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# Polyphasic Characterization of Microbial Community from the Surface of *Brassica oleracea* (Cabbage) Head

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**Fresh produces (fruits and vegetables) harbor large and diverse type of microbes on their surface. *Brassica oleracea L.* var. *capitata* (Cabbage) has a dense green leafy head which is generally used as cooked vegetable but some people consume it as a raw salad. Therefore, there are more chances of pathogenic microbes may come in contact with gastrointestinal system that may directly cause disease. Also, the non-pathogenic microbes may also harm indirectly by acting as a source of allergy when consumed or may act as new lineages of commensal organisms. In the present study different mediums i.e. Tryptic soy agar (TSA), diluted tryptic soy agar (TSAD), R2A medium, Sabouraud dextrose agar (SDA) and MacConkey agar (MAC) were used to isolate diverse type of microbes by serial dilution technique. The highest count was obtained on TSAD medium (157.66 x $10^4$ CFU/ml) followed by MAC (150.33 x $10^4$ CFU/ml) and least was observed on TSA (134 x $10^4$ CFU/ml). The selected 95 isolates were subjected for identification by Matrix Assisted Laser Adsorption/Desorption Time of Flight Mass Spectrometry (MALDI-TOF MS) which showed that these microbes belonged to nine genera i.e. *Enterobacter, Acinetobacter, Klebsiella, Pseudomonas, Pantoea, Achromobacter, Staphylococcus, Microbacterium* and *Acidovorax*. The results clearly showed that the most prominent and diverse genera was *Acinetobacter* followed by *Enterobacter* and *Pantoea*, all belonged to gammaproteobacteria. Further selected 26 isolates were subjected to Fatty Acid Methyl Esters (FAME) identification and results showed that almost all the tested bacterial isolates contain same five types of fatty acids which are straight chain fatty acids with single bond. The 26 isolates were further identified on the basis of 16S rRNA gene sequencing, as such no pathogenic microbe was identified but some opportunistic human pathogens were found. The metagenomic DNA isolated from the cabbage surface head was electrophoresis by Denaturation Gradient Gel Electrophoresis (DGGE) which showed seven DNA bands of which three bands were very prominent which showed that communities of these three bands were more abundant as compared to other four bands community.**

**Key words:** *Brassica oleracea*, Metagenomic DNA, Microbial community, Polyphasic characterization.

Unprocessed bulk foods are one of the most effective ways to stay fit and healthy (Duquesne *et al*. 2005). Among these foods, fresh fruits and vegetables have become popular now a days since it require no or minimal time of preparation (Tournas 2005). The fresh fruits and vegetables are not only the source of nutrients but also known to harbor a large number of microbial diversity on their surface (Rastogi *et al*. 2012; Leff and Fierer 2013; Hofmann *et al*. 2014). This microbial population may be neutral or pathogenic to human beings when consumed (Enya *et al*. 2007; Teplitski *et al*. 2011). The microbial diversity on the surface of produce varies with season, crop cultivar, stage of growth and stages

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between farm to end consumer (Ponce *et al*. 2008; Ottesen *et al*. 2009; Rastogi *et al*. 2012). A huge microbial population is carried by fresh produce which may be pathogenic and since fresh produce is generally consumed raw it may lead to the disease outbreak (Caro-Astorga *et al*. 2015). The non-pathogenic micro-flora may also harm indirectly to human beings by acting as a source of allergy when consumed or may act as new lineages of commensal organisms in the human gastrointestinal tract (Hanski *et al*. 2012; Leff and Fierer 2013).

Cabbage (*Brassica oleracea L*. var. *capitata*) is a biennial leafy vegetable plant which grows annually for his dense overlapping leafy green head. This green leafy head is a rich source of dietary fiber, vitamin C and vitamin K. In addition to these nutrients, cabbage heads also carries a huge microbial population. Beside its use as cooked vegetable, it also been consumed as raw salad. Thus, the consumption of raw cabbage could introduce several microbes to human, which may cause various food-borne illnesses/ allergies/new lineages of commensal organisms. So, there is need to understand the microbial community prevailing on the cabbage heads.

The most of previous studies are generally based on culture dependent techniques and targeting mostly the pathogenic microbial community. The culture dependent studies generally miss the large number of microbial community as approximately 99% organisms are unculturable (Pham and Kim 2012), also these culture based approaches are influenced by the time of incubation, type of media used (Jackson *et al*. 2013). No doubt culture dependent techniques provide the physiological information of the organism but it is unable to provide the comprehensive details of the microbial communities composition (Orphan *et al*. 2000; Carraro *et al*. 2011). Furthermore, the organisms which may have important role in the community has been overlooked (Vartoukian *et al*. 2010). To overcome these problems molecular biology is a magic tool which provide deep insight into the community and provides comprehensive details. The new era of microbial ecology is started with the advances in genomics and sequencing technologies (Rastogi and Sani 2011). Now days, molecular approaches like genetic fingerprinting, metagenomics, metaproteomics etc are important tools to study the community structure and its interaction with biotic and abiotic factors as these techniques does not require cultured microorganism for analysis. So, the culture-dependent and culture-independent techniques are complimentary and should be used simultaneously for the better understanding of community structure and its function. Therefore, the present work was conducted to study the microbial diversity from the surface of cabbage head surface by using various techniques.

## MATERIALS AND METHODS

### Sampling

Sample of cabbage (*Brassica oleracea L*. var. *capitata*) head was purchased from the local vegetable market at Pashan, Sus Road (Pune, India) in a sterilized plastic bag and immediately brought to the laboratory for processing.

### Isolation of culturable and non-culturable microbial diversity

The cabbage head was washed thoroughly in the sterilized plastic bag with sterile phosphate buffer saline (PBS) for 10 min. After washing, the 1ml of PBS buffer was used for the isolation of culturable diversity on five different media i.e. Tryptic soy agar (TSA), diluted tryptic soy agar [10 times dilution of TSA (TSAD)], R2A medium, Sabouraud dextrose agar (SDS) and MacConkey agar (MAC) by serial dilution technique. All the media plates after spreading were incubated at 30±1 ºC for 24 hrs whereas, MacConkey agar plates were incubated at 37±1 ºC for 24 hrs. All the experiments were done in triplicates. The left PBS buffer was filtered by a sterilized vacuum filter using 0.22µ membrane filter. The filtrate was discarded and filter paper was kept in sterilized eppendorf tube at -20 ºC until use. All the work was done under aspectic conditions. After incubation microbial count was taken and microbial diversity was grouped based on their morphology. For the isolation of metagenomic DNA from the membrane filter paper disc, Quamp stool DNA mini kit (Qiagen) was used by following the instructions of the manufacturer. The DNA so obtained was quantified in NanoDrop (Nanodrop Technologies, Wilmingtom, Delaware) as well as checked in the 0.8% agarose gel.

**Culturable Diversity Analysis**

**Matrix Assisted Laser Adsorption/Desorption Time of Flight Mass Spectrometry (MALDI-TOF MS)**

Diversity analysis of 95 representative bacteria of cabbage head surface was done by using Matrix Assisted Laser Adsorption/ Desorption Time of Flight Mass Spectrometry (Autoflex speed, Bruker Daltonics). Mass spectra were acquired in a linear positive ion extraction mode at a laser frequency of 200 Hz within a mass range from 2,000 to 20,000 Da. The spectrum was obtained by hitting 1000 laser shots at 10 different locations in the same isolate. The ion source 1 voltage was 19.5 kV, ion source 2 voltage was maintained at 18.2 kV, lens voltage at 7 kV, and the extraction delay time was 240 ns. The spectra was calibrated externally using the standard calibrant mixture (*Escherichia coli* extracts including the additional proteins RNase A and myoglobin, Bruker Daltonics).The MALDI Biotyper software 3.0 (Bruker Daltonik) was used to identify the isolates and to visualize the mass spectra.

**Fatty Acid Methyl Esters (FAME)**

Based on MALDI-TOF MS results out of 95 isolates, only 26 representative isolates were further subjected to FAME analysis as described by Sasser (2009). For this 20-25mg of fresh cultures (24 hrs ± 2 hrs) grown on TSA from the third quadrant were taken in glass tubes (heated in muffle furnace at 450 ºC for 2 hrs before use) with screw cap in a laminar air flow. The fatty acid was isolated from each bacterial sample as described earlier by Sasser (2009). The samples were processed in gas chromatograph (Agilent Technologies 7890A, USA) with a flame ionization detector (FID) and capillary silica column (25cm x 0.22µm with 0.33µm thickness). Hydrogen was used as carrier gas at the rate of 30 ml/min and Nitrogen as carrier gas with flow rate of 30.2ml/min. The overall pressure was maintained at 21.5 psi and split ratio was 1:40. The initial oven temperature was 170 ºC and raised to 270 ºC at the rate of 5 ºC/min. The detector and injector temperature were maintained at 300 ºC and 250 ºC, respectively.

**16S rRNA gene sequencing**

The bacterial DNA was isolated by using the phenol-chloroform-isoamyl alcohol method as described earlier by Zoetendal *et al* (2006). For the amplification of 16S rRNA gene, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Meier *et al*. 2012) primers were used with the following recipe for 25ìl reaction: Taq buffer (10X) 2.5ìl, primers 1ìl each, dNTPs 1ìl, Taq polymerase 0.2ìl, template 1ìl and PCR water 18.3ìl. Amplification was done with the following PCR conditions: Initial denaturation at 95 °C for 3 min, 35 amplification cycles with denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 7 min. The PCR product was purified, quantified in Nanodrop and sequenced in a 3730xl DNA Analyzer (Applied Biosystems, CA, USA). The sequencing data so obtained was searched in a NCBI data bank for the identification of bacterial samples.

**Unculturable Diversity Analysis**

For the analysis of unculturable bacterial diversity from the cabbage head surface, denaturation gradient gel electrophoresis (DGGE) was done for the isolated metagenomics DNA. Initially, V3 region of 16S rRNA gene fragment was amplified by using primers 341F(GC) and 516R (Dar *et al*. 2005) by using touchdown PCR (annealing temperature decreased from 66 °C to 56 °C in 10 cycles) with some modification. The PCR recipe contained 5 ìl Taq buffer, 5ìl dNTPs, 2ìl each primer, 0.6ìl Taq and final volume was made to 50ìl with PCR water and run under following PCR conditions: initial denaturation at 95 °C for 5 min, 35 amplification cycle with denaturation at 95 °C for 1 min, annealing at 66 °C for 30 sec, extension at 72 °C for 45 sec and final extension was done at 72 °C for 10 min. The PCR product so obtained was purified and run in 10% bis-acrylamide (40%) containing urea and formamide gradient from 40% to 60%. The DNA sample was loaded in the well and run at 80V for 16 hrs at 60 °C in 1X TAE buffer. Following electrophorosis, the gel was stained in solution containing SyberGold stain for 40 minutes and visualized for band patterns under gel-doc system to capture the image.

## RESULTS AND DISCUSSION

**Isolation of culturable microbial diversity**

The phyllosphere is the arial part of the plant that harbors huge microbial diversity (Whipps *et al*. 2008; Hunter *et al*. 2010). This diversity is influenced by rainfall, temperature,

radiation, type of plant species etc (Hunter *et al*. 2010) and even on the surface of same leaf the distribution pattern of microbes is uneven (Mariano and McCarter 1993; Vorholt 2012). The phyllosphere is just like a desert where nutrients availability is scarce. So, to survive under such conditions microbes has to tolerate various abiotic and biotic stresses. To cope-up and to survive under these stresses microbes secrets various species specific as well as general stress proteins, regulate carbon metabolism, regulate transporter proteins etc. (Boch *et al*. 2002; Okinaka *et al*. 2002; Knief *et al*. 2012). Cabbage (*Brassica oleracea L*. var. *capitata*) head has densely packed leaf which is generally used as cooked vegetable but it also used as a salad under raw conditions. So, the consumption of raw cabbage gives the opportunity to associated microbial diversity to get in direct contact with human gastro-intestinal tract. This direct contact of microbes may be harmful causing disease or introducing new lineages of commensal organisms in the gastro-intestinal tract or sometimes it may cause some allergies (Hanski *et al*. 2012; Leff and Fierer 2013). Keeping all this in view, different media were used in the present study to isolate the microorganisms from the cabbage head surface. As far microbial count was concerned, the highest count was obtained on diluted TSAD medium (157.66 x $10^4$ CFU/ml) followed by MAC (150.33 x $10^4$ CFU/ml) and least was observed on TSA (134 x $10^4$ CFU/ml) as depicted in Figure 1. Earlier workers also study the microbial diversity from the surface of fruits/ vegetables and isolate different genera (Jackson *et al*. 2013; Leff and Fierer 2013). Statistically there was no significant difference in microbial count ($p = 0.47$) in all the tested five different media. Based on morphology the isolates were selected from each medium for further study. The number of morphotypes obtained were 7 each on R2A, SDA, TSA and TSAD, and 9 on MAC. Based on morphotypes 95 isolates were selected for further study. In the present study nutrient rich (TSA, SDA), nutrient poor (R2A, TSAD) and enteric bacteria specific media (MAC) were used to maximize the isolation of diverse organism present

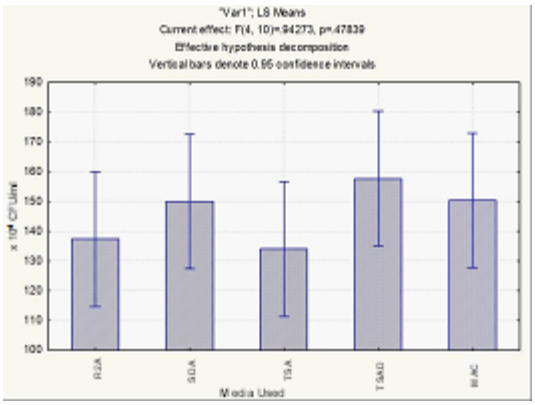


**Fig. 1.** Culturable bacterial count on the different media used

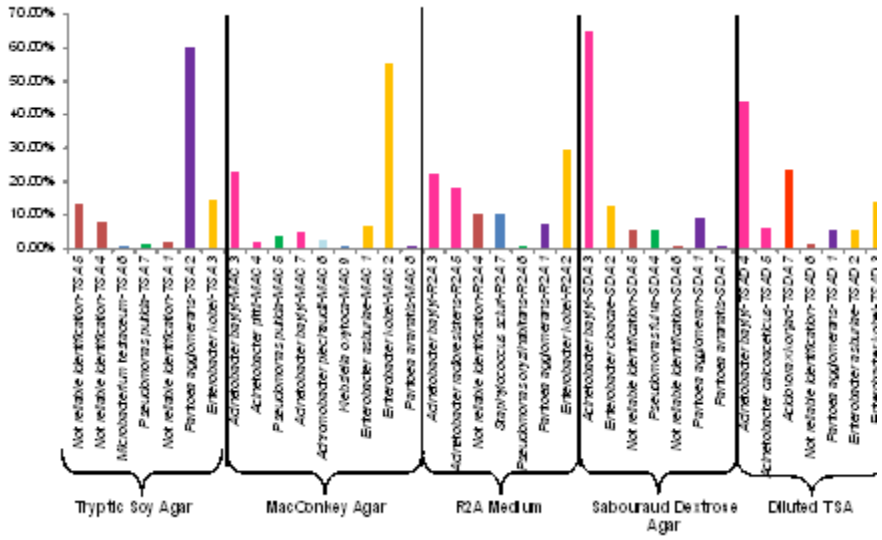


**Fig. 3.** Percent distribution of bacteria on different media

in the sample. As all the isolates were capable to grow on TSA medium so, all the cultures were maintained on this medium for further study.

**MALDI-TOF based identification**

Taxonomical classification is a very important aspect of any microbiological study. Generally, traditional methods like microscopy, biochemical characteristics, immunological features etc. are used for the identification. But these techniques are time consuming and even do not depict the reliable/reproducible results (Alvarez 2004; Salplachta *et al*. 2013). On the other hand, molecular tools are accurate but these tools are expensive, time consuming and trained man power is required. To overcome these problems MALDI-TOF is a cheap, rapid, sensitive, high resolution and efficient tool for characterizing the bacteria at species level (Welker and Moore 2011; Salplachta *et al*. 2013). In the present study, 95 isolates were subjected to MALDI-TOF analysis for the identification. The results showed that these belonged to nine genera i.e. 19 belonged to genus *Enterobacter* (gammaproteobacteria), 18 to *Acinetobacter* (gammaproteobacteria), 1 to *Klebsiella* (gammaproteobacteria), 5 to *Pseudomonas* (gammaproteobacteria), 9 to *Pantoea* (gammaproteobacteria), 1 to *Achromobacter* (betaproteobacteria), 3 to *Staphylococcus* (firmicutes), 1 to *Microbacterium* (Actinobacteria), 2 to *Acidovorax* (betaproteobacteria) and for 22 isolates not reliable identification was found. Whereas, 14 isolates did not showed any protein spectrum upon ionization with laser. As evident from the results that highest morphotypes (9) were obtained on MacConkey agar followed by SDA, R2A, TSA and TSAD which had 7 each. It was observed that the most prominent and diverse genera was *Acinetobacter* followed by *Enterobacter* and *Pantoea* (Figure 2). The results showed that most of the isolated bacteria belonged to gammaproteobacteria which shows that this class of bacteria are very efficient and can survive under adverse conditions on pyllosphere. In the study by Jackson *et al*. (2013) they also found that gammaproteobacteria and betaproteobacteria were dominant in all green leafy vegetables they have tested. Similarly, Costa *et al*. (2012) also found gammaproteobacteria high among proteobacteria from the leaves of common beans.

In MALDI-TOF soft ionization results into detection of small as well as large unfragmented intact protein molecules (Dreisewerd 2003) which

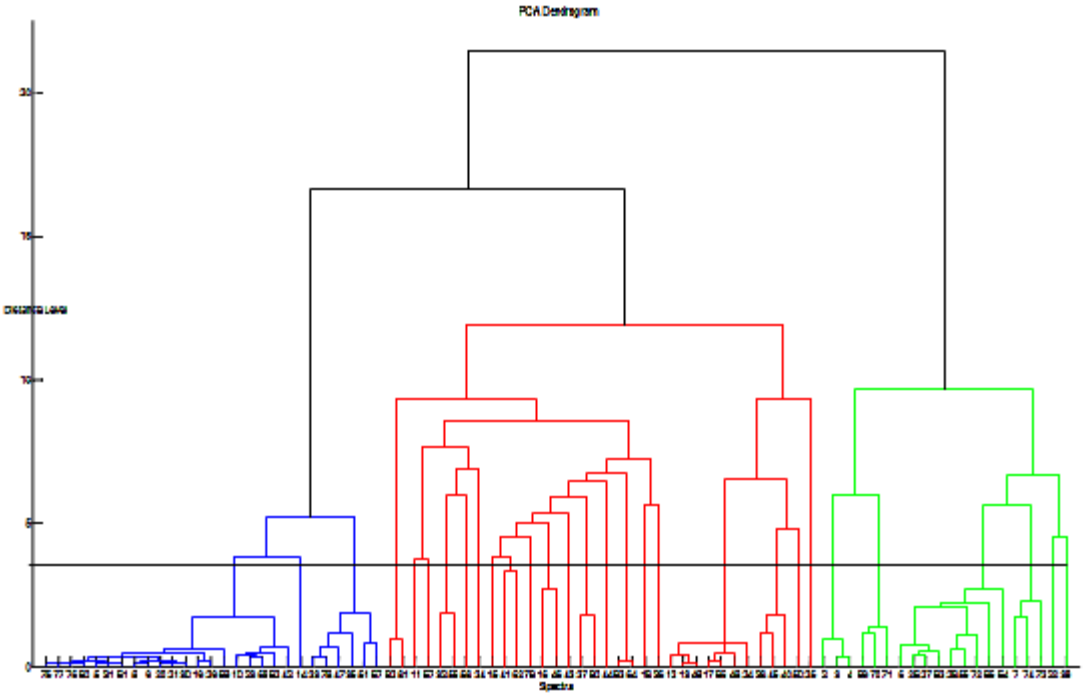


**Fig. 3.** Dendrogram based on protein profile spectra generated by MALDI-TOF MS of 81 bacterial isolates

represent the specific peak profile that is unique to that particular genus/species (Welker and Moore 2011). Based on the protein spectra of each bacterium, a dendrogram was constructed (Figure 3) at 3.5% level which grouped 81 isolates into 29 clusters. Out of these 29 clusters, representative bacteria from each cluster were studied further. Since, cluster 24, 25, 26 and 27 contained *Enterobacter* therefore only one bacteria was selected from these four clusters. For this reason 26 isolates were selected for further study (Table 1).

**FAME analysis**

Our classical identification methods are not sufficient/efficient to classify and identify microorganisms accurately as well as these methods are time consuming. So, with the advancement in the field of bioengineering many new technologies are developed which enhance our accuracy to identify and classify the microbes. One such technique is fatty acid methyl ester (FAME) analysis which is cheap, easy and automated (Slabbinck *et al*. 2009). Fatty acids are generally present in the cell wall of bacteria and are conserved for each species in respect of chain length, presence/location of double bond and presence of specific functional groups which make fatty acids excellent taxonomic marker (Dawyndt *et al*. 2006; Slabbinck *et al*. 2009). Therefore, in the present study the selected 26 isolates were further subjected to FAME analysis and their identification based on the fatty acid profile is given in Table 1. Based on fatty acid profile a dendrogram was prepared as shown in Figure 4. The isolates which grouped together in a dendrogram showed almost the same fatty acid profile. It was also evident from the results that five fatty acids i.e. 12:00, 14:00, 16:00, 18:00 and Summed Feature 3 were present in almost all the tested bacterial isolates. This showed that the all the tested bacterial isolates contain straight chain fatty acids with single bond. The isolate TSAD7(1), TSA4(2) and R2A6 showed unique composition of fatty acids that is why these isolates formed independent and separate group as shown in Figure 4.

**16S rRNA gene sequencing**

Sequencing of 16S rRNA gene has revolutionaries our knowledge about the microbial communities and helped in overcoming limitations of other traditional techniques for the identification of microorganisms (Frank *et al*. 2008). This is most common housekeeping gene which is present in

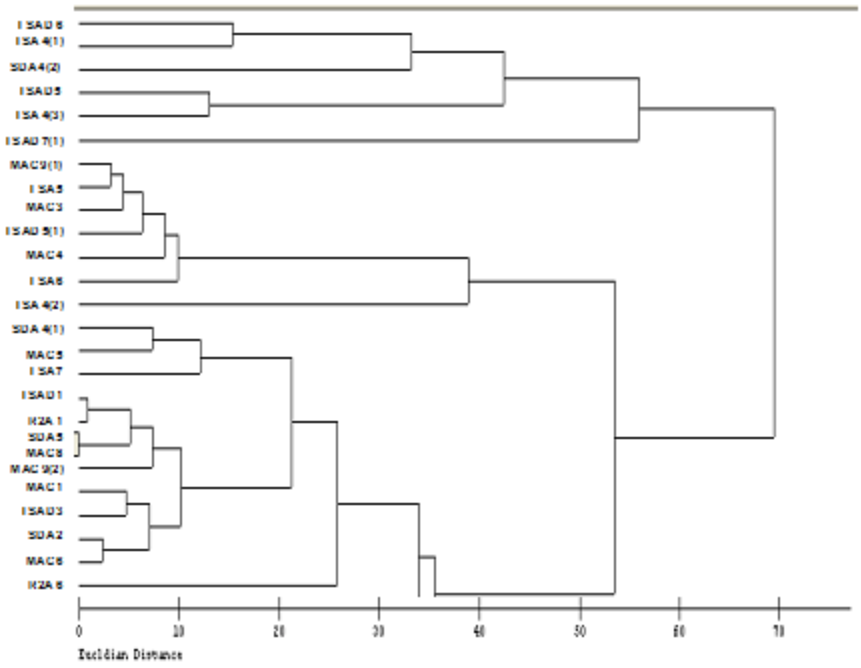


**Fig. 4.** Dendrogram of 26 isolates isolated from cabbage head surface based on their fatty acid profile

**Table 1.** Identification of 26 isolates based on fatty acid profile (FAME), protein spectra (MALDI-TOF MS) and 16S rRNA gene sequencing

| S.No. | Isolate | FAME | Similarity Index | MALDI-TOF MS | Score Value | 16S rRNA gene | % similarity | Seq. (bp) |
|---|---|---|---|---|---|---|---|---|
| 1. | MAC 8 | *Cedecea davisae* | 0.765 | *Pantoea ananatis* | 1.90 | No amplification was obtained | - | - |
| 2. | MAC 6 | *Achromobacter xylosoxidans* | 0.555 | *Achromobacter piechaudii* | 1.93 | *Achromobacter mucicolens* | 98.70 | 844 |
| 3. | MAC 5 | *Pseudomonas putida* | 0.253 | *Pseudomonas putida* | 1.99 | No amplification was obtained | - | - |
| 4. | MAC 4 | *Acinetobacter genomospecies 3* | 0.545 | *Acinetobacter pittii* | 2.21 | *Acinetobacter oleivorans* | 99.73 | 741 |
| 5. | MAC 3 | *Acinetobacter calcoaceticus* | 0.520 | *Not Reliable Identification* | - | No amplification was obtained | - | - |
| 6. | MAC 1 | *Salmonella enterica* | 0.754 | *Enterobacter cloacae* | 2.32 | *Enterobacter cancerogenus* | 99.62 | 796 |
| 7. | SDA 5 | *Escherichia coli* | 0.840 | *Not Reliable Identification* | - | *Pantoea eucrina* | 100.00 | 989 |
| 8. | SDA 4 R3 | *Staphylococcus epidermidis* | 0.228 | *Not Reliable Identification* | - | No amplification was obtained | - | - |
| 9. | SDA 2 | *Shigella boydii* | 0.843 | *Enterobacter cloacae* | 2.31 | *Enterobacter asburiae* | 99.19 | 861 |
| 10. | SDA 4 R1 | *Pseudomonas putida* | 0.387 | *Pseudomonas fulva* | | *Pseudomonas cremoricolorata* | 100.00 | 570 |
| 11. | R2A 1 | *Morganella morganii* | 0.764 | *Pantoea agglomerans* | 2.20 | No amplification was obtained | - | - |
| 12. | R2A 6 | *Flavimonas oryzihabitans* | 0.879 | *Pseudomonas oryzihabitans* | 2.35 | *Pseudomonas oryzihabitans* | 97.85 | 319 |
| 13. | TSA 4 R1 | *Cellulosimicrobium cellulans* | 0.382 | *Staphylococcus hominis* | 2.01 | No amplification was obtained | - | - |
| 14. | TSA 4 R2 | *No Match Found* | - | *Not Reliable Identification* | - | No amplification was obtained | - | - |
| 15. | TSA 4 R3 | *Microbacterium imperiale* | 0.163 | *Microbacterium testaceum* | 2.13 | No amplification was obtained | - | - |
| 16. | TSA 5 | *Acinetobacter calcoaceticus* | 0.479 | *Acinetobacter baylyi* | 2.08 | No amplification was obtained | - | - |
| 17. | TSA 6 | *Acinetobacter calcoaceticus* | 0.648 | *Acinetobacter baumannii* | 2.08 | No amplification was obtained | - | - |
| 18. | TSA 7 | *Pseudomonas putida* | 0.565 | *Pseudomonas putida* | 2.47 | No amplification was obtained | - | - |
| 19. | TSAD 1 | *Cedecea davisae* | 0.733 | *Pantoea agglomerans* | 2.11 | *Pantoea anthophila* | 98.75 | 802 |
| 20. | TSAD 3 | *Shigella-boydii* | 0.851 | *Enterobacter kobei* | 2.35 | *Enterobacter asburiae* | 98.92 | 798 |
| 21. | TSAD 5 R1 | *Acinetobacter calcoaceticus* | 0.340 | *Acinetobacter radioresistens* | 1.76 | *Acinetobacter soli* | 99.65 | 1139 |
| 22. | TSAD 6 | *Arthrobacter nicotianae* | 0.696 | *Not Reliable Identification* | - | *Arthrobacter nicotianae* | 100.00 | 906 |
| 23. | TSAD 7 R1 | *Staphylococcus lentus* | 0.660 | *Staphylococcus sciuri* | 2.04 | *Staphylococcus sciuri* | 99.26 | 678 |
| 24. | TSAD 7 R3 | *Acidovorax avenae* | 0.960 | *Acidovorax avenae* | 1.81 | *Acidovorax oryzae* | 99.32 | 1172 |
| 25. | TSAD 5 R2 | *No Match Found* | - | *Microbacterium testaceum* | 2.04 | *Microbacterium hominis* | 97.36 | 1375 |
| 26. | MAC 9 | *Salmonella enterica* | 0.824 | *Klebsiella oxytoca* | 2.30 | *Klebsiella michiganensis* | 100.00 | 661 |

all bacteria, conserved in nature and large enough to provide sufficient information for the bacterial phylogeny and taxonomy (Janda and Abbott 2007). The studies of this universal gene i.e. small subunit rRNA gene not only provide the identification of culturable organisms but also provide deep insight into non-culturable bacteria (DeLong 2004; Frank *et al*. 2008). This marker is used extensively worldwide therefore, in the present study 26 isolates were further identified by 16S rRNA gene sequencing and only for 15 isolates the sequencing results were obtained (Table 1). As evident from the Table 1 that bacterial isolates identified by MALDI-TOF MS, FAME or 16S rRNA gene sequencing were almost matched at the genus level but some discrepancies were their specially between FAME and 16S rRNA gene sequencing identification. The isolate MAC1 was identified as *Enterobacter cancerogenus* on the basis of 16S rRNA gene sequencing but FAME based identification was *Salmonella enterica*. Similar discrepancies were also observed for isolates SDA5, SDA2, TSAD3 and MAC9. The reasons for this could be that the FAME technique is dependent on commercial identification system such as Sherlock Microbial Identification System (MIDI) and the back end libraries of these systems are updated after every few years therefore the accuracy of identification of this system is suspicious (Slabbinc *et al*. 2009). In the present study based on 16S rRNA identification few isolates were found to be opportunistic human pathogens i.e. *Enterobacter cancerogenus* may sometime associated with wound infection and bacteremia (Abbott and Janda 1997), *Enterobacter asburiae* may cause wound infection (Koth *et al*. 2012), *Staphylococcus sciuri* may be responsible for septic shocks, endocarditis, wound infection, peritonitis (Chen *et al*. 2007).


**Fig. 5.** DGGE profile of V3 region of 16S rRNA gene fragment of bacterial community from the cabbage head surface (S: Sample)

**DGGE Profiling**

It is well known fact that very small fraction of microbial isolates can be cultured under lab conditions (Muyzer and Smalla 1998). So, for the better understanding of microbial diversity in any community traditional approaches should be complemented with new approaches. In recent year's advances in molecular tools helped in the study of culturable as well as non-culturable diversity which helped in the exploration and analyzing the microbial community structure (Sun *et al*. 2013). Among these molecular tools, genetic fingerprinting technique is one of the important tools but to analyze the whole microbial community (culturable and non-culturable) structure, denaturing gradient gel electrophoresis (DGGE) is excellent technique to get the overall idea regarding community structure (Muyzer and Smalla 1998). In DGGE, PCR amplified product of the universal conserved region (16S rDNA, *rpoB*, ITS etc.) is used for generating community profile (Casamayor *et al*. 2002; Blackwood *et al*. 2005; Rosier *et al*. 2015). DGGE is capable of separating the two DNA molecules of same length with different sequence, even single base pair change can be detected by it (Muyzer and Smalla 1998). In case of DGGE the separation is based upon electophoretic mobility of the DNA in a PAGE which has linear gradient of denaturants (urea and formamide). The DNA molecule when reaches its melting temperature at a particular position in PAGE, its helical and partially melted structure is stuck at that particular location. So, the DNA molecules with different sequences have different melting temperature and they will halt at different position in PAGE. The number of DNA bands in DGGE represent the microbial diversity whereas, the intensity of bands represent their abundance (Brons and van Elsas 2008; Marzorati *et al*. 2008). The PCR-DGGE is capable to detect the 95-99% of bacterial community diversity. So, in the present study, PCR amplified product of V3 region (200 bp) of 16S rDNA was

subjected to DGGE. The results (Figure 5) clearly showed that the cabbage head surface was dominated by seven genera but the intensity of 3 bands (band 1, band 2 and band 4) was higher than the other four bands. Band 2 showed highest intensity followed by band 4 and then band 1 which showed their relative abundance in the tested sample.

In conclusion, the culture dependent and culture independent techniques are very important to reveal the diverse microbial community present in the sample. In the present study some opportunistic human pathogens were detected so care should be taken while consuming the raw vegetables especially as salad. So, fresh produce (fruits and vegetables) should be properly washed before consuming as it may harbor pathogenic/ opportunistic microbes on their surface.

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