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RESVERATROL AND ITS IMPACT ON PROSTATE CANCER

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ABSTRACT

Cancer is a serious threat to human health in populations across the globe. Chances are that at some point cancer will have an effect on every person’s life in one form or another. Current treatments are incredibly inefficient because of the disease’s unique adaptive capabilities, which render many treatments useless. Still other treatment methods, such as chemotherapy, often make the patient more ill than the disease itself. Resveratrol (RES), a polyphenolic compound naturally found in a variety of red fruits and some vegetables presents an effective non-toxic alternative, which would allow physicians to treat the disease non-intermittently. Recently, much research has been conducted on the cancer-fighting properties of RES, but very little is known about the mechanism(s) in which RES utilizes to express its numerous anti-cancer properties. In this review, we are focusing on some of the mechanisms utilized by RES to sensitize Prostate cancer (PCa) cells.

Key words: Prostate cancer, Resveratrol, Aryl Hydrocarbon Receptor

1. Introduction

Cancer is a major threat to the health of human populations all over the world, but it is especially prevalent in populations of the western hemisphere. Cancer is extremely tough to prevent because there are so many factors that contribute to its different types including: lung, breast, cervical, and prostate. In this review paper, we will be focusing on the mechanisms used in the sensitization of mainly prostate cancer cells, and how scientists are uncovering new hope for a cure to this type of cancer, utilizing natural remedies. Prostate cancer (PCa) is the most common form of cancer among U.S. men 50 years of age or older. It is most commonly presents as an adenocarcinoma (a tumor formed on the glandular structures of epithelial tissue) of the male prostate, which changes the morphology of the gland [1-3].

This can lead to complications of the urinary system and if not treated early can spread to other parts of the body [4, 5]. Studies are still being conducted as to what are the direct causative factors for the disease. Thus far, not much progress has been made in the search for a solution and researchers in both the scientific arena as well as the medical establishment are becoming increasingly aware of the relationship between a proper diet and good health [6-8]. Researchers and physicians are beginning to investigate the potency of more natural compounds against diseases such as cancer. One such compound is resveratrol (RES). RES is a polyphenolic compound found in the skins of red grapes (which are used to make red wine), some berries, select vegetables, and even peanuts. The chemical has natural anti-oxidative, anti-inflammatory, and anti-aging properties [9-14]. In its most pristine form the compound protects the plant(s), which produces it, from harmful invasive pathogenic organisms. Researchers seek to harness RES’s disease/pathogen fighting power in hopes of revolutionizing the way cancer is treated. Ambitious research endeavors such as the conjugation of RES with various carbon nanoparticles (poly epsilon-caprolactone, PCL and poly lactic glycolic acid, PLGA) illustrates this point. The resulting data from such work have shown the...
significant increase in the overall stability and target efficiency of the compound. The study also demonstrated that conjugated RES increased cytotoxic conditions in cancer cell lines more so than non-conjugated RES [15]. Furthermore, RES is attractive to researchers because of its low probability of harmful side effects. Current cancer treatment methodologies put the patient at risk often making the patient more ill than the cancer itself. When someone is diagnosed with cancer the individual is often put through a rigorous regimen of chemotherapy. The chemotherapy treatment is toxic to both cancerous and non-cancerous cells, which causes severe morbidity in the patient to the point where the treatment must be halted intermittently to allow the patient to recover [16-18]. This period of intermission also allows the cancer cells to recover often with more resistant properties than the preceding generation. This is why current cancer treatment methodologies have had such a historically low rate of success. In recent years, research has shown that RES is an effective inhibitor of several types of cancers including prostate. Not much is understood about how RES mediates the tumor sensitization, but it is clear that RES acts to prevent cancer proliferation on a variety of levels including nuclear/genetic. This review paper is an attempt to highlight the mechanism(s) of tumor clearance by RES.

2. RES inhibition at DNA/nuclear level

Several studies have reported that RES acts through a variety of biochemical pathways, both intrinsic and extrinsic to the cell to inhibit PCa development. Perhaps amongst the most intriguing finding is the fact that RES has the ability to illicit changes within the target cell at the DNA/nuclear level [19-24]. There are a variety of ways in which RES achieves this but the general idea is that the chemotherapeutic agent acts as a ligand and binds to a number of different cellular receptors initiating sequential phosphorylating events inside the cell that eventually make their way into the nucleus. Once inside the nucleus, it activates pro-apoptotic transcription factor(s) which help in expressing anti-cancer genes that result in cell death (figure 1). A perfect example would be a previous study involving a novel RES analogue (HS-1793) which exhibited the ability to inhibit the expression of the proteins: hypoxia-inducible factor-1alpha (HIF-1α) and vascular endothelial growth factor (VEGF) in human prostate cancer cells (PC-3). These transcriptional factors have been implicated for their role in the metastasis and angiogenesis of tumor cells [25-27]. Some of the molecules involved in DNA damage-dependent apoptosis are described below.

2.1. Role of PTEN/AKT Pathway

One such example is the regulation of the phosphatase and tensin homolog deleted on chromosome 10 (PTEN)/AKT pathway by RES [28, 29]. In this pathway, RES directly binds to the Epidermal Growth Factor Receptor (EGFR) rapidly inhibiting EGFR phosphorylation, which results in decreased AKT phosphorylation and activity. It must be understood that inside the cell there are many pathways that are initiated simultaneously that often intersect and produce the same biochemical results. PTEN is a known tumor-suppressor gene and it is concurrently up-regulated when RES binds to its receptor. Another one of those receptors is the Androgen Receptor (AR) [11, 30-32]. RES inhibits the phosphorylation of this receptor which subsequently results in PTEN transcriptional activity and the suppression of tumor formation. Binding to AR yields the same result as in the previously described EGFR pathway [33-35]. Studies indicate that an imbalance of AR activity is a major factor in tumor formation; PTEN, through the activation of its pathway by RES, facilitates a balance for this
receptor’s activity. RES produces a conformational change of AR (Hsp90/70) in the cytosol of the target cell, which then translocates into the nucleus to then express specific anti-cancer genes. In short, RES induces PTEN activity by AR modulation in PCa cells [28].

In one study, RES was used to target cells with PTEN mutations. The inactivation of AKT by the binding of RES and the up-regulation of PTEN induces the activation of FOXO gene transcription factors [29, 36]. FOXO gene transcription factors are proteins that were conserved throughout evolution. These genes are common to mammalian cells and are known to scientist as “housekeeping” genes. These genes regulate fundamental cellular functions such as DNA damage repair, cell cycle arrest, cellular stress responses, and apoptosis. Researchers found that FOXO genes are functionally redundant; this simply means that several genes can regulate the same pathway and produce similar transcription factors [29, 37]. During this study when the FOXO gene was knocked down murine (mouse) model cells exhibited the first stages of tumor development. When FOXO expression was artificially induced the model cells did not exhibit tumorigenesis. This indicates that the FOXO gene could be an important mediator for cellular apoptosis and RES activity. This study shows that RES induces apoptosis and growth arrest through yet another cellular mechanism, this time via the activation of FOXO transcription factors. For many human PCa models the PTEN gene is inactivated through some type of mutation this causes phosphorylation/activation of pro-cancer pathways such as PI3K/AKT, which as a result are catalysts for unregulated cell growth and tumor progression [29].

2.2. Role of P53

Another widely known gene concerning cancer and its progression is p53. This gene is associated with many cancers; in fact, p53 mutations are present in over half of all human cancers [38-43]. P53 is responsible for mediating cell cycle arrest and apoptosis in response to specific stimuli such as cellular and genetic stress. Prior studies have shown that the administration of RES produced an effect on all cell lines involved suggesting that RES has the capacity to mediate anti-cancer effects through a wide array of biochemical pathways [38-40, 44]. However, the effect of RES was somewhat truncated in the cell line with the partially knocked down p53 gene. Constitutive p53 mRNA expression was observed at much higher levels in the cell lines without partially deleted p53 [44]. This indicates that the dietary compound acts in concert with p53 on some molecular level, which is not yet fully understood.

2.3. Role of micro RNAs

Recently, RES has shown the capability to regulate several microRNAs. MicroRNAs are small non-coding RNAs, which regulate coding RNAs at the post-transcriptional level [45]. MicroRNAs originate from primary transcripts converted in the nucleus into precursor miRNAs [46-49]. They are then exported to the cytoplasm where they are cleaved and transcribed. MicroRNAs are associated in many gene regulatory functions/networks and their malfunction has been linked to a bevy of different cancers. The over expression microRNAs have been implicated in the progression of several types of leukemias, lymphomas, and PCa [50-52]. In certain cases of chronic inflammation, the inflammation has been found to be a significant factor in the increased expression of some microRNAs. By far the most researched of these mysterious bits of non-coding RNA is miR21 [50, 53]. Because of its impactful role in oncogenesis researchers often use miR21 as a biomarker for cancer identification. These regulatory RNAs induce oncogenesis by down regulating tumor-suppressor genes such as programmed cell death 4 (PDCD-4), mapsin, and the previously mentioned PTEN; the down regulation of these gene’s products creates an environment perfect for the onset of cancer proliferation. Studies have shown miR21 to be a major catalyst in the metastasis of some forms of colon cancer by disrupting apoptosis via the mechanism previously stated [45-47, 53]. Apoptosis in human glioblastoma cells was restored when the miR21 gene was knocked down providing further evidence of miR21’s role in oncogenesis. In this particular study, researchers found that when model cells were treated with RES, the tumor formation was greatly inhibited. In this finding, RES actually down regulated AKT, which is an upstream regulator of the miR21 gene. This of course decreased the formation of miR21’s anti-apoptotic products including Bcl-2 proteins and nuclear factor (NF-kB) [54-56]. In addition to this mechanism RES also showed the capacity to upregulate miR663, a microRNA that acts antagonistically to the anti-apoptotic effects of miR21 [45, 57].
2.4. Role of Aryl Hydrocarbon Pathway

Polycyclic aromatic hydrocarbons (PAHs) are environmental poisons such as those produced from byproducts of the chemical, nuclear, and textile industries. PAHs and other environmental toxicants (such as cigarette smoke) act as ligands for the Aryl Hydrocarbon Receptor (AhR) [58-61]. When bound to the cellular receptors, these toxins can cause immunosuppression and endocrine malfunction, which has been linked to the development of some cancers [62-64]. Prior studies have been carried out to examine what component of red wine was responsible for its anti-cancer properties [4, 10, 12, 19, 20, 65, 66]. One group of researchers isolated a variation of RES-trihydroxystilbene resveratrol (3, 5, 4’-trihydroxystilbene resveratrol RES), which is found in red wine, and according to their study, is a competitive antagonist of PAHs for AhR binding [22, 23, 67]. In addition, this form of RES is very potent and vegetables it is also nontoxic which is key to its and since it is naturally found in some edible fruits potential treatment of human cancer. This study demonstrated that RES binds to AhR in the cytosol and translocates the RES-AhR complex into the cell’s nucleus. A whole-cell binding competitive assay was designed in vitro to compare the binding efficiencies of RES, TCDD (tetrachlorodibenzo-p-dioxin-a type of PAH), and indole-3-carbinol (an established natural AhR ligand) to AhR [23]. The study revealed that RES did not displace the other two ligands from binding with AhR. All three compounds bound to the receptor at a similar rate. This indicated that the inhibitory effect of RES took place at the DNA level between both the RES-AhR and transcriptional complexes within the nucleus. To confirm this a GFP fluorescent tag was placed on an AhR vector to track the movement and destination of the ligand-receptor complexes within the cell [67]. Thus, RES mechanisms have been shown to work through AhR pathway.

3. RES sensitization of cancer cells

Cancer is notoriously difficult to treat because of the innate ability of its cells to develop multiple forms of resistance to modern treatments. Among other mechanisms cancer cells possess an efflux pump that is responsible for immediately pumping out any agents that are toxic to the cell. Cancer cells have also been documented to possess enhanced DNA repair mechanisms, which in effect neutralize the main mode of effectiveness of many available cancer-fighting drugs [68-73]. The expression of glutathione/glutathione S-transferase (GSH/GST) within the cancer cell presents yet another problem to cancer researchers because this allows the cancer cell to inactivate chemotherapeutic agents through the cleavage of chemical structures. To put it concisely, the medical establishment is losing an arms race with an innate cellular intelligence that wishes to survive and proliferate at all costs. That is why dietary compounds such as RES present such promise because it has shown the versatility to combat this disease through multiple channels [72, 74-76]. Research has documented RES’s ability to modulate apoptotic pathways, and down-regulate both proteins responsible for molecular transportation and tumor proliferation. RES sensitizes cancer cells to anti-cancer agents by modulating or changing the effects of certain proteins responsible for the survival of the cell thereby making the cell more susceptible to the induction of apoptosis [29, 40, 77-79]. As we have previously discussed, RES has the ability to up-regulate pro-apoptotic genes, such as p53, PTEN, and PDCD-4. RES has the ability to bypass or eliminate chemoresistance mechanisms by modulating the actions of transmembrane and multi-drug resistance proteins (MRPs). Additionally, RES mediated the activation of caspase proteins, which are responsible for the initial stages of cellular degradation and DNA fragmentation [80].

4. RES and the mitochondrial pathway

Additional studies demonstrated that RES sensitizes mammalian cells to apoptosis through the mitochondrial pathway. In healthy cells the mitochondrial membrane contains proteins from the Bcl-2 family [56, 81, 82]. These proteins help maintain the integrity of the mitochondrial membrane. In response to internal cell stressors Bax proteins make their way to the mitochondrial membrane and deactivate the Bcl-2 proteins thereby releasing pro-apoptotic factors such as, cytochrome c. These apoptotic factors then bind to initiator caspases and then later effector caspases, which subsequently induce cell death [66, 83-85]. Unpublished experimental data from our group demonstrated the active involvement of mitochondrial and caspase
RES produced cell death not only through the mitochondrial pathway, which was more active, but also through a caspase-dependent pathway. This suggests a synergistic role of caspase-dependent and caspase-independent mechanisms in RES-dependent sensitization of tumor cells.

5. Summary/Conclusion

RES is truly a remarkable compound that utilizes many different biochemical pathways to inhibit a number of cancers. Its versatility far exceeds anything ever manufactured in man-made laboratories. The question now becomes, how does this amazingly effective dietary compound mediate its incredible inhibitory effects on cancer cells? And by what mechanism(s) does this chemotherapeutic agent use to induce apoptosis in cancer cells exclusively. Advancements in this area of research have the potential to revolutionize cancer diagnosis and treatment.

References


PARTICIPATORY GENOMICS IN THE ERA OF PERSONAL GENOMES

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ABSTRACT

The recent advancements in the field of nucleotide sequencing has tremendously improved the scale of genome sequencing and consequent drastic reduction in the costs, making whole genome/exome sequencing affordable. Though most of the genomic data presently resides in well-protected silos, prohibiting cross-comparison and efficient data mining. This significantly limits the realisation of the wealth of information encoded in the genome. In this commentary, the author argues how a participatory approach could significantly impact healthcare and research.

Key words: Next-Generation Sequencing, Personal genomics, Exomes

1. Introduction

Tremendous developments in the scale, throughput and accuracy of nucleotide sequencing in the recent years have accelerated the understanding of genome sequence, genome structure and biology. These new technologies have been popularly dubbed as next-generation sequencing technologies, differentiating it from the earlier sequencing technologies which were time-limiting, costly and many times laborious. Next-generation sequencing technologies has today found applications beyond human genomics in a wide variety of areas, including research applications, microbial identification, biosurveillance and industrial quality control.

Apart from the advances in scale and throughput of whole genome sequencing, a number of technologies and datasets developed immediately after the availability of the reference Human genome sequence has provided immense insights into the genetic variations in human populations and their associations with diseases or in general human traits.

This include miniaturisation of genotyping assays in the form of genotyping microarrays clubbed with statistical genetics methods to identify genetic variations associated with human traits. Presently there are over 80 diseases and traits with genome-wide estimates of genetic risk. The recent years have seen an increasing number of traits studied for their genetic predisposition has and independent revalidation of the risk in different genetically unrelated populations, significantly adding to the repertoire of genetic variants showing association with human traits.

Presently technologies allow a wide variety of scales and resolutions of understanding genomic variations. On one extreme is typing individual variations or a set of variations in genomes. The next higher resolution is offered by exome capture and sequencing which offers complete sequences of the exons of protein-coding genes. The highest resolution is by and large achieved through whole genome sequencing. Each of the resolution of genome analysis offers characteristic advantages and widely used to address characteristic questions. While the genotyping approach has been widely applied for understanding ancestry and predisposition to traits, exome and whole genome sequencing approaches have been used for understanding rare genetic variations, including somatic variations in cancers. Recent high throughput sequencing expeditions including the 1000 genome sequencing, regional sequencing initiatives including the Pan-Asian...
personal genomics initiative and many country-wide sequencing projects including the Arab genome projects aim to improve the repertoire of the human genetic variants which would the starting point to understand genetic variations and their phenotypic consequences.

The present manuscript discusses how improved scale, accuracy and reducing costs would provide a new opportunity towards understanding genomes at an individual level, and how a participatory approach could significantly impact healthcare and research.

2. Personal Genomics

The advent of sequencing technology which offer fast and efficient sequencing of whole human genomes and consequent drastic reduction in cost has opened up a new opportunity towards understanding the genome sequence, variations and the biological consequences of the variations at an individual level. This has many implications and applications in healthcare and has been one of the widely discussed topics in recent years. The availability of whole genome sequencing technology initially saw the sequencing of personal genomes including individual genomes from different populations around the world.

The availability of whole genome sequencing at affordable costs has made possible sequencing of whole genomes or exomes of a number of individuals. This includes research applications including population scale projects as well as Cancer Genome projects around the world. Genome/exome sequencing is also presently being done routinely for diagnosis of rare genetic diseases. Many of the genomes/ exomes sequenced as part of research projects have been collected from anonymous volunteers and apart from analysis of the variants focussed on the research problem in question. The vast majority of the variations generated as part of the study, though could be of immense use to the subject/volunteer under question, remain behind privacy and security firewalls.

The application of genome sequencing in regular clinical settings, though is its infancy, has shown promises, especially in fine-tuning the diagnosis and therapy. Nevertheless, the routine application would require tremendous investments in handling, long-term storage and analysis of data and possibly complete automation of the analysis and reporting pipeline. This would only be possible with widespread application of genomics through a further reduction in the cost of genome sequencing and better, faster and more informative analysis pipelines for interpretation of the functional genetic variants with faster turnaround times.

3. Evidence/accuracy and interpretation

The evidence on genetic variants and their effects have been improving significantly in the recent past, thanks to genome-wide association studies, and an increasing number of genetic variants identified through exome sequencing of rare genetic diseases. In addition, evidence based collection of genetic variants, for example, pharmacogenetic variants have been systematically curated in databases. As a matter of fact, many pharmacogenetic markers are presently advised and included in the drug labels. Availability of tools and resources in open source and ubiquitous computing has made it possible to analyse and interpret whole genome or exome data sets in clinical settings.

A number of initiatives have spearheaded the standardisation of evidence and interpretation of genetic variations. The foremost in this include the GET-Evidence initiative supported by the Personal Genomics Project which aims to provide resources and tools for analysis of genetic variations. A number of crowdsourcing initiatives including OpenSNPedia, annotating and curating genetic evidence from literature, OpenPGx which has been manually curating pharmacogenetic variant information and OpenSNP which has been curating personal genotype datasets are initiatives worth mentioning.

4. Cost of genome sequencing

It is widely assumed that genome sequencing or genotyping is an expensive proposition and accessible to a very few, well-to-do and technology savvy individuals. This elitist concept is not founded on hard facts. In fact the cost of whole genome sequencing has exponentially fallen over the last decade, with good quality human exomes/genomes presently available at near 1000-3000 US$ in commercial settings and is expected to significantly fall in the coming decade, with the wide acceptability and applicability of genome sequencing for clinical diagnostics.

Though hard evidence do not exist on the potential cost-advantage of genome sequencing in terms of the value it brings to healthcare improvement, nevertheless a number of ongoing studies aim to fill in this gap. In many markets, routine exome/genome sequencing for genetic diseases have been covered by
insurance, suggesting a potential economic benefit of sequencing.

5. Disclosure, Privacy and Ethics

Privacy of genetic information forms the key in any genomics study or analysis. Nevertheless, a significant number of individuals have made available the genome sequences in public domain. There is no hard evidence to show that disclosure of genome information in public domain could be harmful. And in fact more revealing information regarding an individual could be potentially gleaned through social networks and other online sources. The advantages of disclosure including potential availability of a higher level of annotation through application of novel methodologies and tools far outweighs the potential harm implicated by making the genome sequences available in public domain. The disclosure and availability of genome sequences in public domain also attains enormous importance as no single organisation would be able to provide all the potential applications and annotations possible on genomes as any point in time. The dynamic nature of the analysis pipelines and annotation data sets used for the analysis limits a comprehensive analysis of genome at any point in time. Nevertheless a continuous update and re-analysis would be impossible to be performed every time a new toolkit or annotation dataset is made available. Nevertheless availability of a dataset in public domain, including the Human reference genome and other personal genomes has made possible in-depth analysis of datasets on a continuous basis as they would form a corpus for testing toolkits and annotation data sets. The advantages and implications of making available genome sequences of not just individuals, but even other members of a family has been recently discussed in much great detail.

6. Availability of data in public domain

The availability of genome sequences in public domain would significantly add to the available repertoire of genome variations and would be the much needed starting point towards standardising and applying tools and annotation data sets. This could potentially help in a variety of ways not limited to personal benefits. For example a corpus of data sets from a particular population or group would help to understand variations prevalent in the particular population, say, for example, a pharmacogenetic variant. This would help physicians to plan treatment regimen for the patient group as a whole, which has the potential to drastically improve the quality of healthcare by avoiding adverse drug reactions, for example and reducing the cost by avoiding individual testing. Many ethnic groups already have such population level predispositions known at least for some diseases, which could be improved with high-quality genomics datasets.

7. Future perspectives

The routine application of personal genomics in clinical settings and participatory genomics would in the future be dependent on five key factors which include technological advancements and changes in the social outlook.

1) Cost of genome sequencing: A significant reduction in the cost of genome sequencing commensurate with the economic benefits accrued through genome sequencing and also tagged to the affordability in markets where healthcare insurance does not support genome sequencing would decide the tipping point for widespread adoption and practice of genome sequencing in regular clinical settings.

2) Scale and turnaround time: One of the major technical limitations of whole genome sequencing is the time taken for the sequencing and analysis of data, which needs to significantly improve to provide clinical turnaround times for data generation and interpretation. This would require significant investments in the area of information technology and availability of rich resources for evidence on clinical actionability of genetic variations.

3) Genetic education and conducive social outlook: Genetic education on the pitfalls and advantages of personal genomics is absolutely essential to abrogate mistrust and sharing of information on free-will.

4) Economic benefit for genome sequencing: The long term sustenance of any technology and its application would depend on the economic benefits accrued by screening apparently normal individuals. In depth studies on the personal benefits of whole genome sequencing and benefits of sharing data versus confidentiality of the data needs to be estimated to estimate the cost at which genome sequencing would be economically sustainable in routine clinical applications. The economic benefit would also in turn depend on the depth of annotation possible and the accuracy of the analysis and clinical applicability in preventive strategies.
5) Availability of large secure and interoperable databases and resources: The key to participatory personal genomics is the availability of a common and accessible repository of genomic variations available for researchers. Initiatives in this direction including the Personal Genome Project and OpenSNP which collects personal datasets and limited phenotype information in public domain are worth mention.

References


9. www.papgi.org


17. http://evidence.personalgenomes.org/


drug metabolism genes in an ethnically diverse population. *Pharmacogenomics, 5*(7), 895-931.


"MIR”ACLES IN NEURO MOLECULAR MEDICINE

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ABSTRACT

MicroRNAs (miRNAs) are transcriptional and post-transcriptional regulators that negatively control the expression of a gene. These small non-coding RNAs are 20 to 26 nucleotides long and regulate gene expression by binding specifically to the 3′-prime untranslated region of a gene transcript, leading to its translational repression or degradation. Studies demonstrate that miRNAs form an integral part of gene regulatory system of multicellular organisms and consequently play a pivotal role in the expression of protein coding genes. Many of the miRNAs are reportedly involved in mammalian Central Nervous System (CNS) and mediate the function and regulation of the nervous system. The disruption of miRNA based gene regulatory system has been implicated in numerous CNS disorders as a single miRNA can control the expression of several downstream mRNA targets. In the present study we report a bioinformatics pipeline for identification of miRNAs from the EST dataset of medicinal plant Curcuma longa. The putative miRNAs identified can be exploited in molecular therapeutics for CNS disorders.

Key words: miRNA, CNS, gene-silencing, Bioinformatics

1. Introduction

Translational control and mRNA transcript degradation is an imperative part of gene regulation machinery of a cell. Small non-coding RNA molecules are effective modulators of transcriptional activities of a gene [1]. These RNA molecules are reportedly involved in the cytoplasmic control of mRNA translation and degradation, a phenomenon known as RNA interference or RNA silencing. A cell exhibits RNA interference with the help of either endogenous or exogenous micro RNAs (miRNAs) and by short interfering RNAs (siRNAs). These 20 to 26 nucleotides long, single-stranded effector molecule alters the transcriptional rate [2]. A stable mRNA transcript can be decapped by the interfering RNAs or can be cleaved by an endonuclease [3]. Since their discovery in Caenorhabditis elegans, hundreds of miRNAs are now being reported in genomes of plants and animals [4]. Studies prove that upto 30 % of human genes are regulated by miRNAs [5].

Increasing evidence suggests that miRNAs play a key role in cell growth, tissue differentiation, embryonic development, cell proliferation, and apoptosis [6]. The dysregulation and impaired function of miRNAs and their targets may result in various diseases including cancer [7], cardiovascular diseases [8], schizophrenia and Fragile- X mental retardation [9] and AIDS [10]. Recent studies carried out on rat spinal cord reveals the presence of 350 miRNAs. This suggests that many miRNAs are implied in the regulation and development of the Central Nervous System [11-12]. miRNAs govern diverse set of neurobiological functions such as neuronal growth and apoptosis. Krichevsky et al. suggests that the expression of miR-124 and miR-9 increases during differentiation of mouse embryonic stem (ES) cell-derived neural progenitors [13]. Micro RNAs have been found to be associated in CNS and progressive neurodegenerative disorders. In Parkinson’s disease which is characterised by the progressive neurodegeneration of dopaminergic neurons in the substantia nigra (a structure located in the mid brain) a micro RNA miR-133b is under-expressed [14]. miR-133b regulates the function and maturation of dopaminergic neurons by negative feedback circuit including a paired like homeodomain transcription factor Pitx3. Decreased levels of miR-133b in the mid brain disrupts the negative feedback loop of Pitx.
leading to degradation of dopaminergic neurons and producing the multitude of symptoms associated with Parkinson Disease [14]. Huntington’s disease is caused mainly by a mutation in the gene which codes for the protein huntingtin. This mutation is responsible for the occurrence of excess of Glutamine residues in a protein thus disrupting its normal function. The disruption in the function of huntingtin protein causes abnormal neuronal cell death by an unknown mechanism. Extensive research on testing of RNAi (RNA interference) technology for reducing the abnormal production of huntingtin protein is underway as a plausible cure for Huntington’s disease. Preclinical studies on animal models in which the mutant huntingtin was suppressed showed considerable improvement in motor dysfunction and neuro-pathology. A group of researchers have collaborated with CHDI Foundation (Cure Huntington’s Disease Initiative) to develop an implantable infusion system to deliver a siRNA molecule which will target mutated huntingtin directly in the brain of patients with Huntington’s disease. Similarly in Spinocerebellar ataxia, a mutation in a gene called SCA1 increases the number of CAG repeats on the DNA leading to the production of an abnormal ataxin-1 protein. Hence, downregulating the expression of such mutated genes could reduce the effect of spino-cerebellar ataxia. Mouse stage III preclinical trials involving a miRNA silencing the mutated SCA1 gene showed improved motor co-ordination as compared to untreated mice [15]. Similar associations of miRNAs and their possible RNAi are reported in diseases like schizophrenia, Alzheimer’s and Parkinson’s [12]. Various genes like MAPK12, NOP56 are known to associate with CNS and neurodegenerative disorders. Thus, present study aimed to explore different miRNAs that govern the silencing of genes that are responsible for CNS disorders. We found 12 miRNAs from medicinal plant Curcuma longa (Indian Turmeric) which were able to silencing human gene transcripts involved in CNS and other Neuro-degenerative disorders. Curcuma longa is a medicinal plant which is known to treat various ailments for centuries in Ayurvedic medicine, the traditional Indian system of healing. The major component of Curcuma longa Curcumin is responsible for its biological activities. In-vitro data suggests that curcumin, the active component of the Zingiberaceae family plant has antioxidant, anti-inflammatory and anti-amyloid activity. Commonly known as the blood-purifier turmeric preparations are used to cure a variety of diseases like dyspepsia, flatulence, liver disease, urinary tract disease [16]. Due to the anti-oxidant properties of Curcumin, the plant preparations have also been known to prevent CNS disorders [16-17]. In addition to its anti-oxidant properties, the homeostatic, the cholesterol lowering properties and the anti-amyloid properties of Curcumin make it an attractive source for treating CNS and neurodegenerative disorders. A recent study in animal models reports a decrease in amyloid pathology in Alzheimer Disease by the use of Curcumin [18]. Hence Curcuma longa can be explored as a potential molecular therapeutic agent in the treatment of neurodegenerative and CNS disorders.

In the present study, we have attempted to explore the medicinal uses of Curcuma longa by the in-silico identification of miRNAs from the Expressed Sequence Tags dataset of the plant. These putative miRNAs identified by bioinformatics approaches and in-house developed perl scripts were screened for the potential human targets. Computational identification of miRNAs exploits the fact that 3-prime UTR of human mRNAs contains a 2-8 nucleotides long seed region which shows complementarity with the 5-prime region of their respective miRNAs. Hence, most computational algorithms designed to predict the miRNA targets rely upon the basic rule of base-complementarity. The human targets which were identified by the in-silico pipeline were then screened for their involvement in CNS disorders and the linked pathways. We report 12 putative miRNAs potentially involved in neurodegenerative and CNS disorder pathways. These RNAs can prove to be a potent tool in neuro-molecular therapeutics.

2. Materials and Methods

2.1 Curcuma longa EST dataset

As of January 1st 2013 dbEST (database of Expressed Sequence Tags) [19]; contained 74,186,692 ESTs in the database. Among them, 12,593 EST’s of Curcuma longa were downloaded from dbEST database of NCBI. The dbEST has the highest set of impurities associated with it including the vectors, linkers, adaptors and the primer sequences which has to trimmed before further analysis [20]. Seqclean was used to remove impurities and redundant sequences. Seqclean [21] utilizes BLAST (Basic Local Alignment Search Tool) [22] to screen sequences highly similar (default parameter: 94% identity) to a given list of vectors, linkers, adaptors and primer sequences. Seqclean takes care of the polyA repeats

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that associated with the mRNA transcripts and uses low complexity filtering for picking similar vector segments in sample ESTs [22]. The cleaned and trimmed ESTs were then processed via RepeatMasker [23]. Sequencing process of ESTs may yield some artifacts and many distinct ESTs may be a part of the same mRNA of an organism. To undermine this sequencing error, assembling of the ESTs into contiguous contigs and scaffolds was done by TGICL clustering tool [24].

2.2 microRNA sequence dataset

Mature and precursor plant miRNAs were downloaded from Plant MicroRNA Database (PMRD). PMRD is a database of validated and published plant miRNAs [25].

2.3 Searching miRNAs homologs and their secondary structure prediction

Contigs and Singletons obtained as an output of TGICL were blasted against PMRD miRNA dataset of plant miRNA to identify EST homologs in our set. Initial blast search was done with stringent parameters of eval 2e-4, word size 7 and 90% identity. EST database was also blasted with e-value 1000 and 90% identity to remove false-negatives predicted in first blast search. Secondary structures of predicted miRNAs were calculated and their Minimum Folding Energy (MFE) scores were recorded using mfold and RNAfold web servers [26].

2.4 Hybridization with human 3'UTRs using miRanda

The non-redundant human UTRs were downloaded from UTRdb database. The targets for putative miRNAs were then scanned in the 3’UTR with the help of miRanda software. Hybridization energy and percent identity for each hit EST were calculated and used to further screen the possible mRNA-miRNA targets. The target genes were then scanned by KEGG database for their involvement in CNS and neurodegenerative disorders.

Figure 1 Bioinformatics Pipeline for in-silico identification of miRNAs from EST datasets

3. Results and Discussions

Out of the numerous miRNAs satisfying the BLAST search and the minimum energy folding criteria, a total of 23 putative miRNAs were found to hybridize with the human 3’UTR sequences downloaded from human UTR database. The hybridization was carried out using miRanda software which runs a simple base complementarity algorithm at its back end. The targets were screened based on blast score and hybridization energy parameters of miRanda software. The hybridized UTRs of human genes as predicted by miRanda were fed into Kyoto Encyclopedia of Genes and Genomes (KEGG) to track the metabolic pathways of the target genes. 12 miRNAs were found to be directly involved in pathways of CNS disorders and neuro-degenerative disorders. The result is in accordance to the known anti-anxiety, anti-amyloid and anti-oxidant activity of Curcumin, as about 50 % of the predicted miRNAs were potent at silencing the expression of target genes involved jointly or independently in CNS disorders. Table 1 depicts the sequence, the length and the minimum folding energy of the predicted miRNAs.
Table 1: Statistics/Details of the predicted miRNAs. With the increase in length of the mature miRNA, the minimum folding energy tends to decrease. LGMD, Limb-Girdle Muscular Dystrophy; ALS, Amyotrophic Lateral Sclerosis; PME, Progressive Myoclonus Epilepsy; MPS3, Mucopolysaccharidosis Type III; CMT, Charcot-Marie-Tooth disease.

<table>
<thead>
<tr>
<th>miRNA Identifier</th>
<th>Sequence</th>
<th>Length</th>
<th>Minimum Folding Energy</th>
<th>AU Content (%)</th>
<th>Gene Target</th>
<th>Linked Disease/Chatter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UCAUCU</td>
<td>16</td>
<td>47.6</td>
<td>49.5</td>
<td>PERK</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>2</td>
<td>UCAUCU</td>
<td>16</td>
<td>45.8</td>
<td>54.2</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>3</td>
<td>UCAUCU</td>
<td>16</td>
<td>43.9</td>
<td>55.3</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>4</td>
<td>UCAUCU</td>
<td>16</td>
<td>42.1</td>
<td>56.4</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>5</td>
<td>UCAUCU</td>
<td>16</td>
<td>40.3</td>
<td>57.5</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>6</td>
<td>UCAUCU</td>
<td>16</td>
<td>38.5</td>
<td>58.6</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>7</td>
<td>UCAUCU</td>
<td>16</td>
<td>36.6</td>
<td>59.7</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>8</td>
<td>UCAUCU</td>
<td>16</td>
<td>34.8</td>
<td>60.8</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>9</td>
<td>UCAUCU</td>
<td>16</td>
<td>33.0</td>
<td>61.9</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
<tr>
<td>10</td>
<td>UCAUCU</td>
<td>16</td>
<td>31.1</td>
<td>63.0</td>
<td>MARK2</td>
<td>LGMD, ALS, PME, MPS3</td>
</tr>
</tbody>
</table>

The AU content of pre-miRNA should range within 30% to 70% as unstable pre-miRNA structures are essential to produce mature single stranded miRNAs by endonuclease decay [27]. Our results are thus in accordance with thermodynamic properties of secondary structure and the total AU acceptable content. Figure 2 shows the secondary structures of the putative miRNAs. These structures were predicted using RNAfold and Mfold webserver. The consensus Minimum Folding Energy (MFE) was then chosen for further analysis of miRNAs.

Figure 2 Secondary Structures of putative miRNAs generated by RNAfold web server.

ath-miRf10621-akr was found to be complementary to the 2 to 8 nucleotide seed region of human PERK gene. Recent Studies on PERK pathways indicate that PERK can be a potential neuro molecular target for the treatment of Alzheimer’s disease [28]. miRNA ptc-miRf12223-akr was found to be complementary to the ATPO gene (ATP oligomycin sensitivity conferral protein: OSCP). OSCP is a mitochondrial matrix protein and is a subunit of F1F0-ATPase complex. Accumulation of ubiquitinated OSCP in mitochondria of patients suffering from neurodegenerative diseases is common. A growing body of evidence suggests that defect in the ubiquitination-proteosome pathway of cells may lead to accumulation of matrix associated proteins in mitochondria of cells, which consequently may lead to cancer and neurodegenerative diseases [29]. Figure 3 shows the involvement of ATPO gene in neurodegenerative diseases. Specifically targeting this gene can ameliorate the neurodegenerative tendency of the CNS disorders.

Figure 3 ATPO and neurodegenerative disorders. (picture adopted from KEGG)
Several theories prove the evolutionary conservation of microRNA non-coding sequences among plant families. To substantiate this fact, a phylogenetic tree was constructed from the 12 putative miRNAs. Figure 5 shows the phylogenetic tree obtained by MEGA software [30].

MicroRNAs regulating a particular set of disorders share considerable sequence similarity. This is due to the fact that the genes governing a particular set of disorders are linked together at the pathway level. For example the pathway of Alzheimer’s disease reveals a connection between the human ATPO gene (ATP Synthase O subunit) and the human PERK gene. This suggests that genes involved in CNS or neurodegenerative disorders are linked together and are thus regulated together by a set of closely related miRNAs. Analysis of phylogenetic tree (Figure 5) of predicted miRNAs showed that these miRNAs are closely related to each other. The result of the phylogenetic tree analysis strongly reveals that predicted miRNAs are regulating a set of genes associated with the CNS Disorders.

4. Conclusion

A revolution in CNS drug discovery was anticipated with the mapping of the human genome. It was believed that the data would account for understanding disease pathophysiology and for discovery of certain disease associated drugs [31]. But the neuropsychiatric conditions have dozens of discrete, casual, and susceptibility related genetic associations. For schizophrenia, a total of 30 gene associations are reported, none of which have been proved to be a potent drug target [32]. Interestingly the currently used target for treatment of schizophrenia, the dopamine D2 receptor, was not present among these reported genetic associations. The catechol-O-methyltransferase V158 polymorphism found in some patients with schizophrenia have been associated with a variety of disease conditions like breast cancer, myocardial infarction, anxiety and Alzheimer’s disease, limiting its use as a drug target for treating any of these conditions [32]. Even the most apparently simple genetic disorder can be caused by a variety of mutations. Mutations can vary in type. One mutation can alter the maximum velocity of a protein, other, the Michaelis constant, and lastly may produce a functional enzyme or a receptor with different stability or a propensity to aggregate. The chance that a single drug target could reverse these three different mutations is very meagre. Thus, collective tools of translational and regulational medicine, pharmacogenomics, neuroimaging, and molecular therapeutics can be considered as better promise for multi-target disorders [33].

The present study was aimed at in silico prediction of miRNAs in Curcuma longa and their implication in CNS and neuro-degenerative disorders. The methodology predicted 12 putative miRNAs, the human gene targets of which were directly involved in pathways of neuro-degenerative and CNS disorders.

The putative miRNAs can be effective RNAi based drugs in the following CNS and neuro-degenerative disorders:
1. **Amyotrophic lateral sclerosis (ALS)**

The predicted miRNAs ‘ath-miRf10590’ and ‘ath-miRf11012-akr’ in *Curcuma longa* were found to silence human MAPK12 gene which induces the abnormal activation of SOD1 gene responsible for causing Amyotrophic lateral sclerosis disease (adopted from Kyoto Encyclopedia of genes and genomes KEGG). The inhibition of MAPK12 signalling pathway in brains of ALS disease patients would provide a breakthrough in ALS prevention and cure.

2. **Spinocerebellar ataxia (SA)**

Spinocerebellar ataxia is a type of neuro-degenerative disease which is characterized by slow progressive incoordination of gait and is often associated with poor coordination of hands, speech, and eye movements. It can be caused by mutations in many genes known to regulate body co-ordination. For example *NOP56* (nucleolar protein 56) gene mutation in Spinocerebellar ataxia causes a large expansion of an intronic GGCCTG hexanucleotide repeat which leads to abnormal ribosome biogenesis and genetic information processing in eukaryotes [34]. Spinocerebellar ataxia is also caused by a mutation in the gene *SCA7* which leads to CAG repeats in the DNA and in turn augments the production of abnormal ataxin-7 protein. miRanda results showed that predicted miRNAs ‘ath-miRf10730-akr’ and ‘ptc-miRf12248-akr’ bind to *NOP56* and *SCA7* gene respectively and can thus be exploited in RNAi based therapeutics for spinocerebellar ataxia.

3. **Lissencephaly**

Lissencephaly is a severe neuronal migration disorder that ranges from agyria/pachygyria to subcortical band heterotopia. Human PAFAH (platelet-activating factor acetylhydrolase IB subunit alpha) gene is a key mediator in lipid metabolism pathway of humans. The mutated PAFAH gene causes dysfunctional lipid metabolism which is responsible for Lissencephaly and Miller-Dieker syndrome. Putative miRNA ‘osa-miRf11323-akr’ silencing mutated PAFAH gene can provide a cure for Lissencephaly.

4. **Alcoholism**

Alcoholism is a term used to describe the obsessive and uncontrollable consumption of alcohol. It’s an addictive or substance-dependent disorder normally referred as the ‘Alcohol Dependence Syndrome’ in medical terms. src homology 2 domain-containing transforming protein C (*SHC3*) gene is a major intermediate in the pathway of human substance dependence. This pathway is responsible for alcohol addiction in humans (adopted from KEGG). The miRNA ‘ath-miRf10147-akr’ showed efficient binding to *SHC3* gene transcript and can be delivered in brains of alcohol addicts to disrupt the addiction pathway of Alcoholism.

5. **Mucopolysaccharidosis type III**

Mucopolysaccharidosis type III is an autosomal recessive lysosomal storage disorder caused by a defect in one of the four enzyme genes involved in glycosaminoglycan degradation. The defect results in the accumulation of heparan sulfate in many organs, as well as elevated metabolite levels in urine. Common signs and symptoms include mental retardation, behavior and aggression problems, and seizures. The mutated N-sulfoglucosamine sulfohydrolase gene (*SGSH*) causes abnormal Glycosaminoglycan degradation which leads to abnormal accumulation of heparan sulphate and the apparent symptoms associated with the disease. The miRNA ‘ath-miRf10354-akr’ based RNAi therapeutic could help in alleviating the symptoms and be a possible cure for the disease.

6. **Progressive myoclonic epilepsy (PME)**

The ceroid-lipofuscinosis neuronal protein 8 (*CLN8*) gene cause fatty acid elongation in mitochondria which is the sole cause of Progressive myoclonic epilepsy. miRNA ‘ath-miRf10158-akr’ could be used to regulate the pathway of fatty acid synthesis in patients suffering from epilepsy.

7. **Hereditary Spastic Paraplegia**

Hereditary Spastic Paraplegia is a nervous system disorder characterized by progressive distal limb weakness and lower extremity spasticity. *APSZ1* gene (adaptor-related protein complex 5, zeta 1 subunit) is an important gene associated with spastic paraplegia. Defects in this gene are the cause of autosomal recessive form of spastic paraplegia. Putative miRNA ‘ptc-miRf12150-akr’ was predicted to silence this gene.
8. Parkinson’s, Alzheimer’s and Huntington’s disease

Human ATPO gene (ATP synthase) is associated with the mitochondrial F1 complex. This complex plays a key role in metabolic pathways and oxidative phosphorylation. Defects in ATPO gene can affect mitochondrial dysfunction, which results in the accumulation of matrix associated proteins in the mitochondria of the cells. The result can be an increased propensity to neuro-degenerative disorders like Alzheimer’s Parkinson’s and Huntington’s disease. The defective ATPO gene is found to be a potential target for silencing by the putative miRNA ‘ptc-miRf12223-akr’. RNAi based therapeutics specially targeting the damaged ATPO gene could pacify the symptoms and slow the rate of progression of the CNS disorders [35].

9. Charcot-Marie-Tooth disease

A recent study states that the mutations in Ganglioside-induced differentiation associated protein-1 (GADPIL1) causes a rare form autosomal recessive Charcot-Marie-Tooth diseases [36]. Charcot-Marie-Tooth disease is a hereditary motor and sensory neuropathy characterised by progressive loss of muscle tissue and touch sensation across the body parts [37]. Putative miRNA ‘ptc-miRf10122-akr’ targets the gene which causes the CMT disease. The miRNA can be an effective drug-target to be explored for human CMT disorder.

miRNA based therapeutics could accelerate the research in CNS disorder prevention and therapy. The putative miRNAs predicted by the in-silico method from EST dataset of Curcuma longa (a well-known panacea for many human ailments) could be excellent drug source for RNAi based neurotherapeutics. In-silico identification of miRNA in medicinal plants could be useful not only in predicting new drug molecules and targets for CNS and neuro-degenerative disorders but also for other fatal multifactorial diseases like cancer, HIV and Tuberculosis.

References


ISOLATION AND SCREENING OF BIOSURFACTANT PRODUCING BACTERIA FROM POLLUTED LAKE

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ABSTRACT

Present work emphasizes on isolation and screening of biosurfactant producing microorganism. Four different strains were isolated for the study AW1, PR2, BF3, PL4. Out of all the strains PL4 which was isolated from a lake polluted with municipal waste was characterized. The isolated bacteria showed high oil displacement activity 7.5 cm, when cultured on minimal salt medium. Surface tension calculated was 21.16 dynes/cm, which was least among all other strains and it has highest emulsifying capability of diesel oil because its emulsification index was maximum i.e. 60%. Glucose and diesel are two important media ingredients essential for production of biosurfactant for this strain.

Key words: Biosurfactant, Bacteria, Polluted lake

1. Introduction

The term surfactant is an abbreviation of the expression “surface active agents” [1]. They are fundamentally distinguished by their amphiphilic and amphipathic characteristics and by their ability to decrease surface and interfacial tensions of liquids. Biosurfactants or microbial surfactants are surface metabolites that produced by bacteria, yeast and fungi having very different chemical structures and properties [2]. They are amphiphilic molecules consisting of hydrophobic and hydrophilic domains that find application in an extremely wide variety of industrial process involving emulsification, foaming, detergency, wetting, dispersing or solubilization [3]. These are produced mainly by hydrocarbon producing microorganisms which includes Arthrobacter spp., Bacillus spp., Candida spp., Clostridium spp., Corynebacterium spp., Nocardia spp., Pseudomonas spp. and more other genera have been reviewed [5-14].

The characteristic property of a biosurfactant is its ability to reduce surface and interfacial tension by accumulating at the interfaces between liquids, solids and gases. They reduce the repulsive forces between two dissimilar phases, which allows them to mix more easily4-6. Major classes of Biosurfactants include glycolipids, phospholipids, lipopeptides, lipoproteins, fatty acids and polymeric biosurfactants [15]. Biosurfactants have many environmental applications such as recovery of hydrocarbons from dregs & muds, removal of heavy metals from sediments, enhancement of oil recovery [16-18]. Other potential applications of biosurfactants relate to food, cosmetics, pharmaceuticals, agriculture, mining, transportation of crude oil [12-14]. Some biosurfactants are known to have therapeutic applications as antibiotics, antiviral and antifungal compounds [19]. They are potential alternatives of chemically synthesized surfactant in a variety of application because of their advantages such as lower toxicity, higher biodegradability, higher foaming, better environmental compatibility, the possibility of their production through fermentation, lower critical micelle concentration, ability to be synthesized from renewable resources, higher selectivity, specific activity at extreme temperatures, pH and salinity [20-28].
Our objective is to isolate hydrocarbon utilizing or biosurfactant producing microorganism from various sites including polluted lake, automobile workshop, refinery, etc and then performing various primary and secondary screening tests. Also the best medium selection and effect of different carbon sources on the growth followed by the production of biosurfactant is studied in this work.

2. Materials and Methods

2.1 Sample collection

Soil and water samples were collected from Automobile workshop located in Punjabi bagh (west), New Delhi (AW1), Hindustan petroleum refinery, Shakurbasti, New Delhi (PR2), Hydrocarbon contaminated Soil near the ETP of Bhomia buttons pvt.ltd., Bahadurgarh, Haryana (BF3), Polluted lake, Noida (PL4). The samples were stored in sterile polythene bags in refrigerator at 4°C.

2.2 Enrichment and isolation of culture

One gram of soil sample was dispensed in 250 ml shake flask containing 100ml of the minimal salt medium (MSM: Glucose 30 g/l, Sodium nitrate 3.6 g/l, Yeast extract 0.5 g/l, Potassium chloride 1.1 g/l, Sodium chloride 1.1 g/l, Ferrous sulphate heptahydrate 0.00028 g/l, Magnesium sulphate heptahydrate 0.5 g/l, Trace elements solution 5ml/l, Zinc sulphate heptahydrate 0.29 g/l, Calcium chloride tetrahydrate 0.24 g/l, Copper sulphate pentahydrate 0.25 g/l, Manganese sulphate monohydrate 0.17 g/l), enriched with 1% diesel. The media was incubated at 37°C on incubator shaker at 150rpm for four days. After four cycles of enrichment, serially diluted (10⁻⁵, 10⁻⁷, 10⁻⁸) cultures were plated on MSM agar. The plates were overlayed with 100µl of diesel oil and incubated at 37°C for 3 days. Similarly, the water samples were also used for the isolation of microorganism by spread plate technique on MSM agar plates and incubated at 37°C for 2-3 days. The single colony from the plates were streaked into nutrient agar plates and incubated overnight. The culture was maintained on nutrient agar plates and periodically subcultured.

2.3 Screening of biosurfactant producing microorganisms

Loopfull of culture was inoculated in nutrient broth from nutrient agar plate and incubated till the OD reaches 0.6. 1ml of the culture was then transferred to 250 ml Erlenmeyer flask containing 50 ml of MSM supplemented with 1ml diesel incubated along with control at 37°C in incubator shaker at 150rpm for 96 hrs. The cells were harvested by centrifugation at 7,000 rpm for 15 minutes at 4°C, dried in oven to determine dry biomass weigh. The culture filtrate was used for further investigations.

Primary screening was done by Cetyl Trimethyl Ammonium Bromide (CTAB) method developed by Siegmund and Wagner,1991 where blue agar plates were prepared by supplementing cetyl trimethyl ammonium bromide (0.5mg/ml) and methylene blue (6µl/50ml) in minimal salt (MS) agar containing 1% diesel. The culture filtrate was loaded into the wells bored in MS agar plate and incubated overnight at 37°C followed by flushing with CTAB-MB solution and incubation at 4°C for 24 hours in order to observe a dark halo around the wells.

Oil spread method (OSM) developed by Morikawa et al, 2000, was also used where, petri plate base was filled with 15ml distilled water followed by addition of 500µl cedar oil to the surface of water. Further, 5µl of supernatant from culture broth was added at different spots on cedar oil. The occurrence of clear zone was an indication of a biosurfactant activity. The diameter of the clear zones was visualized under visible light and measured after 30 seconds, which correlates to the surfactant activity, also known as oil displacement activity.

Secondary screening was done using Emulsification activity by Cooper and Goldenberg, 1987, was measured at 540nm by optical density method. 2ml samples of cell free supernatant were added to a boiling test tubes containing 2ml distilled water and the solution was mixed with 1ml of diesel. After vigorous vortex for 2 minutes, the tubes were allowed to stand for 1 hour to separate aqueous and oil phase. Aqueous phase is carefully removed and OD is measured and compared with uninoculated broth used a negative control. The calculations were done for all the cultures individually and their emulsification activities were compared with each other.

The emulsifying capacity was evaluated by an emulsification index (E24). 2 ml of diesel was added to 2 ml of the cell – free solution in test tube, vortex mixing for 2 min and allowed to stand for 24 hr. The E24 index is given as percentage of the height of emulsified layer (cm) divided by the total height of the liquid column (cm). The percentage of
emulsification index calculated by using the following equation:
\[ E_{24} = \frac{\text{Height of emulsion formed}}{\text{Total height of solution}} \times 100 \]
Surface tension was measured by Stalagmometer. This device is essentially a pipette with a broad flattened tip, which permits large drops of reproducible size to form and finally drop under the action of gravity. The surface tension can be determined on the basis of the number of drops which fall per volume, the density of the sample and the surface tension of a reference liquid, e.g., water.

2.4 Growth kinetics study of isolate (PL4 strain)

500µl inoculum was transferred to 50ml minimal salt medium (MSM) and 50ml nutrient broth (NB) separately. Biomass analysis for every one hour was done spectrophotometrically at 595nm and plotted versus time.

2.5 Medium Selection for biosurfactant producing isolate

Three different media minimal salt medium g/l (MSM: ), Bushnell Hass medium g/l (BH: Magnesium sulphate 0.200, Calcium chloride 0.020, Monopotassium phosphate 1.000, Dipotassium phosphate 1.000, Ammonium nitrate 1.000, Ferric chloride 0.050, Agar 20, at pH 7 ± 0.2 ) and Proteose peptone-glucose-ammonium salts medium g/l (PPGASM: Glucose 5, Ammonium chloride 1, Peptone 10, Potassium chloride 1.5, Magnesium sulphate 0.4 ) were investigated for biosurfactant production.

The biosurfactant producing isolate PL4 was inoculated in nutrient broth and incubated till OD reached 0.6. Then, 1ml of the culture was inoculated to the flasks containing MSM, BH and PPGASM supplemented with 1% diesel and incubated at 37°C at 150 rpm for 96 hours. CTAB, OSM, Emulsification activity, emulsification index and surface tension measurements were carried out and results were analysed for optimum medium.

In order to Optimization Medium effect of combination of glucose and diesel on biosurfactant production was also studied in order to optimize carbon content. Three different combinations i.e, 3% glucose (no diesel) 1% diesel (no glucose) and 3% glucose (1% diesel) were investigated by supplementing them in MSM media separately. One ml of the grown culture (OD 0.6) was inoculated into the flasks and incubated for 96 hour and harvested.

The harvested culture filtrate was analyzed for maximum biosurfactant production in order to obtain the best combination.

Effect of different carbon sources (3%) incorporated in Minimal salt medium (MSM) on biosurfactant production was studied, where sucrose, glucose and starch were investigated. 5% stock solution of each carbon source was prepared and autoclaved separately along with minimal salt broth in three different flasks. 30 ml stock solution was added to 20 ml minimal salt medium (MSM) incorporated with 1% diesel to achieve the desired concentration of 1 % (w/v). 1% of the isolated culture PL4 was inoculated in the MS medium containing flasks respectively and incubated at 37°C for 4 days. After four days, the broth was centrifuged and supernatant was analyzed. All surface activity tests were performed to find the best carbon source.

3. RESULTS AND DISCUSSIONS

3.1 Screening of biosurfactant producers

Out of 12 isolates, 4 were able to grow on MSM media with diesel as a carbon source. Oil spreading for PL4 strain showed a positive result by displacing the oil and hence increasing the diameter by 5 cm as shown by figure 1whereas the other isolates were unable to displace the oil thereby showing negative result. Table 1 shows diameters of Displacement Zone of oil on water by biosurfactant. PL4 strain showed the maximum emulsification activity and emulsification index, table 2 shows the surface tension of culture filtrates of respective isolates. PL4 strain has the highest capability to emulsify the diesel oil because its emulsification index was calculated maximum i.e 60%.

The dark blue halo is observed due to ion pairing complexation. PL4 strain showed a larger dark blue halo than other isolates as shown by figure 2. Hence the isolate is an anionic biosurfactant producer as it was able to form an ion pair complexation with cationic methylene blue and CTAB. Moreover surface tension of cell free broth containing biosurfactant was reduced to 21.16 dynes/cm.

PL4 strain showed higher oil displacement activity, emulsification activity and reduction in the surface tension when grown on MSM medium hence it is the optimum medium for it to culture. Table 3 shows the results on different media. In Optimization of culture medium when both glucose and diesel together used as the sole carbon source for optimum growth and
production of biosurfactant in MSM medium was observed. The isolated strain was cultured on media different media containing carbon substrates such as glucose, starch and sucrose and on glucose maximum production of biosurfactant was observed. Table 4 shows the effect of different carbon sources.

Table 1: Diameter of Displacement Zone of oil on water by biosurfactant

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial diameter (cm)</th>
<th>Diameter after displacement (cm)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW1</td>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>PR2</td>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>BF3</td>
<td>2.3</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>PL4</td>
<td>2.5</td>
<td>7.5</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Surface Tension of culture filtrates of different isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>No. of drops</th>
<th>Surface tension (dynes/cm)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>32</td>
<td>64.33</td>
<td>Control</td>
</tr>
<tr>
<td>AW1</td>
<td>29</td>
<td>76.98</td>
<td>-</td>
</tr>
<tr>
<td>PR2</td>
<td>35</td>
<td>68.76</td>
<td>-</td>
</tr>
<tr>
<td>BF3</td>
<td>41</td>
<td>50.14</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Properties of different media

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Medium</th>
<th>OSM</th>
<th>E&lt;sub&gt;24&lt;/sub&gt; Emulsification activity</th>
<th>Surface tension (dynes/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BH</td>
<td>Negative</td>
<td>1.6</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>PPGASM</td>
<td>Negative</td>
<td>1.7</td>
<td>29.03</td>
</tr>
<tr>
<td>3</td>
<td>MSM</td>
<td>Positive</td>
<td>3.01</td>
<td>24.26</td>
</tr>
</tbody>
</table>

Table 4: Effect of Carbon sources

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Carbon source</th>
<th>OSM</th>
<th>E&lt;sub&gt;24&lt;/sub&gt; Emulsification activity</th>
<th>Surface tension (dynes/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose</td>
<td>Best</td>
<td>60%</td>
<td>0.61</td>
</tr>
<tr>
<td>2</td>
<td>Starch</td>
<td>Negative</td>
<td>46%</td>
<td>0.32</td>
</tr>
<tr>
<td>3</td>
<td>Sucrose</td>
<td>Better</td>
<td>52%</td>
<td>0.47</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The potent biosurfactant producer PL4 was successfully isolated from a lake polluted with municipal waste, and characterized successfully. The bacteria showed high oil displacement activity when cultured on minimal salt medium. Surface tension was also least with this strain which is the most desirable character required for the perfect biosurfactant producing microorganism. It was also observed from the study that both glucose (major carbon source) and diesel are essential for production of biosurfactant. As biosurfactant is produced in the stationary phase of the growth cycle and glucose is required for the growth of the strain followed by the degradation of the diesel thereby producing the biosurfactant extracellularly in the surrounding medium.
References

17. Pesce, L. A biotechnological method for the regeneration of hydrocarbons from dregs and muds, on the base of biosurfactants. World Patent 02/062, 495, 2002.
22. Desai, J. and Banat, I.M. Microbial Production of Surfactant and Their Commercial Potential, American Society for Microbiology, 61(1), 47-64, 1997.
27. Illori, M.O., Amobi, C.J. and Odocha, A.C. Factors affecting biosurfactant production by oil degrading Aeromonas spp. isolated from a