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Bundesrepublik Deutschland

(17)

Urkunde

über die Eintragung des Gebrauchsmusters Nr. 20 2023 101 412

Bezeichnung:

Waschmittel auf Lipasebasis für verbesserte Fett- und Ölreinigung

IPC:

C11D 3/386

Inhaber/Inhaberin:

Borkar, Prita, Nanded, IN
Dhuldhaj, Umesh, Nanded, IN
Gadewad, Manisha, Aurangabad, IN
Gawai, Dilip, Nanded, IN
Joshi, Vikas, Valsad, IN
Totewad, Narayan, Kalyan, IN
Warangkar, Suchita, Nanded, IN

Tag der Anmeldung:

21.03.2023

Tag der Eintragung:

11.04.2023

Die Präsidentin des Deutschen Patent- und Markenamts

Eva Schewior

Eva Schewior

München, 11.04.2023

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80297 München

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Bezeichnung der Erfindung

IPC-Vorschlag des Anmelders

Waschmittel auf Lipasebasis für verbesserte Fett- und Ölreinigung

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Sonstige Anträge

☐ **Aussetzung** der Eintragung und Bekanntmachung für __ Monate (§ 8 Absatz 1 Satz 2 Gebrauchsmustergesetz)
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Erklärungen

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(12) Unterschrift

(13) Funktion des Bearbeiters

(16)

Bundesrepublik Deutschland

Urkunde

über die Eintragung des
Gebrauchsmusters Nr. 20 2023 100 079

Bezeichnung:
Hydrolase-Enzymkomplex für die Behandlung von Abwässern

IPC:
C02F 3/00

Inhaber/Inhaberin:
Borkar, Prita Shamrao, Nanded, IN
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Manjramkar, Vinda, Thane, IN
Totewad, Narayan Dattatraya, Kalyan, IN
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Tag der Anmeldung:
09.01.2023

Tag der Eintragung:
26.01.2023

Die Präsidentin des Deutschen Patent- und Markenamts

Cornelia Rudloff-Schäffer

Cornelia Rudloff-Schäffer

München, 26.01.2023

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Bezeichnung der Erfindung

IPC-Vorschlag des Anmelders

Hydrolase-Enzymkomplex für die Behandlung von Abwässern

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Sources

1	https://link.springer.com/article/10.1007/s00792-020-01180-2 INTERNET 18%
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A DETECTION AND PREVENTION METHOD OF GLIOBLASTOMA

1. ABSTRACT

Glioblastoma is one of the devasting and chronic diseases in day-to-day life. It's a primary brain tumour in case of adult and around 55 %of the total world. If we count total brain cancer is 100% along that 36% is gliomas. It was administrated by United states that around all gliomas can be detected in fourth stage. Grade IV astrocytoma refers to glioblastoma, the most severe and violent form of astrocytoma. Glioblastoma develops in brain in which (>90 percent, primary glioblastoma). Glioblastomas grow slowly in 10% of cases are also suffering from a lower-grade glioma (grade II or III) and Glioblastoma is also known as secondary

glioblastoma. The current standard of treatment for GBM is to conduct the most safe surgical resection possible. The combination of radiotherapy (RT) and temozolomide commonly combined with adjuvant temozolomide therapy has been shown to be very important, but modestly, better than RT alone after surgery in the third phase of clinical trials conducted by the European Association for the Study 9 and Treatment of Cancer and Centre National Cancer. Patients with their cancer have methylation of the developing methylguanine methyltransferase (MGMT) (GenBank 4255) which lasts longer and is more advanced than those with non-methylated lesions.

Main and secondary glioblastoma are histologically distinct, considering their molecular variations. In recent years, mutations in the genes for isocitrate (IDH) 1 and IDH2 have been discovered. In more than 80% of cases of secondary glioblastoma,2 and with the development of a monoclonal antibody that targets identify the R132H mutation in the IDH1 gene. Glioma mutations account for more about 90% of all IDH1 mutations. Bevacizumab (AvastinVR) is an approved drug for the treatment of cardiovascular

disease.Males are more likely than females to produce primary GBM (M:F = 1.3), while sec ondary GBM is the opposite . Younger patients with primary GBM are abnormal.

Dohrmann et al. claimed in the 1970s that GBM accounts for just 8.8% of all childhood

CNS tumours.

[Dohrmann GJ et al, 1976.]

In 2009, recurrent glioblastoma was recorded in differentcountries, including 7 the United States and Switzerland, but not in the European Union.The challenges is to study further to prepare successful and developed biomarker as well as to initiate prepare therapies and anti-glioma agents which gives such beneficial for whole world.

2. INTRODUCTION

There are several aspect in which glioblastoma can be considered as an harmful in world wise. Glioblastoma is a primary or de NOVO brain tumour that accounts for 55 percent of all gliomas. It is also known as glioblastoma multiforme. It's a tumour that's really violent and infiltrative. Glioblastoma is a very aggressive cancer, with a five-year mortality rate of more than 90% and a survival time of between 14.6 months. The survival rate has not improved significantly over time in comparison to other cancers, and no existing therapy is curative. Astrocytoma's are histologically and clinically grouped into four categories, according to the WHO classification: pilocytic astrocytoma, diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma multiforme. Anaplastic astrocytoma and glioblastoma multiforme have an uncontrolled growth pattern, whereas pilocytic astrocytoma and diffuse astrocytoma have a limited growth rate. [Cao, H.; Wang, 2017]. The blood-brain barrier (BBB), which prevents the transport of saved and / or high-level molecules from the blood to the brain, has a barrier gap in view of the actual growth of the tumor. These cells of glioblastoma create a local hypoxia which can cause of angiogenesis. Changes in the production of aquaporin family proteins in BBB segments are linked to the formation of glioblastoma tissue, due to increased angiogenesis. [Nguyen, H.S.; Shabani et al ,2018 & McCoy, E.; Sontheimer et al,2007]. According a research report that glioblastoma occur in all age of person, Tumours in other parts of the brain may cause more subtle symptoms including executive dysnction disorders, weakness and slight memory problems. Tendon that connects the frontal lobe, temporal lobe, and corpus callosumto grow in size after being revealed

Fig gives the brief detail about all type of brain cancer in which glioblastoma is around 37%.

Headaches are a normal first symptom, and they are usually combined with a serious mass effect, either directly from the tumour or by ventricular obstruction. There are several classifications like Sex in which high prevalence among males. Another thing is a common race among white people after 45 years of age. Genetic Tuberous sclerosis (TSC), types of neurofibromatosis 1 and 2, Turcot syndrome, and Li-Fraumeni syndrome are all symptoms of neurofibromatosis. The radiation that separates the second degree of astrocytoma and the third degree of astrocytoma of the anaplastic is the two types of astrocytoma.

For this group, 5 the current standard of treatment is maximally secure surgical resection, followed by radiation therapy (RT) and concurrent temozolomide (TMZ), and finally adjuvant TMZ. There are no clinical trials comparing the effects of more surgery and less surgery. Selection bias is inextricably linked to retrospective results, making it impossible to determine the true degree of resection. It is a standard process which can be applicable in younger than

70age.However, targeted therapies such as antiangiogenic agents, antiepidermal 5 growth factor receptor (EGFR), and phosphoinositide 3kinase (PI3K) inhibitors have been used as adjuvants for second-line care. Reported events vary by age and gender. The median age of diagnosis is 65, with rates rising for people aged 75-84. Glioblastoma is 1.58 times more common in men compared to children, with an annual incidence of 4.00 years compared to 2.53 per 100,000 people, respectively. Non-Hispanic whites were particularly vulnerable, second only to American Indians and Alaska Natives, with a 40% lower incidence. 3 Glioblastoma is most common in North America, Australia, and Northern and Western Europe worldwide. In the United States, the overall prevalence of glioblastoma is 9.23 per 100,000 people[Taphoorn MJ et al , Ostrom QT et al].

1 RACE/ETHNICITY

AVERAGE ANNUAL AGE-ADJUSTED INCIDENCE RATE PER 100,000 POPULATION

1-YEAR RELATIVE SURVIVAL, %

5-YEAR RELATIVE SURVIVAL, %

Overall

Non-Hispanic white

Hispanic white

Black

Asian/Pacific Islander American Indian/Alaska Native

4.23

4.71

3.34

2.24

2.00

1.88

41.4

40.7

42.9

42.0

50.2

Not presented

5.4

4.8

7.8

6.8

8.8

Not presented

Source From : Ostrom QT, Cote DJ, Ascha M, Kruchko C, Barnholtz-Sloan JS. Adult glioma incidence and survival by race or ethnicity in the United States from 2000 to 2014.

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These strategies have only been slightly successful, and the low median survival rate of GBM patients, which is currently 15 months, has not drastically improved. [Schwartzentruber et al., 2012]. When compare the studies of bevacizumab in recurrent GBM showed massively improved response rates and 6-month progression-free survival (PFS). A surgical procedure to make a diagnosis, alleviate pressure on the brain, and safely remove as much tumour as possible is the first step in treating glioblastoma. Glioblastomas are diffuse tumours with finger-like tentacles that invade the brain, making complete removal impossible. The standard of care [60] for newly diagnosed GBM is determined by a number of factors, including age and molecular biomarkers (MGMT status and IDH mutation). The patient's reaction to initial therapies and the progression of the disease are used to establish how to treat recurrent GBM. Radiation and chemotherapy are used to delay the development of residual tumours following surgery and to treat tumours that aren't surgically removed. TTfield's (Tumor Treating Fields) can also [54] be used along with chemotherapy. Several side effect can be shown in this disease like fever , pain , fatigue , inflammation can occur and can be reduce according to severalprocedurelike cyclotron, which is a nuclear reactor that can break atoms to unleash proton, neutron, and helium ion beams, is used in proton therapy. The patient is seated on a table, his or her head covered by a head frame or face mask. Because of the existence of [55] the blood brain barrier (BBB) and the absence of immune cells, the central nervous system (CNS) was once thought to be an immunopurified organ. Lymphatic vessels in their classic form. It has, however, For decades, it has been understood that brain tumours existcan elicit a protective immune response antigens found in tumours.

Despite the fact that the CNS is an immunologically distinct organ, the immune system can still survey CNS antigens. Initially, this procedure entails antigens gaining access to the cerebrospinal fluid (CSF) by one of many routes Disruption of the BBB, for example, is one process direct tumour extension into the CSF space [Iliff JJ, Wang M, Liao Y]. Several therapy like chemoradiotherapy, immunotherapy, viral therapy, vaccine-based therapy,

tumour treating field and different screening technique for scanning of brain tumour and its weight.

A single gene encodes the MGMT protein, which is found on chromosome band 10q26.34. 15 MGMT is a large gene (>150 kb) with five exons, the first of which is noncoding. The TATA free, CAATfree promoter of the MGMT gene contains a CpG island. continuation of the transcript [Natarajan AT, Vermeulen S ,1992]. Transcriptional control of the MGMT gene is mediated by the 50 regulatory regions. The promoter with maximal activity lies 50 of the gene from 953 to þ202 bp (with the transcriptional initiation site as þ1) and consists of minimal promoter (69 to þ19),36 enhancer (þ143 to þ202) [Tano K, Shiota S, Collier J ,1990] MGMT enhancer binding protein (MEBP) binds to this site, as well as several binding sites such as SP1 and AP1.Transcription 9 of the MGMT gene appears to initiate at a single site within a guanosine-cytosine-rich, non-TATA box containing promoter. This promoter contains a CpG island and has been shown by in vivo foot printing. Analysing between methylation and unmethylation to know mutation of CpG easily [Harris LC et al., PotterPM etal,2012].

Modification of isocitrate dehydrogenase (IDH) is one of the most important mutations in patients with low grade and secondary glioblastoma. In codon R132 of the IDH1 gene, more than90% of IDH mutations are identified.

IDH mutation causes epigenetic changes 30 such as DNA global methylation and histone methylation, as well as the oncometabolite "2 hydroxyglutarate." As a result, the IDH mutation encourages the onset of gIlomagenesis. IDH is a small-molecule protein found mostly 2 in the liver, heart muscle, and skeletal muscle [LaPorte DC et al ,1983]. IDH is a participant. a variety of biological activities, including mitochondrial respiration lipogenesis, glutamine metabolism, oxidative phosphorylation glucose sensing and cellular redox state regulation. IDH is important 2 in the reduction of NADP + to NADPH and inhibits the oxidative decarboxylation of isocitrate in alpha-ketoglutarate (KG) (nicotinamide adenine dinucleotide phosphate). In vivo, IDH

exists in two forms: NADP-dependent IDH (IDH1 and IDH2) and NAD-dependent IDH (IDH3) [Reitman ZJ et al ,2010 & Stander M,2004]. IDH1 is one of them. Antioxidant that is found in the cytoplasm and peroxisomes.In eukaryotes, there are a variety of consequences. Additionally, IDH1 both maintains the antioxidant system in vivo and promotes lipid synthesis. IDH1 and IDH2 mutations have been found in acute myelogenous leukaemia, low-grade glioma, and secondary glioblastoma, among other cancers. While IDH3 mutations are not common in glioblastoma, they do exist. Immunotherapy is a form 45 of cancer treatment that strengthens your immune system to fight cancer. Your immune system helps you fight infections and other problems. White blood cells, as well as lymphatic organs and tissues, make up this organ. Immunotherapy is a form of organic medicine.

Immunotherapies have shown additional effects on the treatment of certain cancers, especially melanoma. In 2011, the Food and Drug Administration (FDA) released a statement. The FDA has approved apilimumab, a monoclonal antibody that binds 45 to the immune system. Cytotoxic T lymphocyte antigen-4 is a checkpoint receptor (CTLA-4), when reported to 9 have a significant impact on survival in people with cancer Melanoma is a cancer that affects the skin [Alberts B et al ,2002].

The glioblastoma's immunological environment is even more complicated. 62 Pleomorphic astrocytes, multinucleated giant cells, microglia, peripheral lymphocytes, and make up the tumour milieu. Both tumours and microglia are aided by microglia. Invasion and proliferation of cells. Tumor-associated macrophages (TAMs), which have been pushed into an immunosuppressive M2 phenotype by neighbouring astrocytes, secrete molecules like TGFb to shut down 5 cytotoxic T cells and metalloproteinase (MMP) 14 to breakdown the extracellular matrix impeding tumor growth. 6 For newly diagnosed glioblastoma, postoperative radiation has been standard treatment. Results of six randomised controlled trials of after surgery, radiotherapy was compared to no radiotherapy. Radiotherapy has significant survival benefits. Consideration of immunotherapy in several basis like viral immunotherapy, vaccine based which show

practical great efforts against glioma. Oncolytic virotherapy is based on the use of replicating viruses that can kill infected cancer cells selectively. Virus-induced cell death can be caused by a variety of mechanisms, the majority (if not all) of which are immunogenic [Aurelian L et al, 2016]. During the process of immunogenic cell death (ICD), damage-related cell patterns (DAMPs) and tumor-related antigens (TAAs) are synthesized, including patient-specific neoantigens produced by genetic variation in cancer cells. [Keskin D. B et al ,2019]. Several processes were discussed in detail to its prevention manner and raise of its survival manner.

3. DISCUSSION

Glioblastoma concept is very much expansive and referred as a grade IV astrocytoma is a fast growing and aggressive brain tumour. Various challenges often taken place for its prevention as well as its increase of its survival rate. The main detection can be occurred due to go through the process called biopsy. Biopsy is a sample of tissue taken from body in order to examine more closely. It gives brief description about initiation of condition whether the tissue is normal or becomes abnormal. According to medical term abnormal area may called lesion or tumour. If it shows grade IV condition then after it can be suggested to go for scanning and detecting

its weight and size by using several advanced technologies. If the tumour is in the malignant stage, it is more dangerous than benign. Several risk factor like its affect all age of groups including children, Prior radiation exposure, particularly a history of having radiation therapy to the head or neck, is one of the most noteworthy glioblastoma risk factors. Glioblastoma occurs in people who have had radiation therapy as a treatment for leukaemia, fungal infections of the scalp, or previous brain malignancies. Being a man, being 50 years old or older, and having genetic abnormalities on chromosome 10 or 17 are all risk factors. Neurofibromatosis (type 1 and type 2), tuberous sclerosis, and von HippelLindau disease are all hereditary disorders that increase the risk of developing brain cancer. Headaches, memory loss, speech difficulty, and visual impairments are all common in those who have one or more of these risk factors. Glioma does not usually

run-in families. However, having a family history of glioma increases your chances of getting it. Although a relationship between genes related and glioma has been identified, more research is needed to confirm a link between these genetic variants and brain cancers. Electromagnetic fields from power lines and radiofrequency radiation from microwave ovens, for example, have not been demonstrated to enhance the incidence of glioma. Surgery, chemotherapy, and irradiation can all be used to treat glioblastoma multiforme (GBM). Soft tissue alterations can be seen using computed tomography (CT) and magnetic resonance imaging (MRI) after treatment. [65] Serial CT or MRI scans may be necessary to distinguish between post-treatment inflammation and persistent tumour, causing treatment to be delayed. Molecular imaging offers a higher sensitivity and specificity for remaining tumours, making it beneficial [7] in the management of gliomas after treatment. The success of treatment, can be influenced by a variety of circumstances. IDH mutation [1] status, Karnofsky performance status (KPS), and O-6 methylguanine DNA methyltransferase (MGMT) status are the three factors to consider. As previously stated, those with IDH mutations have less aggressive cancers that respond better to treatment. Mutations of Isocitrate dehydrogenase (IDH) are common gliomas, and often cause epigenetic changes in the genome. IDH1 by its wild nature is involved in lipid biosynthesis and amino acid metabolism, but its role in cancer is not determined. The expression IDH1 and podoplanin (Pdpn) in IDH-mutated and IDH-wild type of gliomas were identified in this study, and their associations in gliomas were also investigated. In addition, luciferase testing and promoter methylation are used to evaluate the effects of wild IDH1 and mutant Pdpn expression.

1 FEATURE

IDH–WILD-TYPE GLIOBLASTOMA

IDH-MUTANT GLIOBLASTOMA

Precursor lesion

Proportion

Median age at diagnosis, y

Location

Histologic variants

Necrosis

Molecular pathogenesis

Develops de novo

Approximately 90%

62

Supratentorial

Giant cell glioblastoma, gliosarcoma, epithelioid glioblastoma

Extensive

TERT promoter mutation, EGFR amplification, LOH 10q, LOH 10p, PTEN deletion, MGMT promoter methylation, BRAF V600E mutationa

Diffuse astrocytoma, anaplastic astrocytoma

Approximately 10%

44

Preferentially frontal

—

Limited

IDH1/IDH2 mutation, TP53 mutation, ATRX mutation, PDGFRA amplification, LOH 10q, LOH 19q

Source from : Louis DN, Perry A, Reifenberger G, et al. 7 The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131:803-82019 and Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. Clin Cancer Res. 2013;19:764-772.20

The initial step in treatment is a complete and safe surgical excision of the glioblastoma. The term "maximum safe surgical resection" refers to 61 removing as much of the tumour as possible while minimising brain damage. Awake craniotomy, fluorescent dye, intraoperative MRI, and endoscopic surgery are some of the procedures utilised to enhance the amount of tumour removed. Awake craniotomy occurs when the tumour is

surgically removed [48] while the patient is awake. For example, if a tumour is found in the portion of the brain responsible for speech, surgery in that area may result in persistent speech impairment. The patient can better guide the procedure and get better results by being awake and conversing with the surgeon. Endoscopic surgery, also known as minimally invasive surgery, involves inserting a small device into the brain through a small incision in skull to detect and remove malignancies. It's vital to remember that glioblastoma surgery isn't a cure. Even if the glioblastoma tumour was completely removed, there are still numerous microscopic, undetected tumour in brain.

3.1. Screening of tumour using different Technology

When it comes to glioma detection methods, [53] FDG is the most widely used positron tracer in clinical practice, with a wide variety of tumor applications. Although normal physical stiffness may interfere with its use in the thinking of brain gliomas, which can hide uptake by lesions, it may have predictive significance, as higher FDG avidity indicates high-grade malignancies and hence a bad prognosis [Louis DN, Ohgak et al , 2007]. FDG PET may be useful in detecting recurrent tumors from high-intensity radiation at high (III and IV) levels, but it has limited use in determining the amount of tumor involvement and low-grade recurrence [Bénard F et al, 2003]. T1-weighted (T1w) and enhanced-enhanced [13] (T1CE), T2-weighted (T2w), and T2-fluid-attenuated inversion recovery (T2-FLAIR) are all available in any clinical scanner and provide important clinical information there are various processes in the plant area. Advanced MRI modalities have become more widely used in the previous decade to better characterise glioblastoma [Abdullah KG et al , 2013]. Multiparametric [13] MRI sequences such as dynamic susceptibility contrast (DSC) and dynamic contrast enhancement (DCE), higher order diffusion techniques such as diffusion tensor imaging (DTI), and MR spectroscopy are only a few instances (MRS). These new imaging modalities are still being integrated into better clinical procedures and individualised therapy methods. Diffusion imaging [13] techniques, such as diffusion tensor imaging (DTI), produce detailed white matter tractography pictures that can aid in neurosurgery planning and discern between post-operative vascular damage and

persistent enhancing malignancy [Lee SK et al ,2012]. Dynamic enhancement sequence (DCE) is used in the case of surgery to look for pharmacokinetic variables for differential diagnosis, which may be associated with abnormalities in the early stages of prognosis. [Kim R et al, 2017]. At this point in time, advanced imaging could help with radiation planning. The discordance between MRI-delineated gross tumour volumes (GTVs) and 3 18F-fluoroethylthyrosine-positron emission tomography (FET-PET), another functional imaging modality, was observed in a Polish investigation; FET-PET was better correlated with the site of final failure, showing that standard target volumes may not be adequate [Harat M et al ,2016]. Some glioblastomas show imaging changes consistent with progression based on the Macdonald criteria, yet no viable tumour can be seen in the resection specimen after a second surgical intervention, implying that adjuvant treatment may be having a beneficial effect that isn't seen on conventional imaging. This tendency, known as "pseudo progression," is most generally seen in individuals with a methylation MGMT promoter region in their cancers [Brandes AA et al ,2008].

3.2. Molecular mechanisms and therapeutic targets

There are certain confusion which can be clarify according to methylation and un-methylation of MGMT. By interacting to certain methylated DNA-binding proteins (e.g., MeCP2), methylation of a promoter inhibits (silences) transcription of the linked gene.MBD2), which form multiprotein complexes with other proteins. acetylated histones, resulting in chromatin condensation and difficulty to bind RNA polymerase as well as transcriptional problems equipment. A 777-bp CpG island with 97 CpG sites found at the MGMT promoter. The methylation differences between MGMT-expressing and non-expressing cell lines are the subject of this study. in four CpG regions, indicating that methylation had occurred .Sites that influence gene expression are not evenly distributed .The CpG island is quite well[Watts GS et al,1997& 30 Pieper RO et al, 1996]. By methylating the oxygen at position 6 of guanine nucleotides, TMZ destroys DNA. The O6 -methylguanine adducts that result pair with thymidine [Tano K et al , 1990]. Mismatch

repair enzymes seek to remove O6 -methylguanine from DNA, resulting in the formation of Apoptosis is caused by single- and double-strand breaks. Enzyme which used in MGMT, on the other hand, restores the damaged DNA.by converting the methyl group from a regular guanine to MGMT's cysteine residue[Tano K et al ,1990]. .3One 34 of the hallmarks of human cancer is cancer DNA methylation alterations. 67 CpG island methylator phenotype (CIMP) was discovered in colorectal tumours for the first time. hypermethylation of several genes' CpG islands in malignancy in a small percentage of tumours. Investigated DNA methylation changes in 272 GBM tumours and confirmed their findings on non-TCGA GBMs. CIMP lesions belonged 16 to the proneural subgroup in their analysis, were more common among lower-grade gliomas, had distinctive copy-number changes, and were tightly packed. IDH1 somatic mutations are linked. In 5 GBM patients treated with alkylating drugs as nitrosureas48 or TMZ, research have shown that MGMT promoter methylation has prognostic and predictive significance. 52,88 Hegi et al52 looked at 15 the MGMT status of patients treated in the EORTC/NCIC phase III clinical trial that established TMZ in combination with radiation therapy as standard of care for newly diagnosed GBM [Hegi ME et al ,2005 & Stupp R et al ,2009]. Methylated MGMT promoter regions in patients Regardless of whether they were given radiation therapy or not, they lived longer. either alone or in combination with TMZ. This was the subject of a 5-year study. MGMT continues to be demonstrated in the same 5 phase III clinical trial. The biggest predictor of outcome and benefit is methylation as a result of TMZ chemotherapy. MGMT has recently been shown to have prognostic and predictive utility in older people in various clinical trials. Improved OS and survival were predicted by MGMT methylation status. Even in patients over the age of 70, PFS 1 and quality of life can be improved. or older, whose Karnofsky performance level was low, and For freshly diagnosed GBM, they were given only TMZ 56 [Gállego Pérez-Larraya J et al , 2011].MGMT methylation is very much important for all age of group patient of glioma detection but in various cases it can be show that 28 the methylation of the promoter getting silence due to certain changes in that region. On 54 GBMs, the functional role of CpG island methylation in MGMT silencing

was studied. Pyrosequencing was utilised by [Everhard et al] to quantify the amount.and linked the methylation of 52 distinct CpGs parameters associated with MGMT mRNA expression[Everhard S et al ,2009].There were 35 CpG sites upstream and 17 CpG sites downstream of 16 the transcription start site. Overall, an 85 percent degree of concordance was reported between methylation and expression. The MGMT gene, which is found on chromosome 10q26, codes for a DNA-repair protein that removes alkyl groups from the O6 position of guanine, which is a key location for DNA alkylation. The MGMT is consumed by DNA restoration. Protein is a nutrient that the cell needs replenish. Chemotherapy-induced lesions, in particular, if left untreated, can be fatal. Cytotoxicity is triggered by O6-methylguanine [Ochs K 5 et al ,2000 & Liu L et al]. MGMT diagnostic testing necessitates sufficient and well-preserved tumour tissue. With cryopreserved tumour specimens, the optimal yields with methylation-specific PCR are obtained, avoiding fixation-related destruction of tumour DNA.

If we considered about IDH mutation, IDH1 can be 68 found in the cytoplasm and peroxisomes, while IDH2 and IDH3 reside in the mitochondrial matrix[Leighton, F et al 1969]. 14 IDH mutations linked to cancer are most commonly found in the arginine residue, which is required for isocitrate recognition (R132 for IDH1, R140 or R172 for IDH2)[Yan, H., Parsons et al ,2009]. Missense mutations in the IDH1 gene create a low-polarity amino acid, such as histidine, lysine, or cysteine, thought of replacing arginine with a solid, well-charged residue at 132, preventing the enzyme from efficiently and established hydrogen bonds and isocitrate carboxyl and carboxyl sites. [Ward, P. S et al,2010 & Ward, P. S et al ,2012]. 9 As a result, the converted IDH enzyme has a higher affinity for NADPH and a lower isocitrate affinity. IDH gene is converted by tumor cells by heterozygous IDH mutation, and the main types 4 of IDH dimers are thought to be heterodimers that contain wild-type IDH1 type and a mutant version of R132H. As a result, part of the wild dimer type is depleted of non-IDH cells converts isocitrate to -KG to make NADPH, and this mutant component of the dimer has neomorphic activity, converting -KG to D-2-hydroxyglutarate (D-2-HG) in a NADPH-dependent manner [Dang, L., White et al

,2009]. Mutations ior prevention n IDH1/2 were discovered to facilitate the formation of the tumour microenvironment by increasing the expression of VEGF, making it ideal for the growth of glioblastoma stem cells[Zhao H, Yang L et al ,2016].

3.3. Therapies for glioblastoma

Glioblastoma is a chronic disease we must say but it have less durability. According to several research it is known that at a less time period it can destroy the whole immune system as well as person's life. Researchers found out several therapies for glioblastoma. The immunosuppressive aspect of glioma, which controls antitumor immune responses, has been demonstrated in numerous studies. Glioma overexpress cells immunosuppressive elements include programmed cell death 1 ligand (PD-L1) and indolamine 2,3-dioxygenase (IDO), which inhibit antigen presentation [D.A. Wainwright et al, 2012 & O. Bloch et al, 2013]. Interleukin (IL)-10 and transforming growth factor (TGF-) are released by glioma-associated macrophages, which inhibit immune cell activity [B. Kaminska et al ,2013 & S. Wagner et al ,1993]. In addition, regulatory T (Treg) cells mediate immunosuppressive effects on glioma microenvelo by eliminating cytotoxic T lymphocytes, which can directly kill tumor cells. A greater understanding of the immunosuppressive environment in glioma will aid in the understanding of immunotherapy mechanisms. The role of TMZ was identified in 2005, when a study found that combined TMZ with radiotherapy massively reduced the median survival of GBM patients. patients as contrast only to those who received radiotherapy [R. Stupp et al ,2005]. In addition to TMZ, the FDA has approved bevacizumab and tumor-treating fields (TTF) for the treatment of glioblastoma. Bevacizumab is a vascular-targeting synthetic monoclonal antibody. endothelial growth factor is a protein that is produced by the endothelial (VEGF). During a phase II clinical trial, it was discovered that the antitumor operation and protection of bevacizumab alone [H.S. Friedman et al, 2009]. Earlier studies have shown that inhibiting IDO, CTLA-4, or PD-L1 in glioma mouse models decreases the number of tumour-infiltrating Treg cells while still improving long-term survival [D.A. Wainwright et al ,2014].

The immune checkpoint molecule PD-L1 is linked to programmed cell death. Activation of PD-L1 inhibits T lymphocyte function and allows cancer cells to evade the immune system [Brandes AA et al ,2008]. The act of expressing Human glioma tissues 57 have been found to contain PD-L1, which has been linked to glioma grade. These results support PD-potential L1's as a therapeutic target.a cancer treatment goal [D.H. Munn et al , 2013 & G.C. Prendergast et al ,2014]. Interferon gamma, tumour necrosis factor-a, and 56 vascular endothelial growth factor are inflammatory cytokines that cause the expression of on tumour cells and various PD-1/PD-L1 inhibitory ligands Dendritic cells and macrophages with immune cells and PD-L2. The immune response is blocked when PD-1 associates with its ligands. T-cell proliferation is reduced, and cytokine production is reduced. enhancing apoptosis and anergy in activated T cells, and inducing apoptosis and anergy in activated T cells Treg operation. lymphocytes that infiltrate tumours [He J, Hu Y et al ,2015 & Nduom EK et al 2016]. Nivolumab (Bristol-Myers Squibb) 34 is one of the immune checkpoint inhibitors that has been extensively studied. This PD-1 monoclonal IgG4 antibody was first approved by the FDA in 2014 for advanced melanoma and has since been approved for other indications. The combination of nivolumab and ipilimumab has also been shown to be effective as a second-line treatment for solid and hematologic tumour. The FDA has approved immediate approval for the advanced treatment of cancer in 2015.After the combination therapy, BRAF V600 wild-type melanoma showed 28 a substantial increase in the objective response rate phase II/III tests, as well as progression-free survival [Postow MA et al, 2015 & Larkin J, 18 Hodi FS et al ,2015].

When CTLA-4 pathway inhibitors are given, T cells in secondary lymphoid organs are isolated. Naive and relaxed lymphocytes elevate the CTLA-4 site where TCR binds to the MHC-treated peptide, based on the binding TCR binding of antigen expressed. The antigen-producing cell is then regulated by CTLA-4, which antagonizes the costimulatory CD28 molecule of ligand B7. If TCR binds too much and therefore CTLA-4 is activated, the costimulatory signal will likely be distributed between the automatic T cells. In glioblastoma, anti-monoclonal antibodies to CTLA-4,

such as ipilimumab and tremelimumab, 9 are currently being studied. Bevacizumab may also have other immune-boosting properties. 39 In one study, ipilimumab was combined with bevacizumab in 20 patients with malignant melanoma Glioma resulted in a partial radiographic response in 31% of cases, with the rest remaining stable. For the remaining 31%, disease is the cause of their illness, and disease progression is the cause of their illness. The percentage is 38%[. 6 Chinot OL et al ,2014 & Carter T et al ,2014]. Vaccination is very important in preventing cancer. Three classes of antigens were tested in glioblastoma tumor antigens (TAAs), tumor-specific antigens (TSAs), and tumor lysate. Non-invasive TSAs include receptor tyrosine kinases, 4 which have long been investigated as major drivers of oncogenesis.

49 The epidermal growth factor receptor (EGFR) has received a lot of attention. Glioblastoma development has been linked to amplification and activation of change. There have been several discoveries of tyrosine kinases. EGFR-target inhibitors 28 have been tested in recurrent diseases, including glioblastoma, but the results have been negative. The high degree of redundancy and heterogeneity in growth factors. Any of these are believed to be caused by EGFR mutations [. 5 Reardon DA et al , 2014 & Okada H et al ,2011]. Targetable TSAs include receptor tyrosine kinases, which have long been studied as important drivers of oncogenesis. In preclinical trials targeting IL13Ra2, Her2, EphA2, and EGFRvIII, CAR T efficacy in glioblastoma has been suggested.78-81 The findings 18 of a number of clinical trials using adoptive T-cell transfer in glioblastoma that started recently are still pending. In 9 a limited number of patients, safety and bioactivity have been identified. CAR T lymphocytes that were programmed to target IL13Ra2.82,83 were given to the patients.IL13Ra2 is a monomeric IL-13 receptor that is expressed primarily in the intestine. Glioblastoma tumour cells, stem-like cells, and tumor-infiltrating cells were found in glioblastoma tumour cells, stem-like cells, and tumor-infiltrating cells. macrophages, 57 which is regarded as a weak predictor of prognosis [Brown CE et al, 2012, Fichtner-Feigl S et al, 2008]. There is substantial evidence that NK cellular therapy could have a positive effect in other solid tumour settings [Christine E, Brown CE, 2013].

NK cells, unlike T cells, have the ability to react to tumour cells that express antigens in a variety of ways. Patients with a lack of functional NK cells may advance through the stages of tumour development faster [Hegde, M., et al, 2013]. DCs' capacity to initiate anti-tumor activities in both 69 the innate and adaptive immune systems appeal to cancer immunotherapy researchers. DC-based vaccines work by exposing patient-derived, ex vivo matured DCs to tumour antigens and then injecting them back into the body. Patients, 5 in the hopes of eliciting a long-lasting T-cell-mediated anti-tumor immune response. Dendron is a company based 7 in the United States. By increasing median prostate survival times, sipuleucel-T (ProvengeTM) established the precedent for this approach. In 5 a Phase III clinical trial, [USC University of Southern California] increased the survival time of carcinoma patients by 4 months [Kantoff, P.W., et al, 2010]. The most recent trial using rindopepimut (RINTEGA®; Celldex Therapeutics), a promising mutant peptide EGFRvIII in combination with DC maturation factor granulocyte-macrophage colony-stimulating factor (GM-CSF), could be a major indicator in treating patients with GBM. GBM patients with genetic mutations have a receptor that is shown approximately 30% of the time. [Thorne, A.H et al ,2016]. The BBB is a layer of endothelial cells that lines the blood vessels in the brain, allowing only some molecules from the bloodstream to move into the fluid containing the neurons and other brain cells. Many small-molecule drugs and macromolecules, such as peptides, proteins, and gene-based drugs, are blocked by the BBB, limiting the treatment of CNS diseases including neurodegenerative disorders, brain tumours, brain infections, and stroke. The blood-brain barrier is considered "leaky" in the heart of glioblastomas (GBMs), cancer therapeutics such as small molecules and antibodies are still unable to move across.

28 One of the most common treatments for glioblastoma is radiotherapy. Radiotherapy has been incorporated into post-operative GBM treatment after initial research has proven its effectiveness in strengthening survival compared to surgery alone 9 [Walker MD et al, 1980].

Source from : Roger Stupp, M.D., Warren P. Mason, M.D., 32 Martin J. van den Bent, M.D., Michael Weller, M.D., Barbara Fisher, M.D., Martin J.B. Taphoorn, M.D., Karl Belanger, M.D., Alba A. Brandes, M.D., Christine Marosi, M.D., Ulrich Bogdahn, M.D., Jürgen Curschmann, M.D., Robert C. Janzer, M.D., et al., for the 6 European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups and the National Cancer Institute of Canada Clinical Trials Group*

Kaplan–Meier Estimates of Overall Survival According to Treatment Group.

The median survival benefit was 2.5 months; with radiotherapy + temozolomide, the median survival was 14.6 months (dependence area of 95, 13.2 to 16.8 percent), and with radiotherapy alone, was 12.1 months (95% confidence interval, 11.2 to -13.0). The two-year survival rate in the group given radiotherapy + temozolomide was 26.5 percent (95 percent confidence interval, 21.2 percent to 31.7 percent), compared with 10.4 percent (95 percent confidence interval, 95 percent 6.8 to 14.1) in the group given radiotherapy only. With radiation + temozolomide, the median-free-free provival survival was 6.9 months (95% confidence interval: 5.8 to 8.2), while with radiotherapy alone, it was 5.0 months (95% confidence interval : 5.8 to 8.2).

Most patients treated with radiotherapy only received salvage chemotherapy repeatedly or continuously in the present study, and about half 5 of those treated with temozolomide initially received additional chemotherapy further; rescue treatment was given to a large number of patients who were initially treated with radiotherapy only. Despite this, survival favours combined care, supporting the conclusion that adding chemotherapy early along with radiotherapy is the best option for incorporating new medications. The local investigator determined the date of progression, and some patients most likely had pseudo progression [Stupp R, Mason WP et al, 2005]. As a result, glioblastoma patients can benefit from treatment approaches that minimise residual tumour burden. In clinical practise, MRI-based tumour response assessments can be hampered.by transient contrast-enhancing tissue responses like pseudo progression or radio necrosis, which are especially common after focal radio necrosis External radiotherapy and radiation

treatments along with the use of certain chemotherapies. Given that the majority of patients will experience a recurrence after standard of care surgery, RT, and TMZ, salvage options for GBM are critical. When a recurrence occurs, treatment options include supportive care, reoperation, and repeat RT (reirradiation), Structural therapy is multimodal therapy. A favourable success status (KPS) is important determinants of prognosis.70) and younger generation, which are associated with better outcomes following the completion of salvage therapy smaller tumours, non-eloquent brain position, and greater age are all less good prognostic factors. The period between initial treatment and recurrence, and the use of corticosteroids reliability and quality [Carson KA et al , 2007]. Numerous retrospective researches on reirradiation have been reported, using a range of treatment strategies such as 3D traditional RT, intensity-modulated radiotherapy (IMRT), brachytherapy, stereotactic fractionated RT, and stereotactic radiosurgery (SRS) with or without systemic therapy. Improvements in RT and imaging technologies have allowed more precise delineation of treatment volumes and improved treatment conformality, reducing toxicity to adjacent normal tissue [Wong ET et al , 2012]. Bevacizumab has also been studied as a potential salvage agent. The Food and Drug Administration (FDA) received its approval in 2009. Irradiation is a term that refers to the process of radiating anything. Bevacizumab is used to treat recurrent GBM. Bevacizumab is an antibody monoclonal antibody that targets the growth of vascular endothelial factor and inhibits microvascular proliferation. Several phase 2 studies have examined the combination of bevacizumab and other chemotherapeutic drugs, including carboplatin, irinotecan, TMZ, etoposide, erlotinib, and nitrosoureas, in one bevacizumab agent, and all showed high toxicity with no significant change in function. A retrospective study found that combining bevacizumab with another chemotherapeutic agent resulted in a 6-month progression-free survival rate of 2% on the second regimen, suggesting that patients who improve on a bevacizumab-containing regimen seldom respond to a second bevacizumab [Friedman HS et al, 2009 & Sathornsumetee S et al ,2010].

Immunotherapy combined with RT has a high level of performance, leading to a high

survival rate. Treatment of melanoma, [18] non-small cell lung cancer, and renal cell carcinoma with cytotoxic T-lymphocyte-binding proteins associated with 4 (CTLA-4) and targeted death receptors (PD-1) showed promising results. [9] The benefit of RT has long been attributed to its cytotoxic, local effect caused by DNA damage. t RT enhances antitumor T cell recruitment and the host's overall antitumor response by increasing antigen presentation and encouraging a proinflammatory tumour microenvironment, resulting in increased immunogenic cell death [Demaria S et al, 2007]. Just a few studies report on performance data or take tumour eloquence into account. In addition, only two studies looking at the status of MGMT methylation: [Adeberg et al]. had a negative effect of starting RT sooner than 24 days, but included only 50 patients, and Spratt et al. have experienced survival survival with delayed surgery after RT for more than six weeks, but only 45.8% of all people have their fixed MGMT status. Non-IDH-1 altered basic GBM treated with surgery for complete tumor removal followed by radio chemotherapy [11] according to the EORTC 22981/26981 protocol while considering the status of the MGMT promotor methylation [Adeberg S et al, 2015 & Spratt DE et al, 2014]. We have used a precise quantitative approach to search for EOR and RTV. [9] The methylation status of the MGMT promoter, which is a well-known predictive indicator, has been added, as well as an increase in Ki-67, which is one of GBM's most well-researched biomarkers. The actual prediction can be known according to show the PFS and OS graph which can be differ from different clinical trials as well as various patients.

4. CONCLUSION

Surgery is the most common treatment for GBM, followed by radiation and chemotherapy. The main purpose [36] of surgery is to remove as much tumor as possible without damaging the normal brain tissue needed for normal nerve function. GBM, on the other hand, is surrounded by a migratory region, infiltrated by hair [44] cells that invade surrounding tissues, making complete removal impossible. Surgery has the potential to reduce the amount of solid brain tissue in the brain, kill radiation-resistant cancer cells and / or chemotherapy, and reduce operating pressure. [28] Numerous studies have suggested that

MGMT to promote methylation improves OS, which is well known in patients undergoing alkylating chemotherapy and radiation with combined drugs. It is unclear how MGMT gets methylated in glioma patients. It is an important aspect of clinical practice evaluation in many organizations, and is regularly reviewed. The overall survival of patients has improved with the addition of concomitant and adjuvant TMZ, and the results of concomitant experiments analyzing deception with TMZ have provided strong care for the elderly without reducing quality of life or compulsory treatment.

5. FUTURE PROSPECTIVE

We can expect the better future research work for advanced theory regarding combination of different immunotherapy and chemo-radiotherapy technique for better durability and better prognosis to eradicate the devasted disease glioblastoma.

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A REVIEW ON PRODUCTION OF FOLATE FROM PROBIOTIC BACTERIA

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ABSTRACT

Folate is an essential vitamin playing a key role in metabolic pathway and it also important for the healthy foetal development. The WHO has recommended the adequate intake of folate by pregnant and lactating woman is necessary for healthy foetal development. To enhance the folate content in foods naturally, probiotic bacteria can be employed to develop the biofortified products. The probiotic bacteria are present in major indigenous fermented food products. The fermentation by folate synthesizing probiotic bacteria enhances the folate content in food which can be determined by HPLC. This process helps to alleviate deficiency of folate and also impart the benefits of probiotic bacteria via balancing the gut environment. The present review focus on the characteristics of probiotic bacteria and the methods to enhance folate in foods. The fusion of fermentation technology with vitamin B9 producing probiotic bacteria can be a novel methodology to develop fortified products to help humanity.

Keywords: Folatitamin B9, Probiotics, Biofortification

INTRODUCTION

Folate is a vital vitamin, which performs key function in synthesizing nucleotides, amino acids and vitamins. The human system is not capable of synthesizing folate on their own, hence it should be acquired from external sources to prevent the occurrence of diseases like neural tube defects, deficiency of folate, Coronary heart defects, cancer and other diseases. This vitamin B9 enacts a significant part in numerous metabolic pathways like functioning as cofactor in methylation cycle, replication of DNA and synthesis of nucleotides, amino acids and vitamins. The recommended dietary allowances of folate by WHO is 400 µg/day for adults, 400-600 µg/day for women who were pregnant and lactating. Though the green vegetables, fruits and cereal are rich in folate, folate deficiency occurs frequently and it is observed worldwide. Intake of chemically synthesized folic acid as supplement could cause adverse side effects like deficiency of vitamin B12. The researchers were interested in developing novel strategies to increase the content of biological folate in foods. The bacterial application in fermentation of foods might be one of the methods to improve the natural level of folates in foods.

8 Probiotics are living microorganisms which when consumed in adequate amount confer health benefits towards host. The lactic acid bacteria (LAB) belong to the group probiotics and are generally regarded as safe (GRAS) for consumption. The other group includes Bifidobacterium, and Saccharomyces boulardii (yeast) belongs to probiotics. Though most probiotics require vitamins for their growth some existing probiotics are adept to synthesize folate naturally either extracellularly or intracellularly. Recent researches show that employing folate producing probiotic as starter culture for fermentation it has enriched the nutritive value and folate concentration in yoghurts. Hence, it has application in nutraceuticals intended to develop bio-enriched food products to improve the health benefits of people and particularly to maternal health benefits for the development of healthy new born which will help and support the sustainable goal three of health and well-

being [1].

PROBIOTIC BACTERIA AS FOLATE PRODUCER

The LAB was usually auxotrophic to several vitamins however, few strains of LAB have the ability to produce vitamins. The common vitamins produced by LAB includes riboflavin, folate, thiamine and cobalamin. The vitamin producing LAB are isolated from different ecological niches like human gastrointestinal tract, 9 fresh milk from horses, dogs and humans, traditionally fermented milk, potato, corn-based products, different flours, grains and other fermented foods. The folate vitamin is applied in yoghurt production and non-dairy materials like oats, barley, pseudo cereals and potato-based foods [2]. The probiotic bacteria Bifidobacteria and LAB were able to convert dietary components into vitamin K and water-soluble B- group vitamins as Thiamine, cyanocobalamin, riboflavin, thiamine, niacin, folate, pyridoxine. L. plantarum, L. reuteri, L. acidophilus, and B. longum were specifically involved in B- Group synthesis [3]. The commercial probiotic bacterial cultures Streptococcus (St.) thermophilus ST-M6 (Christian Hansen, Hørsholm, Denmark) and TA-40 (DuPont Danisco, Dangé, France) and the probiotic bacterial strains St. thermophilus TH-4, Lactobacillus (Lb.) acidophilus LA-5, L. rhamnosus LGG, L. fermentum PCC, and L. reuteri RC-14 (Christian Hansen). The LAB is capable of producing natural folate and this will not cause any adverse effect. The gene involved in expression of folate production in lactic acid bacteria was called as fol gene. The fol gene expressed in 22 Streptococcus gallolyticus subsp. macedonicus CRL415 was examined for the different in vitro growth conditions. The genes involved in synthesis of folate was folE (GTP cyclohydrolase I), folQ (dihydroneopterin triphosphate pyrophosphohydrolase), folk (2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase), folP (dihydropteroate synthase), folC1 (folylpolyglutamate synthase), folC2 (dihydrofolate synthase) and folA (dihydrofolate reductase).

SCREENING METHODS TO ISOLATE FOLATE PRODUCING ORGANISMS

The probiotic bacteria are isolated from dairy and non-dairy products. The curd, cheese, kefir, fermented cereal products are the usual source of fer. The sample was homogenised and 1ml of sample was diluted in 9ml of peptone water. And serially diluted and 0.1ml was inoculated in MRS (deMans Rogosa Sharpe) agar in respective dilutions. Anaerobically incubate the plates at 37ºC for 24 hours. Each isolate would be 11 inoculated into a 2-ml folic acid assay medium and incubated for 18 h at 37 °C. This serves as the pre inoculum for folate production. The microbiological assay 6 was used to determine the folate production by means of folate auxotroph L. casei NCIM 2364 after fermentation for 7 h by 2% of the pre inoculum in folic acid assay medium [4]. The bioactive peptides are able to be produced by L.plantarum and Pediococcus acidolacti, these can be employed to produce the food enhanced with nutrition [5]. The genotypic identification of the isolate was done for high folate producing isolates.

FOLATE DETEMINATION

The concentration of both intracellular and extracellular folate was quantified by microbial assay based on the growth of the indicator strain L. 1 rhamnosus on 96-well microtiter plates (VitaFAST® Folic Acid from R-Biopharm AG, Darmstadt, Germany). The wells were filled by adding 150µl of folic acid assay medium (contained in the kit) and 150µl of an unknown or reference sample in sterile water. For reference samples, folic acid was dissolved in the sterile water at a concentration ranging from 0 to 1.28µg/100 g (ml). Control wells were not inoculated to check for sterility of the procedure. Each sample and standard were analysed in triplicate. The plates were then incubated in the dark at 37°C for 44 – 48h and at 620 nm the growth of bacteria was measured. The metabolism intensity or growth in accordance with the obtained folic acid was measured as absorbance (OD). The folate concentration of the samples was determined by comparing the OD obtained for treated samples with that obtained for the standard curve prepared [6]. The chromatographic analysis would also be used to determine the folate [7].

Further, the probiotic bacteria lactobacillus species consumed folate rather than

synthesizing it but the Lactococcus lactis and S. thermophilus have synthesized higher about of folate in the medium. The total content of folate synthesized by probiotic bacteria whether intracellular or extracellular was detected through microbiological assay and HPLC analysis. 3 L. lactis or S. thermophilus cells were recovered from a cell culture (50 ml) by centrifugation (12,000 µg, 10 min, 4°C) and washed with 20 ml of 50 mM H3PO4 (pH 2.3) containing 1% ascorbic acid. The cells were resuspended in 1 ml of the same buffer. Cell extract was prepared 1 by the addition of 1 g of silica beads to the cell suspension, following cell disruption in an FP120 Fast prep cell disrupter. To release folate that are binding to proteins by precipitating, 10 the cell extract was heated for 3 minutes at 100°C. Following centrifugation, 100 µl of concentrated cell extract was injected into the chromatograph as soon as possible after extraction, though samples were stable over the working day [8].

FERMENTATION

Fermentation has helped to enhance the potential nutrient and taste of cereal-based foods. Cauliflower, white beans and mixture of cauliflower and white beans were were prepared and inoculated with Lactobacillus plantarum obtained from the company Probi AB, Sweden. The total folate, riboflavin content produced by L.plantarum was detected based on European Standard EN1413. They have concluded that fermentation influences the 9 folate and riboflavin content in foods [9]. The bacteria isolated from Cow's milk has enhanced 1 the folate content in skim milk after fermentation process (Gangadharan et al., 2010). The folate producing S. macedonicus probiotic strains used as starter culture to ferment the milk, the obtained curd served as bio-enriched fermented food with high folate content and also it restores the gut environment [10, ,11].

The fermented cereal diets contribute major part to the diets. 2 Lactobacillus plantarum X13, Pediococcus pentosaceus L73, Candida parapsilosis Y77 and Candida tropicalis Y74. These were used as starter culture to prepare fermented food. The L. plantarum X13 and Candida tropicalis Y74 were able to produce 30.97 ± 0.37 µg/ml. The sensory valuation of

fermented Ogi by these starter cultures was greatly acknowledged than the fermented ogi. In which the folate concentration was also enhanced [12]. And also, the inoculation of pearl millet with L.plantarum and L. fermentum has increased the folate concentration in the fermented product. Which has proved that porridge prepared with starter cultures have significantly higher folate concentration compared to porridges prepared by traditional methods. The folate content was detected by microbiological analysis method [13]. As the interest among the consumer has increased, the bean milk was permitted to be fermented with LAB. The quantity of thiamine, riboflavin, pyridoxine, and folic acid present in fermented milk was detected through HPLC analysis by comparing to the standard. Further, the presence of oligosaccharides was also tested. Lactobacillus rhamnosus yoba, Streptococcus thermophilus, Lactobacillus Bulgaricus subs Debulgaricus, Lactobacillus rhamnosus are the isolates used to in different concentration to prepare the fermented bean milk enriched with vitamins and oligosaccharides [14, 12]. The folate level was enhanced in both bread and bun prepared after fermentation by folate producing folate. Further, the folate was found to retained in the noodles also [15]. It has been found that L. plantarum, L. fermentum and L. rhamnosus isolated from kefir grains, salted mustard and breast milk respectively has generated folate in greater concentration in skim milk media. The researchers had found that weakening the cell wall would synthesize higher amount of folate. However, the growth of bacteria should not be affected in this process [16].

ALTERNATIVE TO ANTIBIOTICS

Antibiotics were widely employed not only to treat infections but also to improve the health benefits of farm animals. Application of antibiotics in poultry gains the market benefits however this leads to emergence of multiple drug resistant pathogens and zoonotic infection to humans. The antibiotic avoparcin similar to glycopeptide vancomycin was banned and it decrease the prevalence of vancomycin resistant enterococci (VRE). Thus, probiotics interestingly serves as a safe alternative to antibiotics and can be used

as animal feeders to improve its health benefits. In their study the chicken was sacrificed by overdose of anesthesia (barbiturate) and the cecum was aseptically transferred to the laboratory, homogenized in stomacher with peptone water followed by serial dilution in 0.85% saline and inoculation in MRS agar, Bifidus selective medium and M17 agar supplemented with 0.1g/L cylcloheximе and incubated at 37 °C for 24h. The gram-positive catalase negative cultures were selected and tested for antagonistic activity against S. heidelberg a pathogen isolated from chicken carcasses. The probiotic isolates produce bacteriocin like inhibitory compound. Further, 2 the folate production by LAB was evaluated with bacteriological assay and folate concentration was quantified with HPLC. Moreover, the LAB isolates were identified by MALDI-TOF. The probiotic characteristics like 15 acid and bile tolerance, coexistence activity, adherence, antibiotic susceptibility test and hemolytic activity of the isolated LAB were examined [17].

6. CLINICAL BENEFITS OF FOLATE PRODUCING PROBIOTICS

Folate is one of the vital vitamins and its deficiency is observed works wide. The folates synthesized by microorganisms could be employed as substitute to chemically synthesized folic acid. In the previous report, Lactococcus lactis subsp. lactis KLDS4.0325 and Lactococcus lactis subsp. lactis KLDS4.0613 to produce folate enriched fermented milk. From the folate deficient murine model, the study proves alleviating the folate deficiency in vivo. Further it also enhances fatty acid and 5-methyltetrahydrofolate content in 23 whole blood and liver and reduced the homocysteine levels [18].

Previous studies shows that vitamin producing probiotic have the capability to treat inflammatory bowel disease. The probiotics that were frequently used belongs to the heterogenous group of bacteria like Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetrogenococcus, Vagococcus and Weisella. The LAB produces lactic acid as end product by metabolizing the carbohydrate 8 which helps to reduce the pathogenic microorganisms. 12 The health benefits of lactic acid bacteria (LAB) include lactose intolerance, anti-carcinogenic effects,

immunostimulatory effects, reversal of depression and anxiety symptoms, anti-obesity and anti-diabetic activity and reduction of serum cholesterol levels.

The one of the main symptoms of intestinal bowel disease is alteration the distribution of microorganisms in gut. The animal models treated with LAB fermented milk have increased the count of lactic acid bacteria in intestinal colonization and decreases the load of Clostridium difficile Further, LAB can reduce the oxidative stress involved in IBD. The LAB alleviates the mucosal destruction triggered by inflammation diseases. In in vitro studies Lactobacillus strains maintains the integrity and tight junctions in the Caco-3 cell line. The cells pretreated with supernatant of Lactobacillus rhamnosus GG prevented the apoptosis of enterocytes treated with 5-Fluoroacil (5-FU). The LAB possesses the ability to synthesize bacteriocin, bioactive peptide, biosurfactants, different flavor compounds and B- group vitamins [2].

The lactic acid bacteria isolated from human GIT possess all the biosynthetic genes required for folate and riboflavin production and they synthesize these compounds in the gut. The folate and folic acids have metabolic activities. Folic acid administration decreases the arterial hypertension and homocysteine level in blood [19].The treatment of obese children and adolescent with metformin and folic acid leads to decrease to insulin resistant and decreases pro inflammatory cytokines TNF alpha, IL-6, IL-7 associated with folic acid. Further, its supplementation can reduce the possible danger of colorectal cancer [2].

The reduced intake of natural folic acid and increased intake of synthetic folic acid have related to development of colorectal cancer. Though a lot of researches are focused on the dietary intake of folate but the folate produced by bacteria colonized in the GI tract also contribute as similar to foods we intake. The folate are water-soluble B-group vitamin names as tetrahydrofolates and plays an essential role in the human metabolisms. The L.plantarum have widely used to study the folate production and some Bifidobacterial which largely depends on its origin and the yeast species are capable of synthesizing folate and B-group vitamins in fermented foods. The folates are usually adsorbed in the small intestine which converts tetrahydrofolates to monohydrofolates. The synthetic folic

acid and bacterial biosynthesized folic acid also adsorbed in small intestine. The epigenetic DNA modification or DNA synthesis leads to cancer development. Hence folate can be used to treat the colorectal cancer [20].

The celiac disease CD is an autoimmune enteropathy which was caused through the gluten 24 in wheat, barley and rye in sensitive individuals. The gluten free grains do not possess all the nutrients. Hence, the Andean grains such as quinoa (Chenopodium quinoa Willd) are gluten free and gained attention for food consumption. Further with has application in medical and nutraceutical fields. The grains have antinutritive compounds like phytic acid, tannins, polyphenols and enzyme inhibitors. The LAB is usually consumed as starter culture for safety, effectiveness, economy and other aspects. The LAB breakdown the phytic acid and increases the minerals bioavailability in the fermented food products. 6 In their study the Leconostoc mesenteries subsp. Mesenteries CRL2131, Lactobacillus plantarum CRL, 1964 and CRL 2107, L. 18 rhamnosus CRL 1963, CRL 1984, and CRL 1983 was collected from culture collection of CERELA. The LAB was grown in MRS broth at 30 °C for 16h. To inoculate the quinoa the bacteria were centrifuged 5 and washed twice with saline, resuspended in tap water to cell density of 8.0 CFU/ml. The quinoa and cell suspension were used to ferment dough and allowed to ferment at 30 °C for 24h. The riboflavin (B2) and folate (F9) were evaluated quantitatively. The determination of B9 was done by microbiological assay with L. rhamnosus NCIMB 10463 as marker strain was compared with standard curve of HPLC. The vitamin B2 was identified by HPLC for different dough concentration and standard curve was obtained by different dilution of commercial riboflavin. The animal model weaned BALB/c mice was used and divided into 6 groups with differs constituents of diet. Finally, the mice were anaesthetized with ketamine and xylazine and the blood samples, plasma and organs were processed. The animals that consume fermented pasta has improved body weight and also concentration of vitamin B9 enhanced the whole blood of nutrient deficient mice model. The histopathological study shows the increased length of villi [21].

The interesting research finding includes the relationship between gut microbiome and

brain a bidirectional communication. The gut microbiota lines the mucosal surface and serves to develop immune system as this is the large surface that exposed to external environment pathogens. Which was validated by mice model born and maintained in the sterile environed has week immunity compared to mice exposed to antigens in gut. The brain function and human behavior was influenced by gut bacteria as it helps to develop the brain regions. Because several psychiatric and neurological disorders were associated with changes in gut microbiota. Scientific evidence was provided by previous study in which the probiotic supplementation has alleviate and helped to treat Parkinson's disease, autism and Alzheimer conditions. Furthermore, stress, anxiety, depression, bipolar disorder, Schizophrenia, gut dysbiosis are connected with composition of gut microbiota. As Hippocrates stated " all disease begins in gut" as the gut microbiome 6 plays an essential role in maintaining the integrity of intestinal barriers and neurological cell functions. Short chain fatty acids were critical for both enterocytes and colonocytes. The SFA directly influence the immune reaction and sustains the anti- inflammatory response. In which, the firmicutes produce butyrate. The probiotic bacteria influence the host cellular property like proliferation, differentiation and maturation functions. The future study involves the different probiotic strains and their metabolites involved in various cellular pathway [22].

The gut dysbacteriosis is associated with lack of folate. The 12 LAB were 25 isolated from Tibetan kefir grains and whole genome sequencing was performed. Yoghurt was prepared and inoculated with LAB and concentration of folate in fermented yoghurt sample was assessed by HPLC. The folate was overproduced with stressing methotrexate and calcium ions [23]. The in vivo study was performed in folate deficient mice model and the blood sample was collected to evaluate the folate concentration and homocysteine level in blood. The recon sample was examined for the composition of bacteria colonized in the intestine. Related to other isolates the L.plantarum produced satisfactory 5 amount of folate and possess the complete folate biosynthetic gene. The increased calcium concentration in milk produces 1 high amount of folate during fermentation. The folate enriched yoghurt

and synthetic folic acid has brought the folate content and homocysteine level to normal in folate deficient bone model [24]. The intake of folate producing probiotic bacteria has clinical importance in preventing certain disease conditions was depicted in Fig.1.

Fig.1: The consumption of folate producing probiotic bacteria can prevent the occurrence of disease in human system.

Consumption of fermented foods impart in numerous health benefits in which it maintains the normal microflora 8 of the gut and in turn the brain functions normally as the neurological and mental disorder patients have observed to possess disturbed gut microbiota. Moreover, the consumption of probiotic enriched food helps to prevent the invasion of harmful pathogen 17 in the intestine and their metabolic product helps to heighten the immune response [25].

7. APPLICATIONS OF FOLATE PRODUCING PROBIOTICS

Buffalo milk is boiled in a bigger iron vessel and a small portion of this is transferred to a smaller vessel. The coagulant was usually mixed with to hot milk and blended with a scoop until coagulation was finished. Then the content in the vessel was emptied into the cloth in order to drain the whey. Entire process would be replicated until the coagulated milk was obtained. The curd was acquired subsequently removing the excess whey through pressing. And at the end, the product would be dropped in cold water. Modification in the tradition method by adding folate producing probiotic culture to form curd instead of sour whey in an innovative way for manufacture of panner.

The 26 L. bulgaricus and S. thermophilus bacteria obtained from culture collection of CERELA and studied for folate production. These cultures were grown on MRS agar plate. The isolates were then used as stater culture for yoghurt fermentation after 6h of incubation at 42 °C, the yoghurt sample was refrigerated. After 28 days the folate concentration and cell viability of LAB was evaluated the content of folate remained unchanged 27 during 28 days of storage which is an effective naturally bio enriched

cheese reported for the first time [26, 27].

The LAB isolated from sour dough, whey, cabbage and human excreta were examined for the nutraceutical like EPS (Exopolysaccharides) and folate. The isolates were grown on MRS agar and the probiotic characterization like tolerance to inhibitory substance, hydrophobicity, antibacterial activity, antibiotic susceptibility test was performed. The homofermentative LAB was able to produce 80% of lactic acid by utilizing the glucose. Whereas the heterofermentative lactic acid bacteria was able to produce only 50% of lactic acid along with ethanol, acetic acid. In order to detect the folate producing bacteria the cultures were grown on modified MRS agar with dextrose, 4g; ammonium chloride, 0.4g; yeast extract, 0.5g; dipotassium hydrogen phosphate, 0.2g; magnesium sulphate, 0.01g and manganese sulphate, 0.005g. At different time interval the 4, 6, 8 and 10 h the sample was analyzed using HPLC. Further the cultures were also detected whether the folate is synthesized intracellular or extracellular. The isolates also assayed for exopolysaccharide production. These isolates capable of producing the folate and EPS could be employed for the nutraceutical development [7].

Folate are essential for human metabolism the deficiency of folate may leads to the neural tube defects of new-born, melanoblastic anaemia. The LAB was isolated from human colostrum the L.plantarum, L. rhamnosus were obtained. Initially LAB is screened in folic acid free medium. Then folate produced extracellularly was assessed by HPLC. The probiotic characterization was also evaluated in their research. The folate was produced at 40µg/L by L.plantarum. The supplemented foods with this bacterial culture can be given to adults and also weaning infants as a novel food with natural folate [28]. From previous research articles, some bacteria produce folate excellently and also possess probiotic properties. The list of bacteria and their folate concentrations and some sources of origin of bacteria were listed in the table-1.

Table 1: List of Folates producing probiotic bacteria used to develop biofortified products.

Bacteria

Source

Concentration of folate

Folate Enriched product

Reference

Streptococcus thermophilus, Lactobacillus plantarum 16cv

Goat dairy product

321.1 ± 14.1 ng/mL

Fermented Milk

[6]

Enterococcus faecium VC223

1 European Food Safety Authority

123,625.74 ± 8.00 ng/ml

Cheese

[29]

E. lactis BT161

384.22 ± 5.00 ng/m

Lactobacillus plantarum GSLP-7 V

Tibet Kefir

3.72 μg mL−1

Yoghurt

[24]

St. thermophilus TH-4, Lactobacillus (Lb.) acidophilus LA-5, Lb. rhamnosus LGG, Lb. fermentum PCC, Lb. reuteri RC-14

1325 ± 77 ng/mL

Fermented Soymilk

[30]

Lactobacillus johnsonii La-1, L. casei strain Shirota, L. rhamnosus GG

CSCC / Nestle/ Yakut

11.5 ng/ g to 40-50ng/l

Skim Milk

[31]

Bifidobacterium longum subsp. infantis BB-02

13 633 ± 36 ng/mL

Okara soy bean and amaranth flour

[32]

Lb. reuteri RC-14

575 ± 28 ng/mL

Lactobacillus delbrueckii subsp. bulgaricus CRL 863 and S. thermophilus CRL 415 and

CRL 803

CRL

22.3 to 135 µg/L

[33]

Lactococcus subsp. cremoris; 1 Lactococcus lactis subsp. lactis;

Milk

12.5 ng /ml;

14.2 ng/mL

Skim milk medium

[34]

Pichia kudriavzevii

Cereal based traditional food -South Africa

FCAM medium

[35]

Lact. plantarum 15HN; Streptococcus thermophilus; Lactobacillus bulgaricus; Lactobacillus acidophilus LA-5 and Bifidobacterium lactis BB-12.

Traditional dairy sources

1487 ± 96.42 µg/L

Yoghurt

[36]

L. lactis

Cow's milk

129.53 ± 1.2 µg/L

[4]

Bacillus spp

Infants faecal

59 ng/ ml

[37]

L. 28 plantarum CRL 2107 + CRL

Culture Collection of CERLA

Eliminates folate deficiency in mice

Quinao Pasta

[21]

Lactobacillus sakei

Tocosh

1900 ng/g

Vegan spread

[38]

Lactobacillus amylovorus CRL887

Culture Collection of CERLA

81.2 ± 5.4 µg/L

Fermented Milk

[27]

Lactobacillus bulgaricus CRL871, Streptococcus thermophilus CRL803

263.1 ± 2.4 µg/L)

Lc. lactis FP368

Goat Milk

313 ng/ml

Fermented Milk

[39]

The biotechnological processes for vitamin inclusion in foods are more environmentally friendly and an attractive alternative to the chemical synthesis of riboflavin, since they include the use of renew- able sources and lead to a yield of equal or superior quality. Amongst, the prevalent application of microorganisms. Capable of producing vitamins would be a biological and a suitable economical way to yield biologically developed foods, this paved the foundation as an assuring substitute to artificially synthesized vitamins. It should let the developed food products with increased quantity of vitamins and without adverse side effects. Some food-grade LAB are capable of synthesizing B-group vitamins, and this trait, together with their adapt- ability to the 19 fermentation processes, their biosynthetic capacity, and metabolic versatility, makes them good candidates for the development of novel functional beverages for vitamin bio-fortification [40].

The development of fruit and vegetable-based juices has increased the attraction by health-conscious people, function food development and researchers as they can be a substitute for lactose intolerance people [41]. The LAB has numerous benefits like probiotic properties, increases density of nutrients by converting sugar concentrations, antimicrobial

and antioxidant activity, biosynthesis of vitamins, degradation of anti-nutritive compound and enriching the sensory qualities. The pomegranate juice fermented with Lactobacillus bacteria have greater antibacterial activity than unfermented lactic acid bacteria. Though most of LAB are auxotrophic, few strains of LAB and Bifidobacteria were able to convert dietary components into vitamin K or B-group vitamins, like thiamine, riboflavin, niacin, pyridoxine, folates, and cyanocobalamin. The B- group vitamins have major role in human including DNA replication/ nucleotide synthesis, precursors for various enzymes. The bacterial species able to synthesize B-group vitamins are the species of L. plantarum, L. reuteri, L. acidophilus, and B. longum. The cashew apple juice fermentation with different probiotic have shown varying effects on content and biosynthesis of B-group vitamins. Further, Vitamin C content was also tested by fermentation with probiotic strains. The short fermentation of citrus juice by probiotic bacteria have maintained the contes of vitamin C as in pasteurized juices [3].

The LAB was used for in-situ fortification by increasing the folate concentration. The lactic acid bacteria were collected from CNR-ISPA Milano (National Research Council, Institute of Sciences of Food Production, Milan) collections. The bacteria used in their study were Lactobacillus delbruekii subspp bulgaricus, L. paracasei subsp. paracasei, L. plantarum, L. rhamnosus, Lactococcus lactis subsp lactis, Enterococcus faecium, E.lactis and Streptococcus thermophilus [42, 43]. The enterococcus sp. studied does not possess any virulence gene. The lactic acid bacteria were cultivated in MRS and M17 media. The folate acid produced by the lactic acid bacteria were quantified using ELISA kit where folic acid conjugates were coated on the well and the absorbance was measured at 450nm after 30 minutes of incubation. Further, the folic acid produced either intracellular or extracellular was identified by microbiological assay. Then cheese was prepared by inoculating the folate producing lactic acid bacteria and allowed to ferment. Then the cheese was quantified for the amount of folate present after fermentation by LAB. They have concluded that L. Plantarum synthesized higher quantity of folate compared to other lactic acid bacteria used in this study [29, 44]. The possible method for the development

of bio-fortified folate rich food product development process was depicted in Fig.2.

Fig.2. The process of biofortified folate enriched food development process.

8. CONCLUSION

Based on literature the genes concerned in de novo biosynthesis of folate were found to be aroB, aroD, aroE, aroK, aroF, aroA, aroC, pabB, pabA, folE, folQ, folB, folK, folP, folC and folA. The folate biosynthetic genes were present on L. plantarum and Lactococcus lactis strains which are good folate producers. Though the floats are present in natural fruits, green vegetables and nuts it does not meet the dietary requirement and people with folate deficiency was observed world-wide. Thus, the innovation in science and technology we can develop folate rich products with the aid of probiotics moreover the people consume these products can also gain other health benefits along with reducing the folate deficiency in people. Hence, proposed to develop bio-enriched panner products. The probiotic bacteria with the ability to synthesize folate was used as starter culture to prepare fermented yoghurt. The fermented bio-enriched yoghurt contains the adequate concentration of folate present in natural foods. Moreover, it enhances the maternal health and child health with folic acid, also probiotic key role is to maintain gut-brain axis and balanced gut microbiota. The metabolites from probiotic bacteria have numerous health benefits to the host. The probiotics have the ability to boost immune response, maintain balances gut microbiota, improves behaviour through gut-brain axis. Apart from this the production of B-group vitamin B9 by probiotics can be incorporated in foods to make folate available easily to meet dietary requirements which also improve the health benefits. Thus, a bio enriched fermented products with probiotics will alleviate folate deficiency and improves the maternal and infants' health and the adequate folate content in diet can be maintained by the ingesting of bio enriched fermented products.

9. FUTURE PROSPECTIVES

• The application of bio-fortification of vegan products using folate producing probiotic LAB is an interesting alternative to the use of synthetic folic acid in foods and provides a strategy for the development of functional foods with increased nutritional value.

• The proper selection of LAB strains and growth conditions play an essential role for obtaining high levels of folate in fermented milk.

• Lactic acid fermentation is of importance for food preservation, while also having impact on taste and nutritional composition.

• The application of omics can provide new tools to monitor, control, modify or improve and validating models of interactions of the better-known probiotics.

• The invention of innovative technologies for the preparation of probiotic food products for improvement of their nutritional value will be the priority area in the near future.

10. ACKNOWLEDGEMENT

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11. DECLARATION

Author declares there is no conflict of interest.

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A REVIEW ON PRODUCTION OF VITAMIN B9 BY PROBIOTIC BACTERIA

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ABSTRACT Folate is an essential vitamin playing key role in metabolic pathway and it also important for the healthy foetal development. The WHO has recommended the adequate intake of folate by pregnant and lactating woman. To enhance the folate content in foods, the probiotic bacteria can be employed to develop the biofortified products. The probiotic bacteria can be isolated from various sources and then the probiotic characteristics of the isolate need to be evaluated. The probiotic bacteria possess the ability to synthesize folate which can be prepared by fermentation technology. The fermentation by folate synthesizing probiotic bacteria enhances the folate content in food which can be determined by HPLC. This process helps to alleviate folate deficiency and also impart the benefits of probiotic bacteria via balancing the gut environment. The present review focus on the development of fermented products with probiotic bacteria. The fusion of fermentation technology with vitamin B9 producing probiotic bacteria can be a novel methodology to develop fortified products to help humanity.

Keywords: Folate, Vitamin B9, Probiotics, Biofortification, Lactic Acid Bacteria

1. INTRODUCTION

Folate is an essential vitamin, which plays an important role in human life for the synthesis of nucleotides, vitamins and some amino acid. This vitamin cannot be synthesized by humans and must be obtained exogenously to prevent folate deficiency, neural tube defects (NTDs) and other related diseases. This vitamin B9 plays an important role in various metabolic pathways like acting as cofactor for one carbon transferring reactions in methylation cycle, DNA replication as well as biosynthesis of nucleic acids, some amino acids and vitamins. Protective role of folic acid in the reduction of neural tube defects, coronary heart diseases and cancer have renewed the research on folate supplementation

to combat its deficiency. 400 µg.day−1 for adults (FAO and WHO, 2005); 400–600 µg.day−1 is commonly recommended for pregnant & lactic women. Though the green vegetables, fruits and cereal are rich in folate, folate deficiency occurs frequently, and it is observed worldwide. Intake of Folic acid, the chemically synthesized form of folate, is used for fortification and supplementation, but it can cause adverse effects such as vitamin B12 deficiency in high intake. Therefore, novel methods to increase concentrations of naturally occurring folate in foods have grabbed the interest of researchers. The application of bacterial cultures in food fermentation is a novel strategy to increase "natural" folate levels. Probiotics are live microorganisms which when consumed in adequate amount confer health benefits to the host. The lactic acid bacteria (LAB) belong to the group probiotics and are generally regarded as safe (GRAS) for consumption. The other group includes Bifidobacterium, and the yeast Saccharomyces boulardii belongs to probiotics. Though most probiotics require vitamins for their growth some probiotics are able to synthesise folate naturally either extracellularly or intracellularly. Recent research show that employing folate producing probiotic as starter culture for fermentation it has enriched the nutritive value and folate concentration in yoghurts. Hence, it has application in nutraceuticals for the development of bio-enriched food products to improve the health benefits of people and particularly to maternal health benefits for the development of healthy newborn which will help and support the sustainable goal three of health and well-being. 2. Probiotic Bacteria as Vitamin B9 producer. The LAB was usually auxotrophic to several vitamins however, few strains of LAB have the ability to produce vitamins. The common vitamins produced by LAB includes riboflavin, folate, thiamine and cobalamin. The vitamin producing LAB are isolated from different ecological niches like human gastrointestinal tract, fresh milk from horses, dogs and humans, traditionally fermented milk, potato, corn-based products, different flours and grains. The reliable method to select riboflavin overproducers are obtained by choosing roseoflavin resistant strains. The folate vitamin is applied in yoghurt production and non-dairy materials like oats, barley, pseudo cereals and potato-based foods (LeBlanc et al., 2020). The probiotic bacteria

Bifidobacteria and lactic acid bacteria are capable of converting dietary compounds into vitamin K and water-soluble B- group vitamins like Thiamine, cyanocobalamin, riboflavin, thiamine, niacin, folate, pyridoxine. L. plantarum, L. reuteri, L. acidophilus, and B. longum were specifically involved in B- Group synthesis (Szutowska, 2020). 2.1. Screening methods to isolate folate producing organisms The probiotic bacteria are isolated from dairy and non-dairy products. The samples are curd, cheese, kefir, fermented cereal products. The bacterial cultures Streptococcus (St.) thermophilus ST-M6 (Christian Hansen, Hørsholm, Denmark) and TA-40 (DuPont Danisco, Dangé, France) and the probiotic strains St. thermophilus TH-4, Lactobacillus (Lb.) acidophilus LA-5, L. rhamnosus LGG, L. fermentum PCC, and L. reuteri RC-14 (Christian Hansen) was procured and used.The sample was homogenised and 1ml of sample was diluted in 9ml of peptone water. And serially diluted and 0.1ml was inoculated in MRS (deMans Rogosa Sharpe) agar in respective dilutions. The plates were incubated anaerobically at 37C for 24 hours. A single colony of each isolate was inoculated into a 2-ml folic acid assay medium supplemented with 20-µl nutrient solution and incubated at 37 °C for 18 h. This was used as the pre inoculum for folate production. The nutrient solution was composed of (milligrams per millilitre) (L-glutamate, 30; L-alanine, 20; L-glycine, 20; L-histidine, 20; L-serine, 10; L-threonine, 10; L-cysteine, 10; L-arginine, 20; L-asparagine, 10; L- isoleucine, 20; L-methionine, 10; L-valine, 10; ascorbic acid, 50; nicotinamide, 220; calcium pantothenate, 20; pyridoxal HCl, 20; riboflavin, 20; biotin, 10; PABA, 1). The folate production was determined by microbiological assay using the folate auxotroph L. casei NCIM 2364 after 7 h of fermentation by 2% of the pre inoculum in folic acid assay medium (Divya et al., 2012). The bioactive peptides are able to be produced by L.plantarum and Pediococcus acidolacti, these can be employed to produce the food enhanced with nutrition. The phenotypic molecular and proteolytic assays help to determine that the isolates belong to LAB and possess the probiotic characteristics (Matti et al., 2019). The 49 isolates from different sources are evaluated in folate free culture medium (FACM) to screen the slate producing lactic acid bacteria. The folate production by the isolates were determined by the

microbiological assay. The genotypic characterization of the isolate was done for high folate producing isolates. These isolates were allowed to ferment the milk and the obtained surd served as bio-enriched fermented food with high folate content (Aashifaa et al., 2019). 3. Determination of Folate The quantification of extracted folate (total, as well as extra- or intracellular) was carried out by a MA based on the growth of the indicator strain L. rhamnosus on 96-well microtiter plates (VitaFAST® Folic Acid from R-Biopharm AG, Darmstadt, Germany). The wells were filled by adding 150 µl of folic acid assay medium (contained in the kit) and 150 µl of an unknown or reference sample in sterile water. For reference samples, folic acid was dissolved in the sterile water at a concentration ranging from 0 to 1.28 µg/100 g (ml). Control wells were not inoculated to check for sterility of the procedure. Each sample and standard were analysed in triplicate. The plates were then incubated in the dark at 37°C for 44 – 48h and the growth was measured at 620 nm by using a microplate reader (TECAN, Infinite F200 PRO), as the bacteria grow until the vitamin is consumed. The intensity of metabolism or growth in relation to the extracted folic acid was measured as absorbance (OD). The folate concentration of the samples was determined by comparing the OD obtained for treated samples with that obtained for the standard curve prepared (Clara et al., 2020). The chromatographic analysis was performed using a Varian HPLC system (USA). The mobile phase was 20 mM phosphate buffer pH 6.2 and acetonitrile in the ratio 39:1. Mobile phase and samples were filtered through 0.45 µm filters (Millipore) before use. The samples were passed through a Nova Pak C18 column 4 µ (4.6×250 mm cartridge) at a flow rate of 1 ml min−1 at 25 °C and detected by UV detector (Varian) at 280 nm (Aswathy et al., 2008). The probiotic bacteria lactobacillus species consumed folate rather than synthesizing it but the Lactococcus lactis and S. thermophilus have synthesized higher about of folate in the medium. The total content of folate synthesized by probiotic bacteria whether intracellular or extracellular was detected through microbiological assay and HPLC analysis. Concentrated cell extracts were prepared as follows. L. lactis or S. thermophilus cells were recovered from a cell culture (50 ml) by centrifugation (12,000 µg,

10 min, 4°C) and washed with 20 ml of 50 mM H3PO4 (pH 2.3) containing 1% ascorbic acid. The cells were resuspended in 1 ml of the same buffer. Cell extract was prepared by the addition of 1 g of silica beads to the cell suspension, followed by disruption of the cells in an FP120 Fast prep cell disrupter (Savant Instruments Inc., Hol- brook, N.Y.). The cell extract was heated at 100°C for 3 min to release folate from folate binding proteins and to precipitate proteins. Following centrifugation (two times at 12,000 µg, 3 min, 4°C), 100 µl of concentrated cell extract was injected into the chromatograph as soon as possible after extraction, although samples were stable over the working day (Sybesma et al., 2003). The bacteria were isolated from cows' milk and preliminary characterization for the selection of probiotic species. Then the isolates in MRS medium 5% were inoculated in folate assay medium with nutrient solution and incubated. The microbiological assay was performed after 7h. The folate titer was compared to standard L. lactis. The selected probiotic bacteria were inoculated into 5% skim milk medium and allowed to ferment and the folate content was estimated by microbiological assay the culture was centrifuged and the supernatant was heated and mixed with folate extraction buffer and reagents were added and observance was measured at 655 nm (Gangadharan et al., 2010). 4. FERMENTATION Thompson et al. (2020) reported that the fermentation has helped to increase the potential nutrient and taste of cereal-based foods. They have chosen cauliflower, white beans and mixture of cauliflower and white beans in 50:50 for their study. These samples were prepared and inoculated with Lactobacillus plantarum obtained from the company Probi AB, Sweden. The control was refrigerated without inoculum whereas, the samples were incubated for 44h at 30 °C. The pH was detected for after 18h and 44h. pH of cauliflower was observed as less than pH 4 and white beans was pH 4.8 after 18h of incubation. The total folate, riboflavin content produced by L.plantarum was detected based on European Standard EN1413. They conclude that fermentation influences the folate and riboflavin content in foods. The Lactic acid bacteria were able to produce natural form of folate and this will not cause any adverse effect. The fol gene expressed in Streptococcus gallolyticus subsp. macedonicus CRL415 was examined for

the different in vitro growth conditions. The genes examined for folate biosynthesis was folE (GTP cyclohydrolase I), folQ (dihydroneopterin triphosphate pyrophosphohydrolase), folk (2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase), folP (dihydropteroate synthase), folC1 (folylpolyglutamate synthase), folC2 (dihydrofolate synthase) and folA (dihydrofolate reductase). The bacteria S. macedonicus was inoculated in milk to ferment it at 42 °C. This fermented milk has restored the gut environment (Laino et al., 2018). The fermented cereal diets contribute major part to the diets. The isolates were isolated from fermented maize and grown on MRS agar and folate analogue was mixed with cooled media to screen folate producing bacteria. For safety analysis the LAB was analyzed for hemolytic, DNase and gelatinase activity. Genotypic characterization was also performed and identified as Lactobacillus plantarum X13, Pediococcus pentosaceus L73, Candida parapsilosis Y77 and Candida tropicalis Y74. These were used as starter culture to prepare fermented food. The L. plantarum X13 and Candida tropicalis Y74 were able to produce 30.97 ± 0.37 µg/ml. The sensory evaluation of Ogi fermented by starter culture was highly acceptable than the naturally fermented ogi. In which the folate concentration was also enhanced in the Ogi preparation (Okoroafor et al., 2019). The inoculation of pearl millet with L.plantarum and L. fermentum it has increased the folate concentration in the fermented product. Which has proved that porridge prepared using starter cultures have significantly higher concentration of folate compared to porridges prepared by traditional methods. The folate content was detected using the microbiological assay method (Bationo et al., 2019). As the interest among the consumer has increased the bean milk was allowed to ferment with lactic acid bacteria. The concentration of thiamine, riboflavin, pyridoxine, and folic acid in the fermented milk was determined by HPLC analysis by comparing to the standard. Further, the presence of oligosaccharides was also tested. Lactobacillus rhamnosus yoba, Streptococcus thermophilus, Lactobacillus Bulgaricus subs Debulgaricus, Lactobacillus rhamnosus are the isolates used to in different concentration to prepare the fermented bean milk enriched with vitamins and oligosaccharides (Anino et al., 2019). 5. Alternative to antibiotics

Antibiotics were widely employed not only to treat infections but also to improve the health benefits of farm animals. Application of antibiotics in poultry gains the market benefits however this leads to emergence of multiple drug resistant pathogens and zoonotic infection to humans. The antibiotic avoparcin similar to glycopeptide vancomycin was banned and it decrease the prevalence of vancomycin resistant enterococci (VRE). Thus, probiotics interestingly serves as a safe alternative to antibiotics and can be used as animal feeders to improve its health benefits. In their study the chicken was sacrificed by overdose of anesthesia (barbiturate) and the cecum was aseptically transferred to the laboratory, homogenized in stomacher with peptone water followed by serial dilution in 0.85% saline and inoculation in MRS agar, Bifidus selective medium and M17 agar supplemented with 0.1g/L cylclohexime and incubated at 37 °C for 24h. The gram-positive catalase negative cultures were selected and tested for antagonistic activity against S. heidelberg a pathogen isolated from chicken carcasses. The probiotic isolates produce bacteriocin like inhibitory compound. Further, the folate production by LAB was evaluated with bacteriological assay and folate concentration was quantified with HPLC. The riboflavin synthesized by LAB was determined and quantified with chromatography method. Moreover, the LAB isolates were identified by MALDI-TOF. The probiotic properties like acid and bile tolerance, coexistence activity, adherence, antibiotic susceptibility test and hemolytic activity of the isolated LAB were examined (Sabo et al. 2020). 6. Clinical benefits of folate producing probiotics Folate is one of the essential vitamins and its deficiency is observed works wide. The folates synthesized by microorganisms can be used as an alternative to chemically synthesized folic acid. In this study they have used Lactococcus lactis subsp. lactis KLDS4.0325 and Lactococcus lactis subsp. lactis KLDS4.0613 to produce folate enriched fermented milk (FEFM). The folate deficient murine model was used to study FEFM in alleviating the folate deficiency in invo. FEFM also increases fatty acid and 5-methyltetrahydrofolate content in whole blood and liver. Further, it has also reduced the homocysteine levels (Jiao et al. 2020). Vitamin producing probiotic have the ability to treat inflammatory bowel disease (IBD).

The probiotics that were frequently used belongs to the heterogenous group of bacteria like Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetrogenococcus, Vagococcus and Weisella. The lactic acid bacteria produce lactic acid as end product by metabolizing the carbohydrate which helps to reduce the pathogenic microorganisms. The health benefits of LAB include lactose intolerance, anti-carcinogenic effects, immunostimulatory effects, reversal of depression and anxiety symptoms, anti-obesity and anti-diabetic activity and reduction of serum cholesterol levels. The one of the main symptoms of intestinal bowel disease is alteration the distribution of microorganisms in the gut. The animal models treated with lactic acid bacteria / LAB fermented milk have increased the lactic acid bacterial colonization and decreases the load of Clostridium difficile Further, LAB can reduce the oxidative stress involved in IBD. The LAB alleviates the mucosal damage caused by inflammation diseases and in vitro studies Lactobacillus strains maintains the integrity and tight junctions in the Caco-3 cell line. The cells pretreated with supernatant of Lactobacillus rhamnosus GG prevented the apoptosis of enterocytes treated with 5-Fluoroacil (5-FU). The lactic acid bacteria have the capacity to produce bacteriocin, bioactive peptide, biosurfactants, different flavor compounds and B- group vitamins (LeBlanc et al., 2020). The lactic acid bacteria isolated from human GIT possess all the biosynthetic genes required for folate and riboflavin production and they synthesize these compounds in the gut. The folate and folic acids have metabolic activities. Administration of folic acid reduces the arterial hypertension and homocysteine level in blood. The treatment of obese children and adolescent with metformin and folic acid leads to decrease to insulin resistant and decreases pro inflammatory cytokines TNF alpha, IL-6, IL-7 associated with folic acid. Further, supplementation of folic acid can reduce the risk of colorectal cancer (LeBlanc et al., 2020). The reduced intake of natural folic acid and increased intake of synthetic folic acid have related to development of colorectal cancer. Though a lot of researches are focused on the dietary intake of folate but the folate synthesized by bacteria colonized in the GI tract also contribute as similar to foods we

intake. The folate are water-soluble B-group vitamin names as tetrahydrofolates and plays an essential role in the human metabolisms. The L.plantarum have widely used to study the folate synthesis and some Bifidobacterial which largely depends on its origin and the yeast species are able to synthesize folate and B-group vitamins in fermented foods. The folates are usually adsorbed in the small intestine which converts tetrahydrofolates to monohydrofolates. The synthetic folic acid and bacterial biosynthesized folic acid also adsorbed in small intestine. The epigenetic DNA modification or DNA synthesis leads to cancer development. Hence folate can be used to treat the colorectal cancer (Kok et al., 2018). The celiac disease CD is an autoimmune enteropathy which was triggered by gluten in wheat, barley and rye in sensitive individuals. The gluten free grains do not possess all the nutrients. Hence, the Andean grains such as quinoa (Chenopodium quinoa Willd) are gluten free and gained attention for food consumption. Further with has application in medical and nutraceutical fields. The grains have antinutritive compounds like phytic acid, tannins, polyphenols and enzyme inhibitors. The LAB is usually used as starter culture for safety, effectiveness, economy and other aspects. The LAB breakdown the phytic acid and increases the bioavailability of minerals in the fermented food products. In their study the Leconostoc mesenteries subsp. Mesenteries CRL2131, Lactobacillus plantarum CRL, 1964 and CRL 2107, L. rhamnosus CRL 1963, CRL 1984, and CRL 1983 was collected from culture collection of CERELA. The LAB was grown in MRS broth at 30 °C for 16h. To inoculate the quinoa the bacteria were centrifuged and washed twice with saline, resuspended in tap water to cell density of 8.0 CFU/ml. The quinoa and cell suspension were used to ferment dough and allowed to ferment at 30 °C for 24h. The riboflavin (B2) and folate (F9) were evaluated quantitatively. The determination of B9 was done by microbiological assay with L. rhamnosus NCIMB 10463 as indicator strain was compared with standard curve of HPLC. The vitamin B2 was determined by HPLC for different dough concentration and standard curve was obtained by different dilution of commercial commercial riboflavin. The animal model weaned BALB/c mice was used and divided into 6 groups with differs constituents of diet. At the

end the mice was anaesthetized with ketamine and xylazine and the blood samples, plasma and organs were processed. The animals that consume fermented pasta has improved body weight and also concentration of vitamin B9 increased in the whole blood of nutrient deficient mice model. The histopathological study shows the increased length of villi (Carizo et al., 2020). The interesting research finding includes the relationship between gut microbiota and brain a bidirectional communication. The gut microbiota lines the mucosal surface and serves to develop immune system as this is the large surface that exposed to external environment pathogens. Which was validated by mice model born and maintained in the sterile environed has week immunity compared to mice exposed to antigens in gut. The brain function and human behavior was influenced by gut bacteria as it helps to develop the brain regions. Because several psychiatric and neurological disorders were associated with changes in gut microbiota. Scientific evidence was provided by previous study in which the probiotic supplementation has alleviate and helped to treat Parkinson's disease, autism and Alzheimer conditions. Furthermore, stress, anxiety, depression, bipolar disorder, Schizophrenia, gut dysbiosis are associated with gut microbiota composition. As Hippocrates stated " all disease begins in gut" as the composition of gut microbiota plays an essential role in maintaining the integrity of intestinal barrier and neurological cell functions. Short chain fatty acids were critical for both enterocytes and colonocytes. The SFA directly influence the immune response and sustains the anti- inflammatory response. In which the firmicutes produce butyrate. The probiotic bacteria influence the host cellular property like proliferation, differentiation and maturation functions. The future study involves the different probiotic strains and their metabolites involved in various cellular pathway (Conte, et al., 2020). The gut dysbacteriosis is associated with folate deficiency. The 12 lactic acid bacteria were isolated from Tibetan kefir grains and whole genome sequencing was performed. Yoghurt was prepared and inoculated with LAB and concentration of folate in fermented yoghurt sample was evaluated by HPLC. The folate was overproduced with stressing methotrexate and calcium ions. The invivo study was performed in folate deficient mice

model and the blood sample was collected to evaluate the folate concentration and homocysteine level in blood. The recon sample was examined for the composition of bacteria colonized in the intestine. Compared to other isolates the L.plantarum produced satisfactory amount of folate and possess the complete folate biosynthetic gene. The increased calcium concentration in milk produces high amount of folate during fermentation. The folate enriched yoghurt and synthetic folic acid has brought the folate content and homocysteine level to normal in folate deficient bone model (Zhang et al., 2020). The most of the lactic acid bacteria were able to degrade starch by secretion of amylolytic enzymes. The LAB isolated from Chinese fermented cereal-based foods are studied for the amylase content by investing on various parameters (Zu et al., 2019). Consumption of fermented foods impart in numerous health benefits in which it maintains the normal microflora of the gut and in turn the brain functions normally as the neurological and mental disorder patients have observed to possess disturbed gut microbiota. Moreover, the consumption of probiotic enriched food helps to prevent the invasion of harmful pathogen in the intestine and their metabolic product helps to enhance the immune response (Filippis et al., 2020). 7. Applications of folate producing probiotics Buffalo milk is boiled in a bigger iron vessel and a small portion of this is transferred to a smaller vessel. The coagulant (usually sour whey) is added to hot milk and stirred with a ladle till coagulation is completed. The contents of the vessel are emptied over a piece of coarse cloth to drain off whey. The whole process is repeated till all the milk is coagulated. The curd is collected after draining the whey and pressed to remove more whey. Finally, product is then dipped in chilled water. Modification in the tradition method by the addition of folate producing probiotic culture to form curd instead of sour whey in a innovative way for production of panner. The L. bulgaricus and S. thermophilus bacteria obtained from culture collection of CERELA and studied for folate production. These cultures were grown on MRS agar plate. The isolates were then used as stater culture for yoghurt fermentation after 6h of incubation at 42 °C, the yoghurt sample was refrigerated. After 28 days the folate content and cell viability of LAB was evaluated the folate

concentration remained unchanged during 28 days of storage which is an effective naturally bio enriched cheese reported for the first time (Laino et al., 2013). The lactic acid bacteria isolated from sour dough, whey, cabbage and human excreta were examined for the nutraceutical like EPS (Exopolysaccharides) and folate. The isolates were grown on MRS agar and the probiotic characterization like tolerance to inhibitory substance, hydrophobicity, antibacterial activity, antibiotic susceptibility test was performed. The homofermentative LAB was able to produce 80% of lactic acid by utilizing the glucose. Whereas the heterofermentative lactic acid bacteria was able to produce only 50% of lactic acid along with ethanol, acetic acid. In order to detect the folate producing bacteria the cultures were grown on modified MRS agar with dextrose, 4g; ammonium chloride, 0.4g; yeast extract, 0.5g; dipotassium hydrogen phosphate, 0.2g; magnesium sulphate, 0.01g and manganese sulphate, 0.005g. At different time interval the 4, 6, 8 and 10 h the sample was analyzed using HPLC. Further the cultures were also detected whether the folate is synthesized intracellular or extracellular. The isolates also assayed for exopolysaccharide production. These isolates were able to produce the folate and EPS and can be used for the nutraceutical development (Aswathy et al., 2008). Folate are essential for human metabolism the deficiency of folate may leads to the neural tube defects of newborn, melanoblastic anaemia. The LAB was isolated from human colostrum the L.plantarum, L.rhamnosus were obtained. Initially LAB is screened in folic acid free medium. Then extracellular folate production was evaluated by HPLC. The probiotic characterization was also evaluated in their research. The folate was produced at 40µg/L by L.plantarum. The supplemented foods with this bacterial culture can be used to adults and also weaning infants as a novel food with natural folate (Bhagya et al., 2018). From previous research articles it has been found that some bacteria produce folate excellently and also possess probiotic properties. The list of bacteria and their folate concentrations and some sources of origin of bacteria were listed in the table-1. The biotechnological processes for vitamin inclusion in foods are more environmentally friendly and an attractive alternative to the chemical synthesis of riboflavin, since they include the use of renew- able sources and

lead to a yield of equal or superior quality. [15]Among them, the use of vitamin-producing microorganisms is a natural and economically viable way to obtain bio-enriched food products, which constitutes a promising alternative to chemical synthesis of vitamins. It should [6]allow the production of foods with higher concentrations of vitamins and without undesirable side effects. Some food-grade LAB are able to synthesize the B-group vitamins, and this trait, together with their adapt- ability to the fermentation processes, their biosynthetic capacity, and metabolic versatility, makes them good candidates for the development of novel functional beverages for vitamin bio-fortification [15](Llamas-Arriba et al., 2019). The development [9]of fruit and vegetable-based juices has increased the attraction by health-conscious people, function food development and researchers as they can be an alternative for lactose intolerance people. The LAB has numerous benefits like probiotic properties, increases density of nutrients by converting sugar concentrations, [4]antimicrobial and antioxidant activity, biosynthesis of vitamins, degradation of anti-nutritive compound and enriching the sensory qualities. The pomegranate juice fermented with Lactobacillus bacteria have greater antibacterial activity than unfermented lactic acid bacteria. Though most of LAB are auxotrophic, some strains of lactic acid bacteria and Bifidobacteria are capable of converting dietary compounds into vitamin K or water-soluble B-group vitamins, like thiamine, riboflavin, niacin, pyridoxine, folates, and cyanocobalamin. The B- group vitamins have major role in human including DNA replication/ nucleotide synthesis, precursors for various enzymes. The bacterial species [6]able to synthesize B-group vitamins are the species of L. plantarum, L. reuteri, L. acidophilus, [4]and B. longum. The fermentation of cashew apple juice with different probiotic have shown varying effects on content and biosynthesis of B-group vitamins. Further, Vitamin C content was also tested by fermentation with probiotic strains. The short fermentation of citrus juice by probiotic bacteria have maintained the contes of vitamin C as in pasteurized juices (Szutowska, 2020). The [5]lactic acid bacteria were used for in-situ fortification by increasing the folate content. The lactic acid bacteria were collected from CNR-ISPA Milano (National Research Council, Institute of Sciences of Food Production, Milan) collections. The

bacteria used in their study were Lactobacillus delbruekii subspp bulgaricus, L. paracasei subsp. paracasei, L. plantarum, L. rhamnosus, Lactococcus lactis subsp lactis, Enterococcus faecium, E.lactis and Streptococcus thermophilus. The enterococcus species used in the study does not have any virulence gene. The lactic acid bacteria were cultivated in MRS and M17 media. The folate acid produced by the lactic acid bacteria were quantified using ELISA kit where folic acid conjugates were coated on the well and the absorbance was measured at 450nm after 30 minutes of incubation. Further, the production of folic acid is either intracellular or extracellular was identified by microbiological assay. Then cheese was prepared by inoculating the folate producing lactic acid bacteria and allowed to ferment. Then the cheese was quantified for the amount of folate present after fermentation by LAB. They have concluded that L. Plantarum synthesized higher quantity of folate compared to other lactic acid bacteria used in this study (Clara et al. 2020). 8. CONCLUSION Based on literature the genes involved in de novo biosynthesis of folate were found to be aroB, aroD, aroE, aroK, aroF, aroA, aroC, pabB, pabA, folE, folQ, folB, folK, folP, folC and folA. The folate biosynthetic genes were present on L. plantarum and Lactococcus lactis strains which are good folate producers. Though the floats are present in natural fruits, green vegetables and nuts it does not meet the dietary requirement and people with folate deficiency was observed world-wide. Thus, the innovation in science and technology we can develop folate rich dairy products with the aid of probiotics moreover the people consume these products can also gain other health benefits along with reducing the folate deficiency in people. Hence, proposed to develop bio-enriched panner products. The probiotic bacteria with the ability to synthesize folate was used as starter culture to prepare fermented yoghurt. The fermented bio-enriched yoghurt contains the adequate concentration of folate present in natural foods. Moreover, it enhances the maternal health and child health with folic acid, also probiotic key role is to maintain gut-brain axis and balanced gut microbiota. The metabolites from probiotic bacteria have numerous health benefits to the host. The probiotics have the ability to boost immune response, maintain balances gut microbiota,

improves behaviour through gut-brain axis. Apart from this the production of B-group vitamin B9 by probiotics can be incorporated in foods to make folate available easily to meet dietary requirements which also improve the health benefits. Thus, a bio enriched fermented products with probiotics will alleviate folate deficiency and improves the maternal and infants' health and the adequate folate concentration in diet can be maintained by the consumption of bio enriched fermented products. 9. FUTURE PROSPECTIVES • The application of bio-fortification of vegan products using folate producing probiotic LAB is an interesting alternative to the use of synthetic folic acid in foods and provides a strategy for the development of functional foods with increased nutritional value. • The proper selection of LAB strains and growth conditions play an essential role for obtaining high levels of folate in fermented milk. • Lactic acid fermentation is of importance for food preservation, while also having impact on taste and nutritional composition. • The application of omics can provide new tools to monitor, control, modify or improve and validating models of interactions of the better-known probiotics. • The invention of innovative technologies for the preparation of probiotic food products for improvement of their nutritional value will be the priority area in the near future. 10. REFERENCE 1. Clara, A., Silvetti, T., & Milena, B. (2020). Folate production by LAB and their potential for dairy product bio-enrichment by fermentation. FEMS Microbiology Letters. doi:10.1093/femsle/fnaa059. 2. Sabo, S.S., Mendes, M.A., Araújo, E.S., Muradian, L.B.A., Makiyama, E.N., LeBlanc, J.G., pr Borelli, P., et al. (2020). Bioprospecting of probiotics with antimicrobial activities against Salmonella Heidelberg and that produce B-complex vitamins as potential supplements in poultry nutrition. Scientific reports 10, 7235. https://doi.org/10.1038/s41598-020-64038-9. 3. Thompson1, H.O., Önning, G., Holmgren, K., Strandler, H.S., and M. Hultberg. (2020). Fermentation of Cauliflower and White Beans with Lactobacillus plantarum – Impact on Levels of Riboflavin, Folate, Vitamin B12, and Amino Acid Composition. Plant Foods for Human Nutrition 75, 236–242. https://doi.org/10.1007/s11130-020-00806-2. 4. Jiao, W., Wang, S., Guan, J., Shi, J., Evivie, S. E., Yan, F., Na, L., Chen, J., Li, Huo, G. (2020). Milk fermented with

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Table 1: List of folate producing probiotic bacteria used to develop biofortified products. Bacteria Source Concentration of folate Folate Enriched product Reference Streptococcus thermophilus, Lactobacillus plantarum 16cv Goat dairy product 321.1 ± 14.1 ng/mL Fermented Milk Clara et al. (2020)a Enterococcus faecium VC223 European Food Safety Authority 123,625.74 ± 8.00 ng/ml Cheese Clara et al. (2020)b E. lactis BT161 384.22 ± 5.00 ng/m Lactobacillus plantarum GSLP-7 V Tibet Kefir 3.72 µg mL−1 Yoghurt Zhang et al. (2020) St. thermophilus TH-4, Lactobacillus (Lb.) acidophilus LA-5, Lb. rhamnosus LGG, Lb. fermentum PCC, Lb. reuteri RC-14 1325 ± 77 ng/mL Fermented Soymilk Albuquerque et al. (2017) Lactobacillus johnsonii La-1, L. casei strain Shirota, L. rhamnosus GG CSCC / Nestle/ Yakut 11.5 ng/ g to 40-50ng/l Skim Milk Crittenden et al. (2002) Bifidobacterium longum subsp. infantis BB-02 633 ± 36 ng/mL Okara soy bean and amaranth flour Albuquerque et al. (2017) Lb. reuteri RC-14 575 ± 28 ng/mL Lactobacillus delbrueckii subsp. bulgaricus CRL 863 and S. thermophilus CRL 415 and CRL 803 CRL 22.3 to 135 µg/L Laino et al. (2012) Lactococcus subsp. cremoris; Lactococcus lactis subsp. lactis; Milk 12.5 ng /ml; 14.2 ng/mL Skim milk medium Gangadharan et al. (2010) Pichia kudriavzevii Cereal based traditional food -South Africa FCAM medium Greppi et al. (2017) Lact. plantarum 15HN; Streptococcus thermophilus; Lactobacillus bulgaricus; Lactobacillus acidophilus LA-5 and Bifidobacterium lactis BB-12. Traditional dairy sources 1487 ± 96.42 µg/L

Yoghurt Khallili et al. (2019) L. lactis Cow's milk 129.53 ± 1.2 µg/L Divya et al. 2014 Bacillus spp Infants faecal 59 ng/ ml Panda et al. (2014) L. plantarum CRL 2107 + CRL Culture Collection of CERLA Eliminates folate deficiency in mice Quinao Pasta Carrizo et al. (2020) Lactobacillus sakei Tocosh 1900 ng/g Vegan spread Mosso et al. (2020) Lactobacillus amylovorus CRL887 Culture Collection of CERLA 81.2 ± 5.4 µg/L Fermented Milk Laino et al. (2014) Lactobacillus bulgaricus CRL871, Streptococcus thermophilus CRL803 263.1 ± 2.4 µg/L) Lc. lactis FP368 Goat Milk 313 ng/ml Fermented Milk Silva et al. (2016)

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BIODEGRADATION OF XENOBIOTIC COMPOUND USING MICROBES P. Riya Department of Microbiology, B. K. Birla College of Arts, Science & Commerce (Autonomous), Kalyan, Maharashtra, India Introduction During the 1950s and 1960s many low- and middle-income countries were struggling to feed for the growing need of their population which saw a boost due to the industrial revolution and rise of employment. The Green Revolution was introduced in these countries, which saw a sharp rise in the yield of the food grains production making the agriculture produce available for exports due to the use of high yielding varieties of seeds, advanced irrigation technology and chemical pesticides, fertilizers. The Indian economy which is largely based on the agriculture has been immensely benefited by the advent use of pesticides, there has been a fourfold increase in the production of foodgrains by the application of pesticides. The term pesticide encompasses a wide spectrum of components from fungicides, insecticides, nematicides, rodenticides, molluscicides, herbicides among others. But the main players are organophosphate, and organochlorine compounds they have completely transformed the field of agriculture by keeping a control on the population of pests and not allowing the growth of unwanted weeds and herbs near the crop of interest so that nutrient availability is not shared. The excessive use of organochlorine compound as DDT in the earlier times used to significantly reduce the mosquito borne diseases malaria but due to the extreme toxicity and bioaccumulation of such xenobiotic compound the developed countries banned its use in the developing countries. What are these xenobiotic compounds? A substance foreign to the biological system is known as xenobiotic compound. Major sources of xenobiotic compounds enter into the environment are chemical and fertilizers, mining, fossil fuels also oil spills. Due to their potential toxicity to both wildlife and humans, several persistent organic pollutants (POPs) have now been totally banned from production and use in many countries around the world. No population is protected from the pesticide ill effects. What is the effect of this pesticide on soil and environment? Due to the leaching of heavy metals from the fertilizers and related compounds not only contaminate the soil flora but also affect the water table and cause poisoning of the

groundwater [1]The world-wide deaths and chronic diseases due to pesticide poisoning number about 1 million per year. High risk groups exposed to pesticides include production workers, formulators, sprayers, mixers, loaders and agricultural farm workers. During manufacture and formulation, the possibility of hazards may be higher because the processes involved are not risk free. Organochlorine compound is virtually found in the tissues of all life forms due to the phenomenon of Biomagnification: Organophosphorus compound toxicity can be classified into two broad categories namely Axons and Thions the axons include Axons which [5]have a P=O double bond and include phosphotriesterase and phosphotulates. The organophosphate poisoning act by interfering with the neurotransmitters namely acetylcholinesterase activity, OPs covalently bond to the hydroxyl group of serine phosphorylation, inhibit the enzyme's active site and cause the accumulation of acetylcholine at synapses. Also, these Ops impair the reproductive function of humans causing infertility, premature birth to birth defects Solution for accumulation Xenobiotic pesticides and its deleterious effects: Bioremediation, means use of microorganisms to remediate/ destroy or to immobilize pollutant from environment. The fate of pesticides in the environment is determined by both biotic and abiotic factors. Pesticides are degraded in the environment principally by the action of indigenous microorganism. Organophosphate pesticides are generally regarded as safe for use on crops and animals due to their relatively fast degradation rates. The degradation rates vary as a function of microbial composition along with different environmental factors, such as pH, temperature, and availability of sunlight. Some studies have shown that organophosphate pesticides degrade rapidly by hydrolysis on exposure to sunlight and air. Degradation of Xenobiotic compounds by Microorganisms: Most pesticides, such as organophosphate, carbamate, or pyrethroid are biodegradable compounds which can hydrolyze spontaneously at high pH and by enzymes to less toxic materials. [3]Enzymes play a key role in degradation of these toxic pesticides and most of these enzymes are produced by bacteria which are naturally equipped with the mechanisms to deal with these toxins produced by manmade activities. The microbes by means of gene transfer can

acquire the gene necessary for production of enzymes which can be helpful for the degradation ,also the group of bacteria can form a consortium and can together bring about degradation of complex and toxic pesticides which can be utilized by other groups of organisms.

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HEALTH BENEFITS AND NUTRITIONAL FACTS OF FENNEL SEEDS. Soniya Joginder Chauhan Department of Microbiology, B. K. Birla College of Arts, Science and Commerce (Autonomous), Kalyan: 421304, Maharashtra, India.

Introduction: Fennel is a Mediterranean Plant, binomial name of Fennel plant is Foeniculum vulgare and commonly it is known as Saunf in India, which belongs to the carrot family. It comprises [5]434 genera and about 3700 species, among all these the carrot family (Apiaceae) is a significant group of flowering plants. Some similar plants as like fennel are [4]Giant fennel (Ferula communis), a member of the same family, their stems grow to about 3 meters (10 feet) high and are used for tinder. Hog's fennel or sulfurweed (Peucedanum officinale) is uses in traditional medicine in parts of Europe. Bulb like stem base of Florence fennel (variety Azoricum) and blanched shoots are eaten as vegetable. Dill (Anethum graveolens) has been used in Ayurveda medicines since ancient times for carminative, stomachic and diuretic, if dill seeds are applied to the mouth and throat for pain and swelling (inflammation), some exceptional people are allergic to it. The cultivated fennel [2]plant is about 1 metre (3feet) tall and has stalks with finely divided leaves composed of many linear or owl-shaped segments. Fennel (Foeniculum vulgare), perennial herb of the carrot family (Apiaceae) grown for its edible shoots, feathery leaves, yellow flowers and seed. The small dry fruits are greenish brown to yellowish brown oblong ovals about 6mm (0.25inch) long with 5 prominent longitudinal dorsal ridges. Fennel seeds contain 3 to 4 % essential oil; the principle components are Anethole and Fen chon. Extracted oil is suggestive of anise in aroma, taste and is used for scenting soaps and perfumes and for flavouring candies, liqueurs, medicines and foods, particularly pastries, sweet pickles and fish. Native to Southern Europe, and Asia Minor, it is cultivated in temperate regions worldwide and is consider an invasive species in Australia and all the parts of United States. Health Benefits of fennel seeds:

Fennel seeds are most common found in Indian kitchens. It has a cooling effect on the body.It balances Vata, Pita and Kapha. Various Health Benefits of Fennel seeds are it helps to regulates Blood Pressure- (a Chewing fennel seed helps in increasing the level of nitrite

in saliva. Nitrite is a natural element that keeps a check on the blood pressure levels). It reduces Asthma Symptoms. Helps in Purifies Blood-(The vital fibre and essential oils in fennel seeds are useful for flushing out sludge and toxins from our bodies which helps in cleansing the blood and also in optimum absorption of nutrients in the body). These are the Source of Anti-oxidants-(The polyphenol act as anti-oxidants in fennel seeds also have anti-flammatory properties that help in fighting various bacteria and viruses, keep safe from various diseases, Choline is a very important 1and versatile nutrient in fennel that helps with sleep, muscle movement, learning and memory. It also helps to maintain the structure of cellular membranes, aids in the transmission of nerve impulses, assists in the absorption of fat, and reduces chronic inflammation. Vitamin C, Vitamin A, and beta-carotene are powerful antioxidants that can help protect cells against damage from free radicals). Highly demanded benefit of fennel tea is, it helps in weight loss because 3fennel seeds are very rich in dietary fibres, which won't get hungry anytime soon, which can avoid weight gaining process. it also helps in recovery of cancer patient – 1selenium is a mineral, found in fennel seeds but not most other fruits and vegetables. It contributes to liver enzyme function and helps detoxify some cancer causing compounds in the body. It can also prevent inflammation and decrease tumour growth rates. Fiber intake from fruits and vegetables like fennel are associated with a lower risk of colorectal cancer. It also contains folate, which plays a role in DNA synthesis and repair. This might help to prevent cancer cells from forming because of mutations in the DNA). Shows Anti-bacterial activity- Studies show that fennel seeds extract inhibits the growth of potentially harmful bacteria and yeasts 6such as Escherichia coli, Staphylococcus aureus and Candida albicans. Helps in Control the cholesterol levels due to presence of fibre in fennel seeds which keeps cholesterol level low in blood pressure. Have the ability of Anti-flatulent- Fennel seed has aspartic acid which helps in reducing bloating of belly due to flatulence (excessive gas formation). 3Nutritional facts of fennel seeds: Fennel seeds are source of many nutrients that is sugars, minerals, essential fatty acids, vital Vitamins like vitamin A, vitamin C and so on, also contain protein and fibre. They are also rich in essential oil and

many flavonoids such as 1-methoxy-4-[(E)-prop-1-enyl]-benzene; 1- methoxy-4-prop-2-enylbenzene; 1, 3, 3-trimethylbicyclo [2.2.1] heptan-2-one. Nutritional facts of fennel-[1]According to the United States Department of Agriculture (USDA), National Nutrient Database, one raw fennel bulb weighing 234 grams contains- 73calories, 0.47gram of fat, 7.3 gram of dietary fibre, no cholesterol, peroselinic acid- 54.22 percent to 61.25 percent in fennel. Sterol content in it is (4.64 milligram/gram of oil) seeds oil. [1]Fennel also contains phosphorous, zinc, copper manganese, selenium, niacin, pantothenic acid, folate, choline, beta-carotene, lutein, zeaxanthin, Vitamin K, Vitamin E. As well as these, fennel provides high levels of dietary nitrates and is a natural source of estrogen.

According to Ayurveda Fennel is “GREAT FOR ACNE”.

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