World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 8, Issue 9, 2019 ISSN: 2319-5622 USA CODEN: JPROK3

Review Article

METAGENOMICS INSIGHT: ENHANCING FERMENTED FOOD QUALITY FOR HUMAN HEALTH

Vandana Tyagi [1], Harleen Kaur [2], Shriya Agarwal [1], Kanupriya Jha [1], Manisha Singh [1] *

*[1] Department of Biotechnology, Jaypee Institute of Information Technology, JIIT, Noida, U.P, INDIA.

[2] Amity Institute of Biotechnology, Amity University, Noida – 201304, INDIA.

Received on: 22-07-2019; Revised and Accepted on: 17-09-2019

ABSTRACT

Food fermentation process delivers many benefits including enhanced quality of food products, better microbial stability, safety and higher shelf life. Also, there are many examples of fermentation processes which led to an increase in the digestibility and nutritional value of raw food materials. The safety of the substrate can be enhanced by reducing toxins and anti-nutritive factors of the substrate. In addition, recent studies substantiated biological functions of fermented foods made due to functional microbes involved in the course of fermentation which were reported to be responsible for numerous health benefits to the consumers. Thus, fermented foods fulfill requirements of consumer's nutritional and fitness needs. Similarly, it's been reported in many studies that fermentation by lactic acid bacteria allows the storage of food for longer periods of time & thus, enhances the nutrient value which improve the digestibility, quantity, and availability of some dietary nutrients. Therefore, many different approaches are designed to identify and classify unidentified microbes to know about their physiology and genetics. Metagenomics in today's research scenario is considered to be one of the most successful methods for identification of uncultivable microbes for their genomics analysis. This approach helps in isolating the genomic DNA from their natural habitat and culturing them in lab condition and cloning into host microbes for further structural analysis. This approach equally can be used as a method for screening of fermentation processes for improving the quality of fermented foods as limiting impacts of organic substitutes may hinder the microbial cell layer properties. Throughout fermentation of food, the cooperation between the micro flora and the maturing nourishment network makes a dynamic wonder, at both microbial and biochemical levels, portrayed by changes in physicochemical conditions (pH, salt, temperature, and so on). Hence, in the present study we focused on analyzing the impact of metagenomics approach in improving the quality and sustainability of fermented food products.

KEYWORDS: *Fermented Foods, Health Benefits, Nutrition, Metagenomics.*

INTRODUCTION

Fermentation is the oldest technology for the production of food products with desirable properties such as increased shelf life, digestibility, nutritional value, improved aroma, and organoleptic properties along with improved microbial stability, safety and can be stored at ambient temperatures [1, 2]. Additionally, recent studies revealed enhanced biological functions of fermented foods attained due to functional microbes involved in the course of fermentation process hence, leading to numerous health benefits to the consumers. The latest updates about various diversities of fermented foods promises to fulfill almost all the nutritional and fitness requirements of consumer's need shall over the world. Traditionally fermented foods are often used by natives from Asian and African countries in their diets, due to their high functional importance and nutritive value [3]. In these regions, the ethnic or aboriginal population makes fermented food using locally available raw materials from varied sources of plants and animals like millets, bamboo shoots, milk, beans, vegetables, cereals, Fish or shrimp etc. and their preparation methods may vary according to their geographical location and eating habits of natives. The traditional knowledge of making fermented foods is acquired from one generation to another, though in today's scenario it's been more modified as per the industrial needs [4] The methods for the industrialization of fermented food products from substrates such as fruits, meat, milk, cereals, and vegetables are well established now and technical mechanism are actively carried out all over the world [5] Among all classes, a dairy-based fermented food like yogurt and buttermilk bases (sweet/sour) has established a huge fanfare in global food market. Though there are large varieties of millet and cereal-based fermented foods available but only few foods products like kenkey, ogi and sourdough are known as a reliable food sources for making buttermilk bases. There are numerous

*** Corresponding author:**
Manisha Singh
Department of Biotechnology,
Jaypee Institute of Information Technology,
JIIT, Noida, U.P, INDIA.
** E-Mail: manishasingh1295@gmail.com*

DOI: https://doi.org/10.5281/zenodo.3557308

health benefits of fermented foods such as the probiotics drinks (kombucha and kefir)not only introduces good bacteria's in gut which leads to improved and balanced digestive system, bowel health and immunity but also, adequate secretion of digestive enzymes in gut that helps in better absorption of nutrients from fermented foods. Further, they are noted to be less expensive than other nutritional supplements and efficiently provide required nutrition thus, qualifies for being budget friendly healthy functional food. Apart from this, many research studies have reported their anticancer effects mechanisms by reducing the exposure to chemical carcinogens, producing compounds that prevent the development of tumor cells, reducing metabolic activities and bacterial growth responsible for carcinogens. The traditional fermented foods preparation in India consist of millet and cereal-based pancakes, porridges, alcoholic and non-alcoholic beverages, breads etc. [6]. According to Sekar and Mariappan (2007), Indian fermented foods are prepared with potential knowledge of ethnic individuals and have due market value along with their health benefits which are now well understood owing to their prior documentation of native preparations.

Insistence for genomics to metagenomics transformation:

Scientists' ability to bring microbes from their natural surroundings into laboratory culture conditions are studied by Staley and Konopka in 1985. They reviewed the data of the "great plate count anomaly" and identified as the large population of microbial cells that can be seen in a microscope and shown by staining procedures cannot be grown on Petri plates and not viable in lab condition [7]. It is identified that only 0.1-1% population of microbes are cultured in standard conditions. The use of genomics-inspired in high-throughput nutrient selection methods and nontraditional methods for observing the growth of microbes in culture will bring many unmanageable microbes into a culture. There are some microbes which can be cultivated by isolation likely to grow very less and a reason for that will be, for growth they require community. A culture-dependent condition may favor the growth of microbes that are able to proliferate in lab condition, not all organisms which are dominant are influential in the environment.

Researcher's shows evidence which shows that many microbes resist being cultivated in lab condition, to identify those microbes culture-independent methods are used for classifying and computing microbes in the environment which plays a large role from several decades. Main technique among them is ribosomal RNA (rRNA) phylotyping [8]. This method relies on the vast databank of rRNAgene sequences (more than 200,000) that is collected to reconstruct the tree of life and identify the unidentified microbes. By comparing sequences of an organism's rRNAgenes with these database helps to place those microbes in the branch of the tree of life and conclude that its ecology and biology are likely to be related to those of its closest relatives, the nearby subdivisions on the hierarchy. An organism does not have to be cultivable to define its phylotype or taxonomy. The polymerase chain reaction (PCR) allows detection of rRNA (or other) genes and isolated directly from environmental samples, then cloned and sequenced. In an environmental sample, there are different types of microbes, which have different rRNAsequences which are a measure of the complexity of the community which is in the context of the hierarchy of the tree or phylogram which helps in determining "who is there." Phylogenetic analysis and Community rRNA sequencing, in itself, is not considered metagenomics (because it emphases on a single gene, not whole genomes), but it is a useful initial step in a metagenomic analysis and provides a phylogenetic valuation of the diversity of a community.

Metagenomics for fermented foods:

Different methods are designed to classify or identify unknown microbes and to know about their genetics and physiology, metagenomics, are a most successful technique for genomic analysis. Isolating genomic DNA from environment circumvents and culturing the microbes under an optimized condition, and cloning it into a cultures host microbes' helps to study and preserve. Metagenomic have different approaches to study about the microbes that are sequence based and functional based.

The term metagenomics was coined to capture the view of examination of an assembly of similar but not identical things, as in a meta-analysis, which is an analysis of analyses. (Community genomics, environmental genomics, and population genomics are synonyms for the same approach.) In 1991 Pace proposed the concept of cloning DNA directly from environmental samples, the first cloning was reported in phage vector [9]. Advancement in the metagenomic analysis leads to the construction of a metagenomic library with DNA Extracted from the different organism grown in a laboratory on dried grasses. Clones which express cellulolytic activities in host organism referred as zoo libraries, a term that has not been used usually in the field. This field was determined by the group of DeLong's group work when they reported libraries constructed from prokaryotes from seawater. They sequenced 16s rRNAgene from 40-kb clone indicating clone was derived from an Archaea which is not cultured before. Construction of libraries from DNA extracted from soil is difficult which is associated with the maintenance of the integrity of DNA during extraction and purification of DNA.

Table No. 1: Ethnic fermented foods along with their substrate and fermenting microorganisms [10]

S. No.	PRODUCT	PLACE OF ORIGIN	SUBSTRATE	ORGANISM
			CEREAL BASED	
1	**Koozhu (Khanji)**	Tamil Nadu	sorghum, pearl millet, little millet and foxtail millet.	*Weisella paramesenteroides* with probiotic properties and *Lactobacillus fermentum* with antibacterial activity
2	**Fermented rice (Pazhaiya soru)**	Tamil Nadu	Rice, Curd and salt	*Streptococcus faecalis, Pediococcus acidilactici, Bacillus sp. and Microbacterium flavum.*
3	**Idli**	South India	Rice	*Leuconostoc mesenteroides and Streptococcus faecalis Saccharomyces cerevisiae, Debaromyces hansenii, Hansenula anomala, Torulopsis candida, Trichosporon beigelii and Pediococcus cerevisiae.*

4	**Ambali**	India	Ragi flour and rice	*L. mesenteroides, L. fermentum and Streptococcus faecalis.*
5	**Siddhu**	Himachal Pradesh	Wheat flour	*malera* (yeast)
6	**Chilra**	Himachal Pradesh	Wheat/barley and buckwheat flour	*starter- Malera; Saccharomyces cerevisiae, Debaromyces hansenii, Schizosaccharomyces sp*
7	**Marchu**	Himachal Pradesh (Lahaul)	Wheat flour	Inoculum- *Malera microorganims - not reported*
8	**Pinni**	Kullu, Kangra, Mandi, Chamba and Shimla	Barley flour	Not reported
9	**Parotta**	South India	Wheat flour	Not reported
10	**Sez**	Uttaranchal	Rice	Not reported
11	**Chakuli**	Orissa	pulse	Not reported
12	**Jilebi**	south India	Wheat	*L. fermentum, S. lactis, Lactobacillus buchneri S. faecalis and Saccharomyces bayanus*
13	**Gulgule**	Himachal Pradesh	Wheat Flour	Lactic acid bacteria
			PULSE BASED	
14	**Kinema**	Darjeeling and Sikkim	Soybeans	*Bacillus subtilis*, *B. licheniformis, B. circulans, B. thuringiensis and B. sphaericus*
15	**Tungrymbai**	Meghalaya	Soybeans	*Bacillus subtilis, LAB and yeast*
16	**Axone**	Northern east India	Soybeans	*Bacillus subtilis*
17	**Bekang**	Mizoram	Soybeans	*Bacillus Subtilis*
18	**Masyaura**	Darjeeling and Sikkim	Black gram	*Pediococcus pentosaceous, Pediococcus acidilactic, and Lactobacillus sp. Saccharomyces cerevisiae and Candida versatilis, Cladosporium sp., Penicillium sp. and Aspergillus niger*
			MILK BASED	
19	**Curd (Dahi, Thayir) Shrikhand, Lassi, Dahi kusum, Rasgullas, Misti dahi**	India	Milk	*Streptococcus cremoris, S. lactis, S. thermophilus, Lactobacillus bulgaricus, L. acidophilus, L. helveticus, and Lactobacillus cremoris*
20	**Chhurpi**	Arunachal Pradesh	Yak Milk	*Lactobacillus plantarum, L.curvatus, L. fermentum, L. paracasei subsp. pseudoplantarum, L. alimentarius, L. kefir, L. hilgardii, Enterococcus faecium and Leuconostoc mesenteroides*
21	**Chhur chirpen**	Arunachal Pradesh	Yak Milk	*Lactobacillus* species
22	**Chhu**	Sikkim	Yak Milk and Cow milk	*Lactobacillus farciminis, Lactobacillus brevis, Lactobacillus alimentarius* and *Lactococcus lactis* subsp. *cremoris*
23	**Philuk**	Sikkim	Yak Milk and Cow milk	*Lactobacillus casei* subsp. *casei, Lactobacillus bifermentans* and *Enterococcus faecium*
			VEGETABLE BASED	
24	**Gundruk**	Arunachal Pradesh	Leaves of raddish/ cauliflower	*Pediococcus pentasaceous, Lactobacillus cellubiosus and Lactobacillus plantarum*
25	**Sinki**	North east India	Raddish roots	*Lactobacillus fermentum initiates the fermentation in sinki production, followed by Lactobacillus brevis and Lactobacillus plantarum*
26	**Sauerkraut**	India	Cabbage	*Started by L. mesenteroides and completed by L. plantarum.*
27	**Soibum**	Manipur and Nagaland	Bamboo shoots	*Lactobacillusplantarum, L. brevi, L. corniformis, L. delbrueckii, Leuconostoc fallax, L. lactis, L. mesenteroides, Enterococcus durans, Streptococcus lactis, Bacillus subtilis, B.licheniformis, B.coagulans and yeast Candida, Saccharomyces and Torulopsis*
28	**Soidun**	Manipur	Bamboo shoots	*Lactobacillus brevis, Leuconostoc fallax, L. lactis*
29	**Hiring**	North east India	Bamboo shoots	*Lactobacillus plantarum* and *Lactococcus lactis*
30	**Ekung**	Manipur	Bamboo shoots	*Lactic acid bacteria (Lactobacillus plantarum, L. brevis, L. casei, Tetragenococcus halophilus) and yeast*

31	**Eup**	Arunachal Pradesh	Bamboo shoots	*Lactobacillus plantarum* and *L. fermentum*
32	**Khalpi**	Sikkim	Cucumber	Lactic acid bacteria
33	**Goyang**	Darjeeling and Sikkim	Cardamine macrophylla leaves	*Lactobacillus plantarum, L.brevis, Lactococcus lactis, Enterococcus faecium,* and *Pediococcus pentosaceus*
34	**Ziangsang**	Nagaland and Manipur	Mustard leaves	*Lactobacillus plantarum, L. brevis* and *Pediococcusacidilactici*
35	**Kanji**	North India	Carrot crushed mustard seeds and hot chilli powder	Lactic Acid Bacteria

Strategies for Metagenomic analysis:

Annotation of metagenomics includes the collection of sample from their natural environment and DNA isolation of MO's from sample followed by its purification. Afterward its ligation into suitable vector, chimera is then transformed into suitable host bacteria for amplification, the screening of host bacteria containing desired gene (Fig.1). Selection and screening is carried out for the identification of phylogenetic markers such as 16s rRNA, or identification of conserved regions for its taxonomy or phylogenetic analysis. These different methods allowidentifying or understanding the uncultured world of microbes, providing sight into a vast community of microbes that are not known to this world [11].

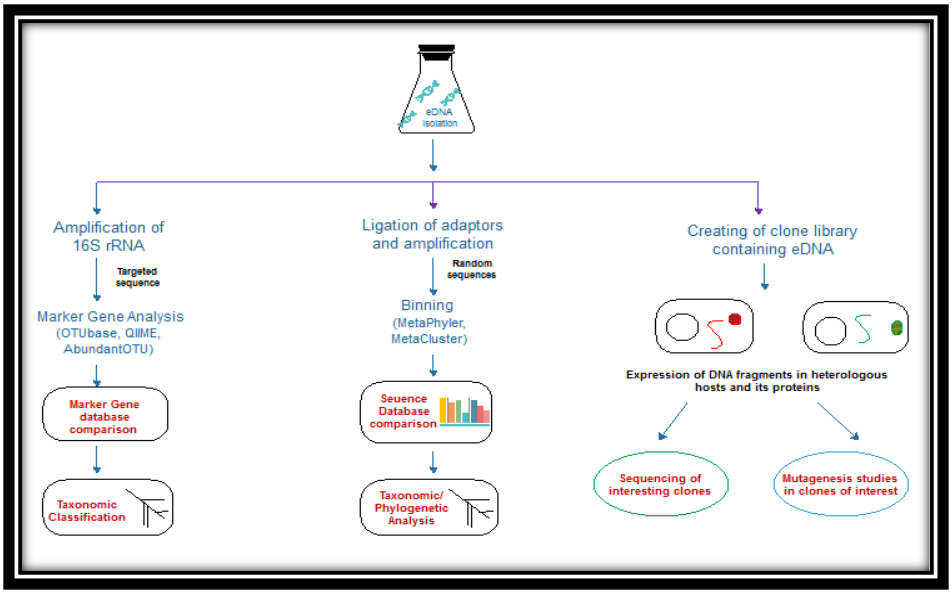

Fig. 1: Metagenomic analysis for the identification of unknown bioactive and biocatalyst from environmental sample

Sequence- Based analysis:

The sequence-based study includes the whole sequencing of clones containing phylogenetic markers which contain the information about taxonomy community which is a source of DNA [12]. On the other hand, to identify the gene of interest random sequencing can be done, once gene of interest is identified, phylogenetic markers are searched into a flanking DNA to provide information of phylogeny with the functional information of gene. DeLongs' group was the first group who identified the phylogenetic markers by sequence analysis and produces the first genomic sequence which is linked to 16S rRNA gene of an uncultured microorganism. Afterward, they recognizedagene from sea water bacteria having 16S rRNAgene that associated with the γ-*Proteobacteria*. Flanking DNA sequence reveals a bacteriorhodopsin-like gene. Its gene product was appeared to be a credible photoreceptor, prompting the understanding that bacteriorhodopsin qualities are not constrained to Archaea but rather are in actuality inexhaustible among the Proteobacteria of the sea [13, 14].

A promising use of phylogenetic marker-directed sequencing is to gather and sequence numerous genomic fragments from one taxon. In more complex environments and taxa, reassembly of a genome may not be possible, but inference about the physiology and ecology of the members of the groups can be collected from sequence data. Sequencing technique has been introduced with clones from different soil samples carrying 16S rRNA genes that associate with the *Acidobacteria* phylum, which is present in large number in soil and highly diverse and less known [15-17]. The whole sequencing of the genome of the estimated ≈500 kb of Acidobacterium DNA in metagenomic libraries may provide understanding about subgroups of bacteria under Acidobacterium phylum which is not cultured before [18].

On the other hand sequencing of random clones is an alternative approach to phylogenetic marker directed approach especially when conducted at large scale. The termination of functions in a community and distribution, relationship of qualities, genomic makeup, and gene transfer can all be identified from the sequence-based analysis. Linkages between phylogeny and function indicate the presence of plenty genes and remodeled the genomes of organisms that cannot be cultured [19].

Phylogenetic markers used either as indicators of taxonomic affiliation for DNA fragments carrying gene of interest or as the initial identifiers of DNA fragments because small number of markers are available to depict their function which provide reliable place in the tree of life. If gene of interest does not carry a dependable marker for identification then origin or organisms remain unknown. The accumulation of phylogenetic markers is developing, and as the assorted variety of markers expands, the energy of this approach will likewise build, making it conceivable to allot more sections of mysterious DNA to the living beings from which they were secluded. Additionally, as more genomes are reproduced, more qualities will be connected to phylogenetic markers despite the fact that they were not cloned at first on a similar part.

Functional metagenomics:

Heterologous expression:

Identification of clones which express their function through metagenomic analysis is a powerful approach. The success of metagenomic analysis depends on successive transcription and translation of the desired gene and required gene product if assay requires it to be extracellular. Functional analysis of genome has identified antibiotic resistance gene, degradative enzymes, novel antibiotics, and Na+(Li+)/H+ transporters. The main benefit of this method is that it does not require the previous analysis of its sequence or comparison to a database it is the approach of metagenomics which discovers the completely new class of genes for new or known functions. The limitation of this approach is that many genes, perhaps most, will not be expressed in any specific host bacterium selected for cloning and express in their natural environment which cannot be studied using functional analysis. In fact, there is an inherent flaw in this approach genes cloned from mysterious organisms to learn about new motifs in biology, and yet these genes need to be expressed in *E. coli* or another host bacterium in order to be detected. An organism whose DNA is successfully expressed in *E.Coli* is identified, but heterologous expression remains an obstacle to mining the concentrated information from functional metagenomics analyses [20].

Identifying active clones screens, selections, and functional anchors:

Expression shown by metagenomic clones if very low. For example, sample collected from German soil in search of lipolytic activity, only 1 in 730,000 clones showed activity [21]. In North America library of DNA sampled from soil, 29 of a total of 25,000 clones shows hemolytic activity [22]. The lack of active clones therefore demands improvement of efficient selection and screening method for discovery of new molecules and new activities. Just as microorganism genetic science depends on choices to notice low-frequency events, advancement in metagenomics looking for selectable phenotypes to enhance the collection of active clones which can be analyzed, compared and used to frame a functional analysis [23].

Numerouscollections proved to be successful. For example, the Daniel group designed a clever selection for $Na^+(Li^+)/H^+$ antiporters that requires complementation of an *E. coli* mutant deficient in the three Na^+/H^+ antiporters (*nhaA*, *nhaB*, and *chaA*) enabling growth on medium containing 7.5 mM LiCl [24]. This powerful selection facilitated the discovery of two novel antiporter proteins in a library of 1,480,000 clones containing DNA isolated from soil. Another selection strategy involved complementation of an *E. coli* mutant deficient in biotin production, which led to the isolation of seven new operons for biotin synthesis from enrichment cultures derived from samples of horse excrement or soil [25].

Choice for anti-microbial protection prompted the detachment of an antibiotic medication protection determinant from tests of the microbiota from the human mouth [26] and aminoglycoside protection determinants from soil [27]. The determination for aminoglycoside protection distinguished nine clones, six of which encoded 6′-acetyltransferases that framed another group in light of arrangement examination. These qualities were found in libraries containing a sum of 4 Gb of DNA, or around 1 million qualities, and subsequently their occasional portrayal would have made it restrictively arduous to find them by a screen without a choice. This case shows the energy of practical metagenomics qualities that are communicated in a conventional host, for example, E. coli might be exceptional and novel.

When selection cannot be carried out by functions of interest then it is substituted by High-throughput screens. For example, some active clones display a functional chacteristic when applied with a specific indicator and can distinguished by their appearance on a plate. Tetrazolium chloride indicator dye used on screened clones which consume 4-hydroxybutyrate in libraries of DNA from river valley soil and agricultural sample [28]. Very less clones of lipolytic activity in the same libraries were detected by making of clear halos on media containing triolein or tributyrin and rhodamine [29].

The revelation of new natural themes will depend to a limited extent on a practical investigation of metagenomic clones. Functional screens of metagenomic libraries have prompted the task of capacities to various "theoretical proteins" in the databases. Advancement will be required to distinguish and beaten the hindrances to heterologous quality articulation and to identify uncommon clones productively in the massive libraries that are expected to speak to the majority of the genomes in complex conditions, for example, soil. A rising and capable course for metagenomic examination is the utilization of useful markers, which are the practical analogs of phylogenetic stays. Functional markers have capacities that can be surveyed quickly in the greater part of the clones in a library. At the point when an accumulation of clones with a typical capacity is amassed, they can be sequenced to discover phylogenetic markers and genomic structure in the flanking DNA. Such an examination can give a cut of the metagenome that cuts crosswise over clones with an alternate specific device, deciding the diverse variety of genomes that contain a specific capacity that can be communicated in the host conveying the library. Innovative improvements that advance useful articulation and screening will propel this new wilderness of useful genomics [30].

Application of Metagenomics to Fermented Foods:

Metagenomics for monitoring fermentation process:

Aging, for the most part, begins with a high microbial decent variety which relates to the microbial contamination of crude materials, human control, and process equipment. Fermentation is described by change cations of the nourishment organization: alcoholic aging outcomes from the transformation of sugars into ethanol and carbon dioxide; Lactic acid fermentation produces lactic acids and perhaps, carbon dioxide, acetic acid, and ethanol; acidic corrosive aging outcomes principally in acidic corrosive development. All through the aging procedure, the cooperation between the microflora and the maturing nourishment network makes a dynamic wonder, at both microbial and biochemical levels, portrayed by changes in physicochemical conditions (pH, salt, temperature, and so on). All these biochemical alter cations share the normal element of creating pressure factors for microorganisms. The

unfriendly impacts of ethanol result from hindrance of microbial cell layer properties [25]. An expansion of natural corrosive levels produces a scattering of the proton slope crosswise over cell films [26]. The creation of the crude material may remove a portion of the ecological worry: for instance, high sugar levels result in an osmotic pressure. Additionally, sustenance planning, for example, salt option performed toward the start of the aging procedure, may add to the pressure impact. As an outcome, a reduction of microbial decent variety is for the most part seen over maturation advance, and a prevailing microflora shows up. This is represented by numerous cases through various methodologies.

For instance, bread is customarily produced using sourdough which is a blend of flour and water containing yeasts and microorganisms. The principal contemplates that considered sourdough assorted variety depended on social methodologies finished by sub-atomic ID of overwhelming species. RAPD (Randomly Amplified Polymorphic DNA) and 16S ribosomal RNA coding district sequencing were utilized to decide the predominant types of lactic acid microorganisms[27] Thereafter, PCR-DGGE (Denaturant Gradient Gel Electrophoresis) was utilized as a part of a few examinations [28].

Over glutenfree sourdough elaboration process, a steady biota with an expansive range of autochthonous lactic acidmicrobes and yeast species was set up, as this biota was adjusted and focused in these conditions. Ruling species were regularly secluded from different kinds of sourdough (rye or wheat) or from other tropical item maturations. For instance, *Pediococcus pentosaceus, Leuconostoc holzapfelii, Lactobacillus gallinarum, Lactobacillus graminis, Lactobacillus vaginalis, Weissella cibaria* were identified however they were not considered as regular of sourdough maturation. Different techniques like PCR-TGGE (Thermal Gradient Gel Electrophoresis) and High Resolution Melting quantitative PCR (HRM-qPCR) were utilized as a part of different examinations [29]. The utilization of PCR-TGGE was exhibited as another productive way to deal with a screen the principle fermentative species in sourdough. The creators underlined the need for a watchful decision of enhancement locales and inspecting, and of basic and reproducible DNA extraction, PCR intensification and investigation before reaching any inference. The upsides of HRM-qPCR depend on the effortlessness of the strategy and its capacity to identify single nucleotide contrasts in target groupings. Notwithstanding, this approach would not be suitable for complex microbiota whereby numerous pinnacles are available, relating to various dissolving temperatures of target arrangements.

For the most part, the last aged item comes about because of countless responses caused by microorganisms. The case of cocoa bean maturation is especially persuading as alcoholic aging, lactic aging lastly acidic aging happen progressively. The underlying anaerobic conditions at low pH (3.6) and high sugar substance of the mash encompassing the cocoa beans (got after the breaking of the case) advance yeast movement (normally present or presented by people). In the mash, sugar transformation into liquor and carbon dioxide causes an ascent in temperature and an expansion in pH because of the utilization of the citrus extract by yeasts. These progressions advance the beginning of lactic corrosive microbes (LAB) that oxidize the liquor to lactic corrosive. As conditions turn out to be more vigorous (because of air circulation), the generation of acidic corrosive by acidic corrosive microorganisms (AAB) is favored. The development of acidic corrosive from liquor is an exothermic response and the temperature comes to around 50ºC; this temperature move causes inactivation of acidic corrosive microscopic organisms. What's more, because of the nearness of acidic corrosive, the biochemical responses which deliver forerunners of chocolate enhance mixes can happen in the seed. Towards the finish of the maturation, the solid smell of acidic corrosive reductions logically. Other fragrant particles (alcohols, acids, ketones, sulfur mixes) are additionally created by the microorganisms amid maturation, and present organoleptic characteristics normal for the item[30]The yeasts and bacteria both are engaged in the creation of mixes in charge of the chocolate season [31]. If not performed well, aging can prompt genuine deformities, for example, beans with slate shading without seasoning, intense taste and astringent or purple beans, and in addition ineffectively sweet-smelling or spoiled beans.

In spite of the fact that writing on an intensive portrayal of the microbial species engaged with cocoa maturation is accessible, generally little is thought about their exact commitment to chocolate quality [32]. Late metagenomics approaches on cocoa maturations permitted a re-investigation of the procedure and uncovered new knowledge into microbial elements, assorted variety and collaborations amid the procedure [33]. Quite, uncommon taxons and also popular groups were recognized, giving a far-reaching view on the environment. This demonstrates complex fermentative biological communities, for example, unconstrained coca bean maturation, contemplated by metagenomics approaches enable new highlights to be found [34].

Metagenomics to identify fermentative agent:

Determination of fermentative agent can be determined by focusing on analysis of DNA but it has some limitation. IN fact. DNA has variable half-life. DNA analysis are not able to distinguish between living or dead microbes.

What's more, basic DNA investigation does not permit recognizing living from dead organisms when they are available in a specific focus.

The investigation of sustenance aging concentrating on RNA rather than DNA gives data on the capacity of the microorganisms and how they are affected by various situations. Surely, RNA examination appears to be ready to better feature the microorganisms that add to the maturation procedure. Envoy RNAs have a short lifetime and their extraction from the nourishment grid remains in fact troublesome. Conversely, the utilization of ribosomal RNA appears a decent trade-off. Without a doubt, its lifetime is more noteworthy than mRNA however not as much as that of DNA [35].

Useful properties of the aged sustenance microbiota can likewise be explored by Metagenomics. To be sure practical assorted variety can be investigated through hereditary screening of qualities of premium. For instance, an examination concentrated on 33 qualities associated with probiotic and healthful capacities engaged with survival, gastro tract, starch digestion, folate and riboflavin biosynthesis. Also, a couple of studies have researched the outflow of focused qualities of enthusiasm for nourishment framework. For example, the statement of quality encoding for amylase has been observed in pearl millet slurries [36, 37].

Metatranscriptomic examination of aged sustenance micro biota still displays specialized Locks. For example, the

extraction of RNA from nourishment network stays troublesome. In fact, such complex networks contain fats, polysaccharides, and Inhibitors that make troublesome the extraction of good quality and amount RNA. In Addition, an absence of fundamental succession information and comment remains. Arrangement data Available in databases is as yet constrained to a few microscopic organisms (lactobacilli, staphylococci, and Food pathogens). Since metagenomics information incorporates a gigantic extent of qualities encoding traditional Cell capacities with little enthusiasm for sustenance microbiota, sequencing of metagenomes should center around particular parts (key catalysts for enhance compound generation, poison Synthesis, or particular amino corrosive corruption) to have the capacity to screen, notwithstanding Variations of species decent variety, changes in microbial movement [38].

Metagenomics for starter selection:

For upgrade of dietary and quality properties (surface, time span of usability, enhance, smell, and so on.) or to quicken the training is widely utilized as a part of nourishment industry. Nonetheless, in the mainstream rising nations, the procedure of maturation rely upon exploratory strategies, portrayed by a flawed practice controller. Thus, low generation and distinctive quality items are gotten. Determination of microbial starters depends on the customary aging practices. Choice criteria of organisms are plural. They incorporate utilization of different crude materials, physiology of strain, and the arrangement of digestion items, yet in addition the nature of sustenance item. Microbial starters pick up to be described phenotypically and hereditarily, for innovative, wellbeing and probiotic highlights [39]. The advances in this field towards an improvement of matured nourishment quality regularly include back-slanting or the immunization of crude materials with a buildup from a past bunch, yet now and again stay lacking to guarantee sustenance wellbeing. Also, current uses of these starters are various in the field of nourishment wellbeing and demonstrate an expanding enthusiasm to comprehend producing procedures of nearby aged. It was shown in the event that reviews, for example, yogurt, that organism microorganism collaboration could positively affect item nature of matured nourishment by trade of 'data' and metabolites. For sure, the Quorum Sensing wonder can be shared between species possessing a similar specialty thus can assume a part in complex groups of organisms engaged with the aging procedure [40].

Metagenomics for Safety Improvement of Fermented Food Stress conditions related to nourishment maturations are identified with ecological pressure and to biochemical changes coming about because of microbial digestion. These conditions result in the determination of predominant verdure yet in addition can prompt practical however non culturable (VBNC) as well as non-reasonable conditions of microorganisms. For instance, in wine, the deterioration yeast *Brettanomyce bruxellensis* can enter this stage and apply its fragrant deviation quite a while in the wake of packaging [41-43].

On one hand, the microbial flow saw fermented foods give matured nourishments a successful insurance against pathogens. It is likewise suggested in the expulsion of poisonous mixes from crude materials. Rivalry and additionally enmity between microorganisms can be interceded through inhibitory particles (peptides, bacteriocins, acids) that show particular and possibly fascinating properties [44, 45].

Metagenomics is possibly an apparatus of decision to evaluate the survival of pathogens, toxinogens or decay species over aged sustenance elaboration process. A conceivable cutoff of the metagenomics approach is its inability to identify the less various microorganisms in a situation containing a predominant microbiota [46, 47]. The recognition of *Escherichia coli* and of Bacillus species, which conceivably included *Bacillus cereus*, over item elaboration, scrutinized the survival states of these microbiological risks. Also, in other grain matured sustenance customarily arranged in Africa, the nearness of *Clostridium perfringens* and of *B. cereus* was confirm from a clone library while the recognizable proof from the fundamental groups from PCR-DGGE profile did not demonstrated these species [48-50]. In another investigation, a peril identified with the recognition of Proteus mirabilis and Staphylococcus spp. was brought up by ARDRA examination connected to isolate from soybean aged food and from aged pork tests though PCR-DGGE distinguished uncultivated Clostridium spp. also, Staphylococcus spp. from matured fish items. Therefore, the error of results between established microbiological techniques and sub-atomic strategies ought to be all the more profoundly examined [51, 52]. In the meantime the most solid approach is to utilize both methodologies as reciprocal apparatuses.

Then again, the utilization of metagenomics in assessing the effect of process alteration on maturation microbiota and security issues is profoundly applicable. Salt diminishment methodologies for sauerkraut, in a more broad perspective of lessening sodium admission, depended on the decline of sodium chloride content, with or without its incomplete substitution by other mineral salts (calcium chloride, magnesium chloride and potassium chloride) [53, 54]. The outcomes of these progressions on sensorial properties were examined. In parallel, PCR-DGGE and traditional microbiology were utilized to evaluate fermentative and security issues coming about because of this procedure changes[55]. It was in this way watched fermentative profiles and *Enterobacteriaceae* populace did not fundamentally change with salt substance and organization [56]. Another investigation concentrated on the connections between mycotoxin pollution and the contagious groups related to nourishment items, for example, espresso [57].

H. Wang *et al.*studied the change in soy sauce maturation process. In this study he examined the different stages of koji and mash by using next generation sequencing. An aggregate of 29 genera was identified in the koji arrange, while 34 in the mash stage. After Koji stage, 7 genera vanished and 12 new genera showed up in the squash arrange. The overwhelming microorganisms were *Kurthia, Weissella, and Staphylococcus* in the koji stage and *Staphylococcus, Kurthia, Enterococcus and Leuconostoc* in the mash stage. The outcomes gave bits of knowledge into the microbial groups associated with soy sauce maturation. The most well-known genera between the koji and pound were *Kurthia* and Staphylococcus, trailed by Weissella and Leuconostoc. The relative plenitudes of *Acinetobacter, Corynebacterium, Lactococcus, Macrococcus and Streptococcus* expanded when the aging entered the mash stage. Some bacterial genera, for example, *Streptomyces, Chryseobacterium, Paracoccus, Aquabacterium, Proteus, Providencia, Salmonella, Enhydrobacter, Aeromonas and Vibrio*, were just distinguished in the mash [58-60].

CONCLUSION

Metagenomics approaches are effective in investigating the microbial nature of complex biological systems, for example, fermentative nourishments. Distinctive organic inquiries would now be addressed worldwide or focused on strategies (practical metagenomics or metatranscriptomics). Be that as it may, a few contemplations ought to be considered when undertaking metagenomics studies [60].

Specialized issues ought to be addressed that leads specific end goal to expect predispositions that can happen in the created information. Nucleic acid extraction (DNA/RNA) yields are subjected to varieties as indicated by the structure of the nourishment grid and the microbial species introduce in the environment. To get a dependable perspective of the biological community, the reproducibility of extraction and PCR must be guaranteed. Moreover, before touching base at a determination about a microbial component or mark in a maturation biological system, it is required that few examinations be performed in a similar environment [61].

While entire group NGS approaches are not seriously affected by specialized inclinations, directed methodologies are subjected to predispositions (with respect to each PCR-based strategy). Particular enhancement chooses successions and weakens the representativeness of increased arrangements concerning the underlying example. Both nucleic acid extraction and PCR enhancement response predispositions can deliver a small amount of information. Thus, prescribed information got from such methodologies be supplemented with information got from various methodologies (sub-atomic and social strategies). Taking all things together, the creation of metagenomic information should be corresponded with all information accessible (pH, temperature, water activity, product synthesis, and so on.) to deliver important and valuable information for the appreciation of complex biological communities.

At last, metagenomic approaches have been utilized to consider the cooperations between human gut and sustenance microbiota. Heftiness being related with a specific gut microbiota (exhaustion of Bacteroidetes), the synchronous examination of aged sustenance eating routine and gut microbiota by metagenomics would bring about a superior learning of the connections between these microbiotas and could be useful in battling against corpulence. Additionally, a product that has advantage for human well-being could be produced. Later on, one can dream of the utilization of metagenomics, and all the more particularly metatranscriptomics, to recognize in a biological system, the nearness of mRNA integrated from qualities encoding amino corrosive decarboxylases associated with biogenic amine pathways or encoding compounds engaged with mycotoxin arrangement. Similar instruments could be connected to examine the arrangement of bacteriocins in a biological system [62].

REFERENCES:

1. Stanbury PF, Whitaker A, Hall SJ. Principles of Fermentation Technology. Elsevier **2016**.
2. Simango C. Potential use of traditional fermented foods for Zimbabwe. Livest Res Rural Dev **1997**;8:40–51.
3. Steinkraus K.H. Fermentations in World Food Processing. CRFSFS **2002**;1:1541-4337.
4. Albesharat R, Ehrmann M. A, Korakli M, Yazaji S, et al. Phenotypic and genotypic analyses of lactic acid bacteria in local fermented food, breast milk and faeces of mothers and their babies. Syst Appl Microbiol **2011**;34: 148-155.
5. Sekar and Mariappan. Usage of traditional fermented products by Indian rural folks and IPR. IJTK **2007**;6:111-120.
6. Illeghems K, Weckx S., Vuyst De L. Applying meta-pathway analyses through metagenomics to identify the functional properties of the major bacterial communities of a single spontaneous cocoa bean fermentation process sample. Int J Food Microbiol **2015**;50:54-63.
7. Staley JT and Konopka A, Measurements of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu Rev Microbiol **1985**;39:321-346.
8. Pace NR, A Molecular View of Microbial Diversity and the Biosphere. Sci **1997**;276:734-740.
9. Handelsman J, Metagenomics: Application of genomics of uncultured microorganisms. Microbiol Mol Biol Rev **2005**;68:669-685.
10. Beja O, Aravind L, Koonin EV, et al. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Sci **2000**;289:1902-1906,
11. Beja O, Spudich EN, Spudich JL, et al. Proteorhodopsin phototrophy in the ocean. Nature **2001**;411:786-789.
12. Janssen, Yates PS, Grinton BE, et al. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions *acidobacteria, actinobacteria, proteobacteria*, and *verrucomicrobia*. Appl Environ Microbiol **2007**;68:2391-2396.
13. Sait M, Hugenholtz P, Janssen PH, Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. Environ Microbio **2002**;4:654-666.
14. Quaiser A, Ochsenreiter T, C. Lanz, et al. Acidobacteria form a coherent but highly diverse group within the bacterial domain: evidence from environmental genomics. Mol Microbio **2003**;50:563-575.
15. Tyson GW, Chapman J, Hugenholtz P, et al. Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature **2014**; 428:37-43.
16. Brett B and Banfield JF. Microbial communities in acid mine drainage. FEMS Microbiol Ecol **2003**;44:139-152.
17. Galperin MY. Metagenomics: from acid mine to shining sea. Environ Microbiol **2004**;6:543-545.
18. Henne A, Schmitz RA, Bomeke M, et al. Screening of environmental DNA libraries for the presence of genes conferring lipolytic activity on *Escherichia coli*. Appl Environ Microbiol **2000**;66:3113-3116.
19. Rondon MR, August PR, Bettermann AD, et al. Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol **2000**;66:2541-2547.
20. Majernik A, Gottschalk G, Daniel R, Screening of environmental DNA libraries for the presence of genes conferring $Na^+(Li^+)/H^+$ antiporter activity on *Escherichia coli*: characterization of the recovered genes and the corresponding gene products. J Bacteriol **2001**;183: 6645-6653.
21. Entcheva P, Liebl W, Johann A, et al. Direct cloning from enrichment cultures, a reliable strategy for isolation of complete operons and genes from microbial consortia. Appl Environ Microbiol **2001**;67:89-99.

22. Diaz-Torres ML, McNab R, Spratt DA, et al. Novel tetracycline resistance determinant from the oral metagenome. Antimicrob. Agents Chemother **2003**;47: 1430-1432.
23. Mayo B, Rachid C, Alegría A, et al. Impact of next generation sequencing techniques in food microbiology. Curr genom **2014**;15:293-309.
24. Mirete S, Morgante V, González-Pastor JE, Functional metagenomics of extreme environments. Curr Opin in biotech **2016**;38:143-149.
25. Nam YD, Chang HW, Kim KH, et al. Metatranscriptome analysis of lactic acid bacteria during kimchi fermentation with genome-probing microarrays. Int J Food Microbiol **2009**;130:140-146.
26. Diaz-Torres ML, McNab R, Spratt DA, et al. Novel tetracycline resistance determinant from the oral metagenome. Antimicrob. Agents Chemother **2004**;47: 1430-1432.
27. Riesenfeld CS, Goodman RM, and Handelsman J. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. Environ Microbio **2005**;6:981-989.
28. Smid E and Hugenholtz J. Functional genomics for food fermentation processes. Annu Rev Food Sci T **2010**;1: 497-519.
29. Henne A, Daniel R, Schmitz RA et.al. Construction of environmental DNA libraries in *Escherichia coli* and screening for the presence of genes conferring utilization of 4-hydroxybutyrate. Appl Environ Microbiol **1999**;65: 3901-3907.
30. Henne A, Schmitz RA, Bomeke M, et al. Screening of environmental DNA libraries for the presence of genes conferring lipolytic activity on *Escherichia coli*. Appl Environ Microbiol **2000**;66:3113-3116.
31. Lee FL, Rusch DB, Stewart FJ, et al Saccharide breakdown and fermentation by the honey bee gut microbiome. Environ Microbiol **2015**;17:796-815.
32. H Li, ML, S Luo, et al. Metabolic responses to ethanol in *Saccharomyces cerevisiae* using a gas chromatography tandem mass spectrometry-based metabolomics approach. Int J Biochem Cell B **2012**;44:1087–9612.
33. Russell JB and Gonzalez F. The effects of fermentation acids on bacterial growth. Adv Microb Physiol **1997**;39: 205–234.
34. Vuyst LDe, Schrijvers, Paramithiotis S, et al. The biodiversity of lactic acid bacteria in Greek traditional wheat sourdoughs is reflected in both composition and metabolite formation. AEM **2002**;68:6059–6069.
35. Park EJ, Chun J, Cha CJ, et al. Bacterial community analysis during fermentation of ten representative kinds of kimchi with barcoded pyrosequencing. Int J Food Microbiol **2012**;30:197-204.
36. Pothakos V, Illeghems K, Laureys D, et al. Acetic acid bacteria in fermented food and beverage ecosystems in *Acetic Acid Bacteria*. Springer **2016**;73-99.
37. Moroni AV, Arendt E. K. F. Dal Bello, Biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs. Int J Food Microbiol **2010**;28:497–502.
38. Lin XB and Gänzle MG, Quantitative high-resolution melting PCR analysis for monitoring of fermentation microbiota in sourdough. Int J Food Microbiol **2014**;8: 186.
39. Breitbart M, Salamon P, Andresen B, et al. Genomic analysis of uncultured marine viral communities. PNAS **2002**;99:14250-14255.
40. Cocolin L and Ercolini D, Zooming into food-associated microbial consortia: a 'cultural'evolution. Curr Opin Food Sci **2015**;2:43-50.
41. Sicard D and. Legras JL, Bread, beer and wine: Yeast domestication in the Saccharomyces sensu stricto complex. CR Biol **2011**;334:229–236.
42. Devi TA., Ahmed KR, Jeyaram K, Microbial and endogenous origin of fibrinolyticactivity in traditional fermented foods of Northeast India. Int J Food Microbiol **2014**;55:356–362.
43. R. Saltini, R. Akkerman and S. Frosch. Optimizing chocolate production through traceability: A review of the influence of farming practices on cocoa bean quality. Food Control **201329**:167-18.
44. Cocolin L, Alessandria V, Dolci P, et al. Culture independent methods to assess the diversity and dynamics of microbiota during food fermentation. Int J Food Microbiol **2013**;167:29–43.
45. Capozzi V, Russo P, Spano G, Microbial information regimen in EU geographical indications. World Patent Informa **2012**;3:229–231.
46. Gilbert JA, van D, Zarraonaindia I. Microbial terroir for wine grapes. PNAS **2014**;11:5–6.
47. Zdolec N, Dobranić V, Horvatić A, et al. Selection and application of autochthonous functional starter cultures in traditional Croatian fermented sausages. Int J Food Microbiol **2013**;20:1–6.
48. Van Hijum SA, Vaughan EE, Vogel RF, Application of state-of-art sequencing technologies to indigenous food fermentations. COBIOT **2013**;24:178-186.
49. Beja O, Aravind L, Koonin EV, et al. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Sci **2000**;298:1902-1906.
50. DH Buckley, Schmidt TM. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environ Microbiol **2003**;5:441-452.
51. Bigot C, Meile JC, Remize F, et al. Applications of Metagenomics to Fermented Foods Fermented foods. Part I. Biochem & biotech, CRC Press. **2016**;978:4987-4079.
52. Keel C, Ucurum Z, Michaux P, et al. Deleterious impact of a virulent bacteriophage on survival and biocontrol activity of *Pseudomonas fluorescens* strain CHA0 in natural soil. Mol Plant-Microbe Interact **2002**;15:567-576.
53. Rondon MR, August PR, Bettermann BD, et al. cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol **2000**;66:2541-2547.
54. Wang H, Wei Q, Gui S, et al. Metagenomic Profiling of the Bacterial Community Changes from Koji to Mash Stage in the Brewing of Soy Sauce. Pol J Microbiol **2017**;66:537–541.
55. Forbes JD, Knox NC, Ronholm J, et al. Metagenomics: the next culture-independent game changer. Front Microbio **2017**;8:1069.
56. Greppi A, Ferrocino I, La Storia A, et al. Monitoring of the microbiota of fermented sausages by culture independent rRNA-based approaches. Int J Food Microbiol **2015**;212:67-75.
57. Wolfe BE and Dutton RJ. Fermented foods as experimentally tractable microbial ecosystems. Cell **2015**;161:49-55.
58. Whiteley JS, Jenkins S, Waite I, et al. Microbial 16S rRNA Ion Tag and community metagenome sequencing using

the Ion Torrent (PGM) Platform. J Microbiol Method **2012**;91:80–88.
59. Shangpliang HNJ,Rai R, Keisam S, et al. Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. Scienti Reports **2018**; 1532.
60. Kergourlay G, Taminiau B, Daube G, et al. Metagenomic insights into the dynamics of microbial communities in food. Int J Food Microbio **2015**;213:31-39.
61. Bokulich NA and Mills DA. Next-generation approaches to the microbial ecology of food fermentations. BMB reports **2012**;45:377-389.
62. De Vuyst L and Weckx S. The cocoa bean fermentation process: from ecosystem analysis to starter culture development .J Appl Microbiol **2016**;12:5-17.

How to cite this article:

Manisha Singh, et al. METAGENOMICS INSIGHT: ENHANCING FERMENTED FOOD QUALITY FOR HUMAN HEALTH. J Pharm Res 2019;8(9):653-662. **DOI: https://doi.org/10.5281/zenodo.3557308**

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil