the formation of a distinct red colour, showing the presence of acid. The indicator solution was prepared by dissolving 0.1 gm Bacto-Methyl red in 300 ml of 95% ethyl alcohol and diluting to 500 ml with distilled water.

c- Voges-Proskawer Test

Cultures were inoculated in tubes containing 10 ml of MR-V.P. medium. Tubes were incubated for up to 5 days. To 5 ml of the culture, 5.0 ml of a 10% KOH solution were added, mixed well, allowed to stand, exposed to the air and observed at intervals of 2, 12 and 24 hr for the formation of a pink colour.

d- Citrate Utilization Test

Cultures were inoculated in tubes containing 10 ml of citrate medium which was prepared as follows:

Sodium ammonium phosphate	1.5 gm.
Monopotassium phosphate	1.0 gm.

Sodium citrate	3.0 gm.
Magnesium sulfate	0.2 gm.
Distilled water	1000.0 ml.

Final pH 6.7

Tubes were incubated at 37ºC for 24 hr. The positive test was detected by the presence of a uniform turbidity.

e- Ei jkman test :

After performing the above mentioned IMViC tests, the tested strains were subjected to the Eijkman test. Cultures were inoculated into tubes containing 10 ml of the following medium:

Tryptone	15.0 gm .
Lactose	3.0 gm .
K2HPO4	4.0 gm .
KH2PO4	1.5 gm .
NaCl	5.0 gm .
Distilled water	1000.0 ml.

Final pH 6.8

Tubes were incubated at 44º C for 48 hr. The presence of a uniform turbidity and gas production at the end of the incubation period was taken as a criterion for a positive test.

2.2.1.4. Microbiological Characteristics by API-20E Test Kit STREB System, and API Staph

The API 50CH strip (Biomerieux, Marcy l'Etoile France) was used to identify *Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus thermophilus* cultures. It consists of 50 micro-tubes which contain an anaerobic zone (the tube portion), for the study of fermentation and an aerobic zone (the cupule portion), for the study of oxidation or assimilation. The first tube contains no substrate and is used as a negative control. The remaining tubes contain a defined amount of dehydrated substrate belonging to the carbohydrate family and its derivatives (heterosides, polyalcohols, uronic acids). These substrates may be metabolized by various biochemical pathways:

- Assimilation is indicated by growth of an organism in the cupule, when the substrate is the only source of carbon present.
- Oxidation is shown by a color change in the cupule portion and is due to the aerobic production of acid detected by a pH indicator included in the chosen medium.
- Fermentation is shown by a color change in the tube portion, and is due to the anaerobic production of acid detected by pH indicator included in the chosen medium.
- The API 20 strips (Biomerieux, Marcy l'Etoile France) was used to identify the *Enterococcus* cultures. It consists of 20 microtubes containing dehydrated substrate for the demonstration of enzymatic activity or the fermentation of sugars. A dense suspension made from a pure culture is then used to rehydrate the enzymatic substrates. The metabolic end products produced during the incubation period are either revealed through spontaneous colored reactions or by the addition of reagents.

2.2.1.5. Preparation of Tissue Samples for Scanning Electron Microscopy Examination

1- The samples were fixated by glutheraldhyde 2.5% and dehydrated by serial dilution of ethanol using automatic tissue processor (Leica EM TP).

2-Then the samples drying using CO_2 critical point drier (Tousimis Audosamdri-815).

3- The samples coated by gold sputter.coater(SPI-Module).

4- Finally The samples eximinated by scanning electron microscopy (JEOL-JSM-5500 LV) by using high vaccum mode at the Regional Center of Mycology and Biotechnology, Cairo,Egypt.

3. STATISTICAL ANALYSIS

Results from the facial hedonic scale record sheets were collated and input into a Microsoft Excel 2007 database, mean, standard deviations and p-values were calculated for each sample. P-values less than 0.05 were considered statistically significant.

4. RESULTS AND DISCUSSIONS

Table1: shows 42 predominant colonies morphology isolated on MRS agar, BHI agar, MSA and SF agar from 12, 16 and 14 samples of saliva, teeth and faeces respectively. This found to be 24 cocci cells, 14 rods cells and 4 yeasts cells. The colonies isolated from saliva were 8, 3 and 1 cocci, rods and yeasts, respectively, while these isolated from teeth were 10, 5 and 1 respectively. The corresponding colonies isolated from faeces were 6, 6, and 2 respectively. All bacterial cells were gram positive.

Table-1. Microbiological isolates types

	Bacterial shape		**Yeasts**
	Cocci	Rods	
Saliva	8	3	1
Teeth	10	5	1
Fecese	6	6	2
	24	14	4

Isolated on MRS agar, BHI agar, MSA and SF agar.

As shown in table 2 the identification of 8 cocci cultures isolated from the adult and children saliva. The isolated cultures were 2, 1, 1, 1 and 3 which belong to *Streptococcus pyogenes, Staphylococcus aures , Enterococcus faecalis, Lactococcus lactis and streptococcus mutans* , respectively. The confirmatory test (plasma coagulase tast) has been carried at *Staphylococcus* cultures which were positive for this test indicating that it belongs to *Staphylococcus aures.*

The presence of *Streptococcus pyogens* normally resides in the throat and is one of the most common medical pathogens in the saliva as reported by Stjernquist-Desatnik and Orrling, [19] who detected streptococcus pyogens in approximately 10% of adults and 25% of children, and in as many as 60% of subjects during large outbreaks of streptococcal pharyngotonsillitis.While Tagg et al, [21] found *Streptococcus pyogens* in 5% of saliva samples of young school children in Newzealand, with a suggestion of a child-to-child transmission of the organism.

On other hand Holt et al, [10] reported that oral Streptococci, like *Streptococcus mutans* are associated with pyogenic and other infections in various sites including mouth, heart, joints, skin, muscle, and central nervous system.

This phenomenon approves the presence of *Streptococcus mutans* in the saliva of both adult and children. The *Staphylococcus aureus* presence may be due to some inflamation in the mouth while *Enterococcus faecalis, Enterococcus faceium and Latctococcus lactis* may be resulted from contamination.

Table-2. Identification tests for cocci cultures isolated from salive

Cocci Cultures					
	2	***1***	***1***	***1***	***3***
Identification tests	***Pyogenous***	***Staph. aures***	***Ent. faecales***	***Lact.lactis***	***Strept.mutans***
Growth at 45	-	+	+	+	+
Growth at Nacl % 4		+	+	+	+
6.5	-	+	+	+	-
10		+	-	-	-
Acid from Xylose		-	-	-	-
Arabinose	-	-	-		-
Cellebiose		-	-	-	
Fructose		-			
Melliziotose		-	+		-
Sorbese				+	
Salicin	+	-			
Sorbitol	-		+	-	+
Succrose		+			+
Raffinose	-	-			
Maltose		+			
Mannitol	-	+	+	+	+
Mannose		+			+
Trehalose		+			+
lactose	+	+	+	+	+
Ribose	-	+			
Sorbose			-	+	
Mellibiose			-	+	+
Growth at 15 ^{0}C		+			
Enulin	-				
Esculin	-				
Arginin	+				
Trehalose	-				
Growth at 10^{0}C	-				

Identification of 8 cocci cultures isolated from the adult and children saliva.

On other hand **table 3** revealed the identification of 3 rod cultures isolated also from the adult and children saliva which were found to be one *lactobacillus acidophilus* and 2 *Lactobacillus brevis* which may be come from the consumption of Dairy products.

Table-3. Identification tests for Rods cultures isolated from saliva

Rods Cultures		
	1	**2**
Identification tests	***Lact.acidophilus***	***Lact.brevis***
Growth at 45	+	-
Growth at Nacl % 4		
6.5		
10		
Acid from Xylose	-	-
Arabinose	-	+
Cellebiose	+	-
Fructose	+	+
Melliziotose	-	-
Sorbese		
Salicin	+	-
Sorbitol	-	-
Succrose	+	-
Raffinose	-	-
Maltose	+	+
Mannitol	-	-
Mannose	+	-
Trehalose	-	-
Lactose	+	-
Ribose	-	+
Sorbose		
Mellibiose	-	+
Growth at 15 ^{O}C		+
Enulin		
Esculin	+	-
Arginin		
Trehalose	-	-
Growth at 10^{O}C		

Identification of 3 rod cultures isolated from the adult and children saliva.

Concerming the microflora of teeth, **Table 4** revealed that the identification of 10 cocci cultures isolated from adult and children who were identified as 1, 2, 3, 2 and 2 *Staphylococcus lentus, Staphylococcus xyloses, Staphylococcus aures, Streptococcus salivaries and Streptococcus mutans*, respectively.

Table-4. Identification tests for cocci cultures isolated from teeth

Cocci Cultures					
Identification tests	**2**	**2**	**2**	**2**	**2**
	Staph.Lentus	*Staph.xyloses*	*Staph.aures*	*Strept.salivarius*	*Strept.mutans*
Growth at 45	-	-	+	+	+
Growth at Nacl % 4			+	-	+
6.50%			+	-	-
7%					
10%			+	-	-
Acid from Xylose	-	+	-	-	-
Arabinose	-	+	-		-
Cellebiose	+	-	-		
Fructose	+	+	-		
Melliziotose	-	-	-		-
Sorbese					

Continue

Salicin	-	-	-	+	
Sorbitol				-	+
Succrose	+	+	+		+
Raffinose	+	-	-	+	
Maltose	-	+	+		
mannitol	+	-	+	-	+
mannose	+	+	+		+
Trehalose	+	+	+	ND	+
lactose	-	-	+	+	+
Ribose	+	-	+	-	
Sorbose					
Mellibiose					+
Growth at 15 ^{O}C	-	+	+		
Growth at 40 ^{O}C					
Growth at 55 ^{O}C					
Enulin				+	
Esculin				+	
Arginin				-	
Growth at 10^{O}C	-			-	

Identification of 10 cocci cultures isolated from adult and children.

There were 5 Rods cultures in table 5 and fig 1 found as 2, 2 and 1 were belong to *Bacillus brevis, Bacillus subtilis* and *Lactococcus acidophillus. Bacillus subtilis* and *Bacillus brevis* were identified by PCR tecnique. Gomes et al, [8] and Pinheiro et al, [16] investigated the microbial findings of teeth with failed endodontic treatment and reported that a very limited assortment of microorganisms, with predominantly facultative anaerobic grampositive species, especially *Enterococcus faecalis*.

Marsh, [14] and Desoet et al, [2005] notice that *S. mutans* and *Streptococcus sobrinus* have a central role in the etiology of dental caries, because these can adhere to the enamel salivary pellicle and to other plaque bacteria [12]. Tanzer et al, [22] reported that Mutans *streptococci* and *lactobacilli* are strong acid producers and hence cause an acidic environment creating the risk for cavities.while Mayooran et al, [13] noticed that the appearance of *S. mutans* in the tooth cavities is followed by caries after 6-24 months.

Table-5. Identification tests for Rods cultures isolated from teeth

Rods Cultures			
	2	**2**	**1**
Identification tests	***Bacillus.brevis***	***Bacillus.subtilis***	***Lact.acidophillus***
Growth at 45^{O}C			+
Growth at Nacl % 4	-	+	
6.50%	-	+	
7%		+	
10%			
Acid from Xylose	-	+	-
Arabinose	-	+	-
Cellebiose			+
Fructose			+
Melliziotose			-
Sorbese			
Salicin			+
Sorbitol			-

Continue

Succrose			+
Raffinose			d
Maltose			+
Mannitol	d	+	-
Mannose			+
Trehalose			d
Lactose			+
Ribose			-
Sorbose			
Mellibiose			d
Growth at 15 ^{O}C	-		
Growth at 40 ^{O}C	+	+	+
Growth at 55 ^{O}C	-	-	
Enulin			
Esculin			+
Arginin			
Growth at 10^{O}C	-	d	
Catalase	+	+	
voges- proskauer test	-	+	
Hydrolysis of casein	+	+	
Amygdalin			+
Gelatin	+	+	
Starch	+	+	

Rods isolates.

Ruby et al, [18] reported that Caries is caused by indigenous oral microorganisms, mainly *Streptococcus mutans*, becoming a dynamic biofilm, which in the presence of fermentable sugars; produce organic acids capable of dissolving inorganic enamel and dentin followed by the proteo-lytic destruction of collagen, leaving soft infected dentin**.** Ercan & Tuna, [7] investigate the type of microorganisms isolated from teeth root canals 68% of isolates were gram positive bacteria, *Streptococcus* sp 14.2%, *Enterococcus faecalis* 9.6%, *Lactobacillus acidophilus* 7%, *Bacillus* sp 2% and *E. coli* 1.6%.

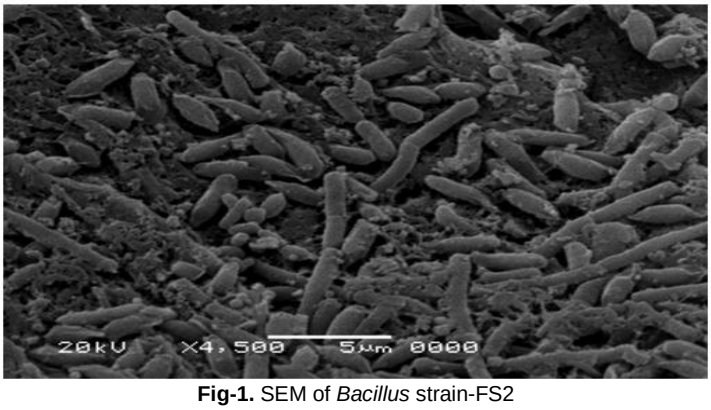

Fig-1. SEM of *Bacillus* strain-FS2

SEM for Bacillus FS2.

As shown in **table 6** it revealed that the identification of 6 cocci cultures isolated from feces were found to be 3Enterococcus faecalis and 3 *Enterococcus faecium*

Table-6. Identification tests for cocci cultures isolated from feces

Cocci Cultures		
	3	**3**
Identification tests	***Ent.faecales***	***Ent.faceium***
Growth at 45	+	+
Growth at Nacl % 4	+	+
6.50%	+	+
10%	-	-
Acid from Xylose	-	-
Arabinose	-	
Cellebiose	-	-
Fructose		
Melliziotose	+	
Sorbese		+
Salicin		
Sorbitol	+	-
Succrose		
Raffinose		
Maltose		
mannitol		
mannose		
galactose		
glucose		
Trehalose		
lactose	+	
Ribose		
Sorbose	-	+
Mellibiose	-	+

Identification of 6 cocci cultures isolated from feces.

While 4 Rod cultures detected in table 7 as one of each culture *Escherichia coli*, *Bacillus brevis*, *Lactobacillus brevis* and *Lactobacillus acidophillus*.

Table-7. Identification tests for Rods cultures isolated from feces

Rods Cultures				
	1	**1**	**1**	**1**
Identification tests	**E.coli**	**Bacillus.brevis**	**Lact.brevis**	**Lact.acidophillus**
Growth at 45			-	+
Growth at Nacl % 4				
6.50%				
10%				
Acid from Xylose		-	-	-
Arabinose		-	+	-
Cellebiose			-	+
Fructose			+	+
Melliziotose			-	-
Sorbese				
Salicin			-	+
Sorbitol			-	-
Succrose	d		-	+
Raffinose			-	d

Continue

Maltose			+	+
Mannitol	+	d	-	-
Mannose			-	+
Galactose			-	+
Glucose		d	+	+
Trehalose			-	d
Lactose	+		-	+
Ribose			+	-
Sorbose				
Mellibiose			+	d
Growth at 15 ^{O}C			+	
Growth at 40 ^{O}C		+		
Growth at 55 ^{O}C		-		
Gluconate			+	-
Esculin			-	+
Catalase		+		
voges- proskauer test	-	-		
Hydrolysis of casein		+		
Amygdalin			-	+
Gelatin		+		
Starch		+		

Identification of 4 rods isolated from feces.

Table 8 shown the Confirmatory identification for eleven isolates by API.

Table-8. The Confirmatory identification for some isolates by API:

Identification using API System	Number of isolates
Staph.lentus 99.6%	1
Staph.xylosus 99.9%	5
B. brevis 96.7%	1
Lactobacillus acidophilus 98.7%	3
Lactobacillus plantarum 97.9%	2
Bacillus sp.	8
Ec.faecalis	18

Confirmatory identification by API.

5. CONCLUSIONS

The endogenous microbial flora plays an important role in health and disease in the human. From our results 42 predominant colonies morphology isolated on MRS agar, BHI agar, MSA and SF agar from 12, 16 and 14 samples of saliva, teeth and faeces respectively. This found to be 24 cocci cells, 14 rods cells and 4 yeasts cells.

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