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Isolation, Molecular Characterization and Antibiotics Susceptibility Pattern of Methylotrophic Bacteria Occuring in the Human Mouth

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Abstract: Methylotrophic bacteria are ubiquitous bacteria that are capable of using one carbon compounds such as methane, methanol, halogenated methane, and methylated amine as sources of carbon and energy for their diverse metabolic activities. Methylotrophic bacteria were isolated from the tongue, and supra- and subgingival plaque in the mouths of volunteers and patients with periodontitis. The isolation, identification, antibiotic susceptibility pattern and molecular characterization of methylotrophic bacteria from 150 volunteers and patients with periodontitis were done using standard method. Nutrient agar fortified with methylamine as a growth factor was used to aid the growth of these bacteria. The result show that a total of twelve (12) bacteria were identified. These are *Bacillus licheniformis, Neisseria flava, Neisseria meningitis, Micrococcus flava, Pseudomonas aeruginosa, Streptococcus pneumoniae*, *Methylobacteria radiotolerance, Methylotrophic thiocynatum, Methylococcus capsulatum, Methylotrophic rubra* and *Branhamella catarhalis.* The antibiotics susceptibility test showed that *B. licheniformis, N. meningitis and P. aeruginosa* exhibited the highest resistance against the antibiotics. The 12 bacteria were characterized molecularly with Polymerase chain reaction PCR amplified method using primers for the virulence genes of Methylotroph. The mxaF gene of methanol dehy- drogenase MxaF was detected in 3 bacteria: pilc of pilin gene in 3 bacteria, stx1 of shiga toxin nana of neuraminidase and pila of pilin gene was detected in all the bacteria. The results obtained in this research showed that methylotrophs may be responsible for periodontitis and that healthy people are carriers.

Keywords: Isolation, Characterization, Methylotrophic, Bacteria, Mouth

1. Introduction

Methylotrophic bacteria are ubiquitous bacteria that are capable of using one carbon compound such as methane, methanol, halogenated methane, and methylated amine as sources of carbon and energy for their growth [1]. These compounds, together with hydrogen sulphide, are products of microbial degradation of free sulphur amino acids and disulphide groups which are released from proteins [2]. Volatile sulphur compound (VSC) in oral cavity is a major cause of halitosis or bad breath [3].

The human mouth is a constant source of volatile one carbon compound, including malodorous methylated sulfides such as methanethiol and dimethylsulfide [4]. These compounds, together with dimethyldisulfide and hydrogen sulfide, are expired by healthy individuals, with between 80 and 90% of the sulfur gases arising in the oral cavity from the microbial degradation of free sulfur amino acids (especially methionine) and from thiol and disulfide groups released from proteins [4].

Oral bacteria have evolved mechanisms to sense their environment and evade or modify the host. Bacteria occupy the ecological niche provided by both the tooth surface and gingival epithelium. However, a highly efficient innate host defense system constantly monitors the bacterial colonization and prevents bacterial invasion of local tissues [5]. A dynamic equilibrium exists between dental plaque bacteria

and the innate host defense system.

Oral bacteria include streptococci, lactobacilli, staphylococci, corynebacteria, and various anaerobes in particular bacteroides. The oral cavity of the new-born baby does not contain bacteria but rapidly becomes colonized with bacteria such as *Streptococcus salivarius*. With the appearance of the teeth during the first year colonization by *Streptococcus mutans* and *Streptococcus sanguinis* occurs as these organisms colonise the dental surface and gingiva. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The gingival crevice area (supporting structures of the teeth) provides a habitat for a variety of anaerobic species. Bacteroides and spirochetes colonize the mouth around puberty [5]. In addition to these bacteria listed or mentioned are the methylotrophs. The majority of these methylotrophs have been exerting an increasing level of resistance to commonly used antibiotics.

Multiple antibiotic resistances (MARs) in bacteria may be commonly associated with the presence of plasmids. Plasmids are circular double stranded extra-chromosomal DNA molecules, and conjugal transfers of plasmids play an important role in the spread of antibiotics resistant genes among methylotrophs and other bacterial strains. The resistance to commonly using antibiotics in bacteria creates a threat to public health in the world. In addition, the size, number and attributes of the plasmid in a bacterium remain the same for a long time and they are transferred to equally daughter cells [5].

The resistance to commonly used antibiotics in bacteria creates a threat to public health in the world. In addition, the size, number and attributes of the plasmid in a bacterium remain the same for a long time and they are transferred to equally daughter cells. Therefore, plasmids have an important role in epidemiological and taxonomic studies [6].

The diversity of methylotrophic bacteria and their resistance to antibiotics has been on the increase and consequently increasing the number of people with mouth odour cases in the society. There is therefore an urgent need to investigate their antimicrobial susceptibility profile [7].

2. Materials and Methods

2.1. Collection of Samples

A total of 150 samples was collected from different parts of mouth of volunteers within one week (30 per days) from students in Microbiology Department in FUTA and Dental Department University Teaching Hospital, Ado Ekiti. The samples was preserved in the prepared mineral broth inside a swab stick container(s). These was transported to the laboratory and cultured on nutrient agar supplemented with mineral salt basal medium to aid the growth of microorganisms. Pure culture of methylotrophs were obtained by streaking of single colonies on agar medium containing methylamine as a growth substrate. Cultures on agar slants in sealed tube were also supplemented with methylamine.

2.2. Media and Procedure for Selective Culture of Methylotrophs

The mineral salt basal medium used contained the following in grams per litre in distilled water: $KH_2PO_4.2H_2O$(1.5), $NA_2HPO_4.2H_2O$(7.9), NH_4CL(0.8), $MGSO_4.7H_2O$(0.1). [8] initial ph 7.3, autoclaved at 121°C for 15minutes, for enrichment culture and the isolation of pure cultures on solid medium, the basal salts medium was supplemented with Oxoid bacteriological agar with methylamine as a growth substrate.

2.3. Methods of Identification of Bacteria Isolates

Characterization of bacterial isolates was based on standard microbiological techniques described by [9]. Gram staining, morphological and cultural characteristics was carried out with various biochemical tests which include: catalase test, coagulase test, oxidase test, motility test, and starch hydrolysis, spore test, methyl red test, citrate utilization and sugar fermentation [10].

2.4. Antibiotic Sensitivity Test

The antibiotic sensitivity test was carried out in order to know the sensitivity of the microorganism to the different commercially available antibiotics. These antibiotics discs include: Augmentin, Amoxacillin, Ofloxacin, Gentamycin, Cotrimoxazole, Nitrofurantoin, Nalidixic acid and Tetracyclin. Disc diffusion method was to determine the effect of standard antibiotics on the bacterial isolates as described by [11]. The experiment was carried out in triplicates.

2.5. Molecular Characterization

Isolates was characterized molecularly using PCR method to amplify a specific gene target in the bacteria) [12].

2.5.1. Extraction of Genomic DNA

Genomic DNA was obtained from pure cultures by lysozyme-proteinase K-sodium dodecyl sulfate (SDS) treatment followed by phenol-chloroform extraction and subsequent ethanol precipitation. The purity and concentration of the DNA preparations were determined spectrophotometrically.

2.5.2. Preparation of Total DNA from an Enrichment Samples

DNA was prepared from five samples. (i) A 500-ml volume of medium 337-B1 with 0.5% (wt/vol) KNO_3 for the enrichment of methylotrophic bacteria was inoculated with 100 µl of activated with Plön (Schleswig-Holstein, Germany). After 4 weeks at 28°C under anaerobic conditions, 500 µl of the enrichment was again inoculated and kept under the same growth conditions. The cells were harvested by centrifugation (6,000 × g for 60 min at 4°C) and resuspended in 400 µl of double-distilled water. The DNA was extracted with Chelex 100. The reagent used Plon (Schleswig-Holstein, Germany) was pelleted (13600 × g for

10 min at 4°C), and the pellet was air dried and resuspended in 0.85% NaCl solution. DNA extraction was followed by an additional hexadecyl trimethyl ammonium bromide (CTAB; Sigma Aldrich, Steinheim, Germany) precipitation step to remove humic acids and carbohydrates was adopted and DNA identified using DNA standard methods.

2.5.3. Sequencing of Amplified Nir Products

For DNA sequencing, amplified PCR products from pure cultures were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) as specified by the manufacturer. DNA sequences were determined by direct sequencing of purified PCR products with the cycle-sequencing kit (GATC, Konstanz, Germany) and Thermosequenase 2.0 (Amersham, Braunschweig, Germany) as specified by the manufacturers. Labeling was performed by terminating the polymerization with biotin-labeled dideoxynucleoside triphosphates. After a denaturing step of 4 min at 94°C, 30 cycles of denaturation for 30 s at 94°C and primer annealing and extension for 30 s at 55°C were performed, followed by an additional extension step of 6 min at 60°C. The sequencing products were blotted with a direct blotting apparatus (GATC) onto a nylon membrane. The separated products were visualized by an enzyme-linked streptavidin-biotin coupling assay with a streptavidin-alkaline phosphatase conjugate (GATC) and NBT/X-phosphate (Boehringer, Mannheim, Germany) as specified by the manufacturers. The sequences obtained were compared with published nirK and nirS sequences in the EMBL Nucleotide Sequence Database by FASTA analysis of the HUSAR program package based on the Genetics Computer Group sequence analysis package.

2.6. Statistical Analysis of Result

Result obtain will be subjected to descriptive one way analyses of variance, SPSS version 16 Microsoft windows 7 and Duncan multiple range test will be used as follow up test.

3. Results

3.1. Bacteria Isolates

Out of the total 150 mouth swabs collected: 75samples from students in Microbiology Department in FUTA and 75 samples from Dental Department Ekiti State University Teaching Hospital. The age and sex distributions of samples collected are shown in table 1.

Growth pattern of the isolates: Generally, samples from students in the department of microbiology had more or heavy growth than the samples from the teaching hospitals and it also reveals that female had highest percentage of Positive growth in the department than female also males had highest growth percentage in the UTH than female. This pattern of growth is shown in table 2.

3.2. Colony Characteristics

A total of 82 isolates were obtained from all the samples cultured. The colonial morphology, biochemical characteristics and sugar fermentation reveals 12 different groups of isolate shown in table 3.

3.3. Biochemical Characteristics

Table 4 and 5 show the colonial morphology, biochemical characteristics, sugar fermentation and probable organisms

Table 1. *Age and Sex Distribution of samples collected from University Teaching Hospital Ado Ekiti and Department of Microbiology FUTA.*

S/N	SAMPLES FROM	NO OF SAMPLES	NO OF MALES	NO OF FEMALES	AGE RANGE(YRS)
1	DEPARTMENT	75	45		
2	UTH ADO	75	20	55	15-70

Table 2. *Growth pattern of methylotrophs from samples collected from University Teaching Hospital Ado Ekiti and Department of Microbiology FUTA.*

S/N	SAMPLE SOURCE	GENDER	NO WITH GROWTH	NO WITHOUT GROWTH	% POSITIVE GROWTH
1	DEPARTMENT	M	32	13	71.11
2	DEPARTMENT	F	29	1	96.67
3	UTH	M	6	14	30.00
4	UTH	F	15	40	27.27

Table 3. *Groups of isolate in relation to sex and source.*

GROUPS OF ISOLATES	NO OF ISOLATES WITH THE SAME MORPHOLOGY	MALE	FEMALE	DEPT	UTH
1	7	4	3	5	2
2	4	2	2	3	1
3	7	5	2	10	2
4	9	5	4	8	1
5	8	6	2	9	-
6	6	4	2	3	4
7	8	5	3	4	4
8	10	5	5	7	3
9	3	3	-	-	3
10	5	2	1	5	2
11	6	1	5	3	3
12	7	1	6	3	4

Table 4. *Colonial morphology of isolates.*

ISOLATE	SHAPE	SIZE	COLOUR	OPACITY	ELEVATION	SURFACE	EDGE	CONSISTENCY	AMOUNT OF GROWTH
1	Calcular	Small	Milky	Transparent	Raised	Smooth	Entire	Glittering	Profuse
2	Fimbriate	Large	Milky	Opaque	Raised	Smooth	Entire	Glittering	Profuse
3	Circular	Tiny	Milky	Transparent	Raised	Smooth	Rhizoid	Glittering	Profuse
4	Circular	Large	White	Transparent	Raised	Smooth	Entire	Glittering	Moderate
5	Circular	Large	White	Transparent	Raised	Rough	Entire	Glittering	Moderate
6	Circular	Large	White	Transparent	Raised	Rough	Entire	Glittering	Moderate
7	Circular	Large	Yellow	Transparent	Raised	Dry	Lobate	Glittering	Scanty
8	Fimbrate	Large	White	Opaque	Flat	Rough	Entire	Dull	Profuse
9	Circular	Tiny	White	Transparent	Flat	Smooth	Entire	Glittering	Profuse
10	Circular	Large	Light green	Transparent	Raised	Smooth	Entire	Shinny	Scanty
11	Circular	Small	White	Opaque	Raised	Smooth	Entire	Glittering	Profuse
12	Undulate	Small	Butter	Transparent	Raised	Smooth	Entire	Dull	Scanty

Table 5a. *Biochemical characteristics and sugar fermentation and probable organisms.*

ISOLATE	GRAM'S REACTION	SPORE TEST	OXIDASE TEST	METHYL RED TEST	STARCH HYDROLYSIS	MOLITY TEST	LACTOSE	SUCROSE	GLUCOSE
1	_ve cocci	_	+	_	+	_	++	+_	++
2	+ve cocci	_	+	+	+	+	+_	+_	++
3	+ve rod	+	_	+	+	_	++	++	++
4	_ve cocci	_	+	+	+	_	++	+_	++
5	_ve rod	_	+	_	_	+	+_	+_	+_
6	+ve cocci	_	_	_	_	_	+_	+ _	++
7	+ve rod		_	_	_	_	++	_ _	_ _
8	+ve rod			+	+	_	+_	++	++
9	+ve cocci	_	+	_	+	+	+_	+_	++
10	+ve cocci	_	+	_	_	_	++	_ _	++
11	_ve rod	_	_	+	+	+	++	++	+_
12	+ve cocci_	_	+	_	_	_	++	_ _	++

Table 5b. *Biochemical characteristics and sugar fermentation and probable organisms.*

XYLOSE	SALICIN	ARABINOSE	MANITOL	CATALASE	PROBABLE ORGANISMS
++	++	+_	_ _	+	*Neisseria flava*
+_	++	_ _	_ _	+	*Micrococcus flava*
+_	+_	++	_ _	_	*Bacillus licheniformis*
_ _	++	_ _	+_	+	*Neisseria meningitis*
_ _	+_	_ _	+_	_	*Pseudomonas aeruginosa*
+_	+_	_ _	_ _	+	*Streptococcus pneumonia*
++	_ _	+_	++	+	*Methylobacterium radiotolerance*
++	_ _	+_	++	+	*Methylobacterium thiocynatum*
_ _	_ _	+_	++	+	*Methylococcus capsulatus*
_ _	++	++	++	+	*Methylococcus rubra*
+_	_ _	+_	+_	+	*Escherichia coli*
++	++	+_	+_	+	*Branhamella catarhalis*

3.4. Antibiotics Susceptibility Pattern

The antibiotics susceptibility test reveals that *Methylobacterium radiotolerance* and *Methylobacterium thyocynatum* had highest susceptibility rate. *Neisseria flava* and *Brahamella catarhalis* was resistant to the antibiotics as shown in figure 1.

3.5. Molecular Characterization

The molecular characterization using polymerase chain reaction revealed five (5) different types of virulence gene which include *stx1*,*nana,pila*, *pilc* and *maxf* as shown in plate

1to 7. It also reveals that occurrence of virulence is higher in *Pseudomonas auroginosa* and *pila* and *pilc* had highest number of occurrence as shown in 6. *Maxf* and *pilc* had highest percentage of methylotrphs possessing the virulence gene as shown in table 7.

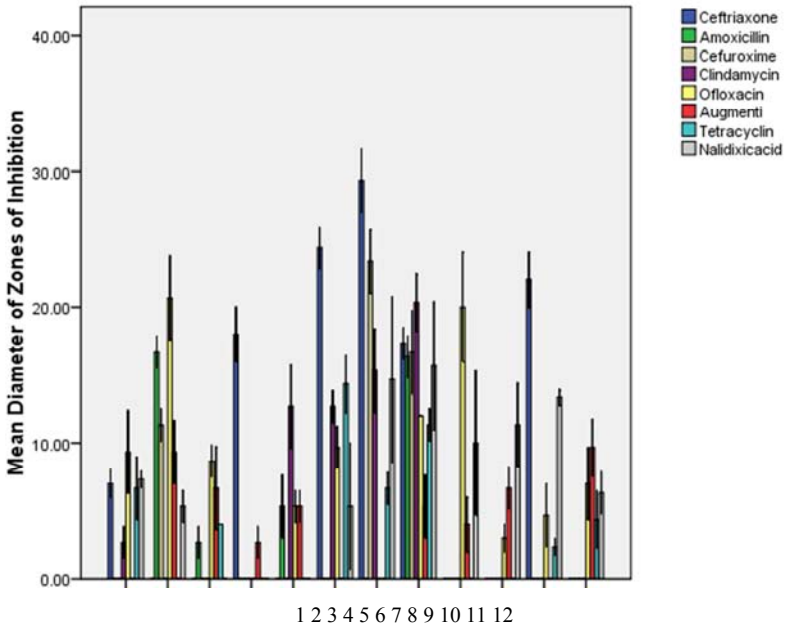

Figure 1. *Diameter of zones of inhibition of standard commercial antibiotics on bacterial isolates.*

Key: 1= *Neisseria flava*
2= *Micrococcus flava*
3= *Bacillus licheniformis*
4= *Neisseria meningitis*
5= *Pseudomonas aeroginosa*
6= *Streptococcus pneumonia*
7= *Methylobacterium radiotolerance*
8= *Methylobacterium thiocynatum*
9= *Methylococcus capsulatus*
10= *Methylococcus rubra*
11= *Escherichia coli*
12 = *Branhamella catarhalis*

Figure 2. *Representative gels for PCR amplification of DNA extracted from E. coli isolated from human mouth showing the presence of virulence genes stx1 Lane M (100bp) = DNA size marker; Lane 1 = E. coli Lane K = Control, respectively.*

Figure 2 Representative gels for PCR amplification of DNA extracted from Methylobacterium radiotolerans isolated from human mouth showing the presence of virulence genes mxaF Lane M (100bp) = DNA size mark Lane 1 =E. coli, Lane K=Control Respectively.

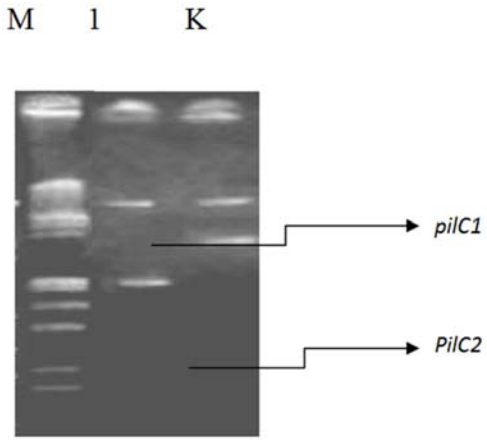

Figure 2. *Representative gels for PCR amplification of DNA extracted from Neisseria flava and Neisseria meningitis isolated from human mouth showing the presence of virulence genes pilC1 pilC2 Lane M (100bp) = DNA size marker; Lane 1 = Neisseria Flava and Neisseria Meningitis Lane K = Control, respectively.*

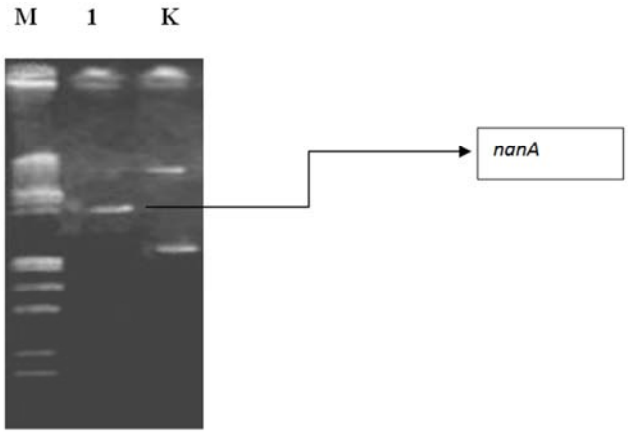

Figure 3. *Representative gels for PCR amplification of DNA extracted from streptococcus pneumonia isolated from human mouth showing the presence of virulence genes nanA Lane M (100bp) = DNA size marker; Lane 1= streptococcus pneumonia Lane K = Control, respectively.*

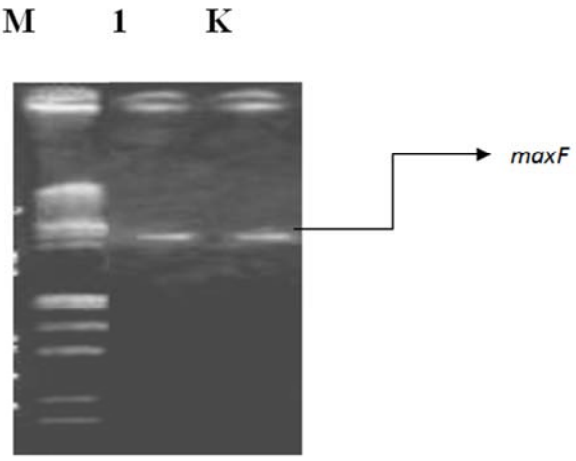

Figure 4. *Representative gels for PCR amplification of DNA extracted from baccillus licheniformis isolated from human mouth showing the presence of virulence genes maxF Lane M (100bp) = DNA size marker; Lane 1 = baccillus licheniformis Lane K = Control, respectively.*

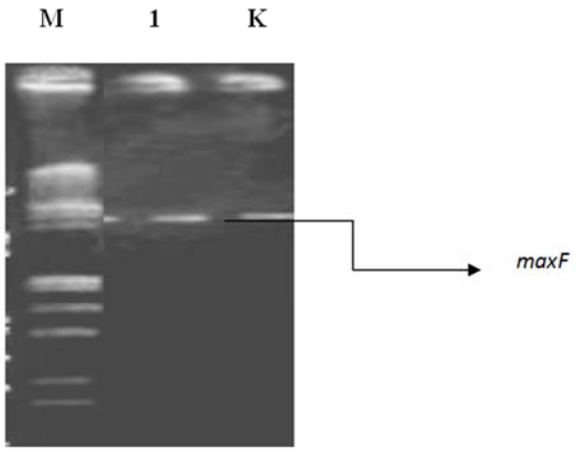

Figure 6. *Representative gels for PCR amplification of DNA extracted from Methylobacteruim thiocynatum isolated from human mouth showing the presence of virulence genes maxF Lane M (100bp)=DNA size marker; Lane 1=Methylobacteruim thiocynatum Lane K=Control, respectively.*

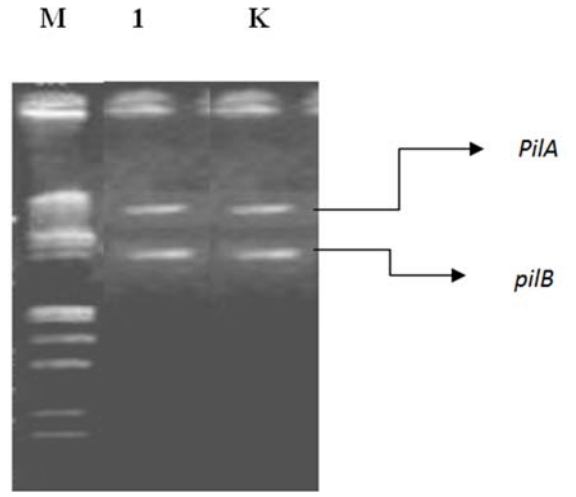

Figure 7. *Representative gels for PCR amplification of DNA extracted from Pseudomonas Aeroginosa isolated from human mouth showing the presence of virulence genes pilA pilC Lane M (100bp) = DNA size marker; Lane 1 = P. Aeroginosa Lane K = Control, respectively.*

Table 6. *Occurence of virulence genes in methylotrophic bacteria isolated from human mouth.*

		stx1	nana	pila	pilc	maxf
1	E. coli	+	-	-	-	-
2	Baccillus licheniformis	-	-	-	-	+
3	Neisseria flava	-	-	-	+	-
4	Neisseria meningitis	-	-	_	+	_
5	Pseudomonas aeroginosa	-	-	+	+	
6	Micrococcus flava	-	-	-	-	-
7	Streptococcus pneumoniae	-	+	-	-	-
8	Methylobacterium radiotolerance	-	-	-	-	+
9	Methylobacterium thiocynatum	-	-	-	-	+
10	Methylococcus capsulatum	-	-	-	-	-
11	Methylotrophic rubra	-	-	-	-	-
12	Branhamella catarhalis	-	-	-	-	-

+ = PRESENT (+ represent 1); - = ABSENT (- represent 0) *stx*1 – Shiga toxin 1; *nana* – Neuraminidase; *pila* – Pilin A 1; *pilc*– Pilin C; *maxf* – methanoldehydrogenase

Table 7. *Number and percentage of methylotroph isolated from human mouth samples carrying the virulence genes.*

stx1	Shiga toxin 1;
Nana	Neuraminidase;
Pila	Pilin A 1;
pilc	Pilin C;
maxf	methanol dehydrogenase gene

4. Discussion

Methylotrophic bacteria are a group of bacteria that are capable in using C1 compounds such as methanol as their only carbon and energy sources, in study we used one carbon compound medium (minimal medium supplemented with 1% methanol) as selective medium for these bacteria and biochemical assays oxidase and catalase assay [2]. A total of twelve (12) bacteria were isolated from the mouth, out of these, all were methylotrophic bacteria as shown in table 5: All strains exhibited fastest growth at about 37°C. All the strains tolerated 2.5% (w/v) NaCl, and strains grew well, alternative nitrogen sources for all these strains were ammonium, nitrate, cyanate and thiocyanate. All strains grew with methanol and methylamine as growth substrate. All were considered methylotrophs because according to [4] microorganisms which are able to grow in media of these types are methylotrophs.

The growth of methylotrophs was noticed to be higher and more from samples collected from the department than the ones from the UTH, Ado-Ekiti as shown in table 2. This probably was due to the fact that those in UTH are already on antibiotics which could have surpressed the growth of the methylotrophs. Isolate, *B. licheniformis* [1] did not exclude the possibility that it might also have been *B. casei rather than a novel species. This is because they have the same identity based on Bergey's manual used for identification.* The reasearch also show that *Pseudomonas aeroginosa* was present in the department but not present in the hospital, this may be because of its susceptibility to antibiotics *Methylobacterium thiocynatum* has highest number of

occurrence both in the department and hospital and this probably was due to its resistance to antibiotics while *Methyloccoccus capsulatum* has least number of occurrences this probably was due to its susceptibility pattern. The finding of methylotrophy in the strains most closely related to *B. licheniformis* adds to the small number of observations of methylotrophic growth by Bacillus species, including the earlier report of a serine-pathway strain of *B. licheniformis*. The strains of methylococcus and Methylobacterium were principally those of known species, and further extend the ecological niches known to harbour these genera [13]. This is also the first report of Escherichia coli in methylotrophic bacteria from mouth, other strains of which have been shown to degrade sulfolanes and butanesulfonate [14], and to possess the unusual property of accumu lating numerous rare-earth elements [15].

Methylococus capsulatus and Methylococcus rubra were closely related to *Gordonia* sp. (99, 98, and 99%), Phenotypic profiles of isolates were similar to that isolated by [16], which was salmon-orange coloured. Moreover, Gordonia isolated by [16] also, both isolates were susceptible to aminoglycoside group such as clindamycin and to β-lactam group such as amoxicillin which is in line with [16] Isolate Micrococcus was closely related to *Microbacterium estearomaticum* (99%) which is a rod shaped Gram positive bacteria, possesses yellow or orange coloured pigmentation, and catalase-positive [17]. There was also no report about the isolation of *Micrococcus* spp. in the human mouth. Previously, this bacteria has been successfully isolated from soil, liquid waste, and clinical specimens such as blood, hospital air, and wounds [18], indicating that *Mircococcus* is an opportunistic pathogen. Susceptibility testing done by [19] also showed a compatible result with this study, that this bacteria is tetracycline-sensitive. Isolate 5 was identified as *Pseudomonas* spp. (100%). A few species from *Pseudomonas* spp. have been reported to be successfully isolated from the human mouth, such a *P. alcalophila* and *P. aleovorans* [19]. Most of *Pseudomonas* is not included in methylotrophic bacteria. However, a few *Pseudomonas* have alcohol dehydrogenase which are able to oxidize methanol, such as *P. aeruginosa*, and *P. putida* [20]. Moreover, *P. fluorescens* has also been reported to effectively degrade VSC compounds, dimethyldisulphide [20].

Further, the diversity of methylotrophic bacteria in human mouth was also shown by polymerase chain reaction analysis to determine the virulence gene. PCR analysis were also distinguish the types of virulence gene in the isolates which have similar phenotypic profile. These results suggested that molecular characterization by PCR analysis technique could be used to show the genetic diversity among the isolates with same phenotypic profiles. However, alternative techniques could be used to increase the discriminative, such as amplification of 16S-23S rDNA, intergenic spacer or genome analysis with Macro restriction Fragment Length Polymorphism (MFLP). Both of these techniques have been used by [21] to analyze the genetic diversity of pink pigmented facultative methylotroph isolated from some Indonesian edible leafy plants. The molecular characterization shows that 8 out of the 12 samples possess virulence gene. The polymerase chair reaction also reveals four different types of virulence gene which include shiga toxin, *pilin*, neuraminidase and dehydrogenase genes.

This research is a good first-hand information source to show that diverse methylotrophic genera exist. This includes, strains of *Neisseria flava, micrococcus flava, Bacillus licheniformis, Neisseria meningitis, Pseudomonas aeruginosa, Streptococcus pneumonia, Methylobacterium radiotolerance, Methylobacterium thiocynatum, Methylococcus capsulatus, Methylococcus rubra, Escherichia coli, Branhamella catarhalis* which have been proven to occur in the human oral cavity. The results will assist in the design and construction of additional oligo-nucleotide probes for the determination of which methy-lotrophic species are present as ubiquitous members of mouth bio-film communities. The result also showed that methylotroph are responsible for periodontitis and even healthy people habour a lot of these methylotroph.

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