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A REVIEW: ANTI-TUMOR EFFECTS OF HALOPHILIC MICROORGANISMS

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ABSTRACT:

Every year, millions of people die from cancer. A fresh strategy for treating cancer is to search for new products that can eliminate these cells. Microbes are a good candidate for the discovery of novel bioactive compounds with anticancer potential because of their brief lifespans. Microorganisms known as moderate halophiles can flourish in saline environments with a salt concentration of between 3 and 15%. These bacteria exhibit tremendous potential for the synthesis of beneficial compounds. In order to treat cancer, a number of therapeutic enzymes have been utilised, including L-asparginase, L-arginase, L-tyrosinase, L-glutaminase, α and β glucosidase, and beta galactosidase. L-asparaginase (ASNase), an enzyme, is used to treat acute lymphoblastic leukaemia. Arginine deiminase (ADI), an arginine-degrading enzyme, has been proposed as a potential anti-tumor agent for the treatment of melanomas and hepatocellular carcinomas (HCCs), both of which are auxotrophic for arginine. Glutaminase has demonstrated the capacity to significantly reduce the proliferation of several cancer cell lines. Some of the halophilic microorganisms like *Naphthomycin A*. Nov. WH26, *Streptomyces* species, *Halobacterium salinarum* IBRC M10715 and *Bacillus* sp.are also used in antitumor activity. This means that halophiles could be used to create more effective and less damaging drugs for cancer treatment.

KEYWORDS: Antitumor, L-asparginase, L-glutaminase, L- arginase, halophiles etc.

INTRODUCTION

Every year, millions of people die from cancer, which is thought to be a fatal illness. Several malignancies recur despite the latest molecular and targeted medicines following multiple treatments (Ramos *et al*., 2017). Subpopulations of cancer known as cancer stem cells are responsible for medication resistance and tumour recurrence. A fresh strategy for treating cancer is to search for new products that can eliminate these cells (De Angelis *et al*., 2018).

Microorganisms are among the many sources of natural compounds, and they are known for producing bioactive compounds quickly and easily. They are a good candidate for the discovery of novel bioactive compounds with anticancer potential because of their brief lifespans (Böhringer *et al.*, 2017). According to estimates, just 0.1% of bacterial species have been identified, and among those that have, only a small number have been screened and examined for the synthesis of bioactive molecules (Sarvari *et. al*., 2015). Over 100,000 secondary metabolites with molecular weights under 2500 have been examined, with about half of these compounds coming from microbes.

Numerous medications were utilised to extend patients' lives, however because to drug resistance, low efficacy, and unresolved side effect problems, efforts to develop other antitumor molecules free of the key problems prompted many scientists to research novel anticancer agents. Actinomycin D, anthracyclines, and doxorubicin are a few examples of microbiological antibiotics that are commonly used in chemotherapy as antitumor agents. Microorganisms known as moderate halophiles can flourish in saline environments with a salt

concentration of between 3 and 15%. These bacteria exhibit tremendous potential for the synthesis of beneficial compounds (Sarvari *et al*., 2015). In order to treat cancer, a number of therapeutic enzymes have been utilised, including L-asparginase, L-arginase, L-tyrosinase, L-glutaminase, α and β glucosidase, and beta galactosidase(Shirazain *et.al.*,2016).

In addition to being salt-stable, halophiles are a great source of enzymes that can tolerate harsh environments and yet carry out reactions well. The suggested uses of L-glutaminase, L-asparginase, and L-arginase in a variety of industries, including the food and pharmaceutical industries, have garnered a lot of attention (GomaaZakariaEman, 2022). Given that halophilic or halotolerant enzymes likely have reduced immunological reactions in patients, all of these enzymes were selected based on cancer cells' amino acid deprivation and evaluated in halophilic and halotolerant bacteria (Zolfaghar *et al.*, 2019).

L-asparaginase (ASNase), an enzyme, is used to treat acute lymphoblastic leukaemia. It is a vital component of chemotherapy and is a member of the N-terminal nucleophile family. Between Gly167 and Thr168, the amido hydrolase ASNase can become catalytically competent. Other hematopoietic cancers need to be autocleaved. It catalyses the synthesis of aspartate (Asp) and ammonia as by-products of the asparagine (Asn) deamidation reaction (Medeiros *et al*., 2018).

Since Asn is auxotrophic for leukemic cells, lowering blood levels of this amino acid as a result of ASNase activity is a successful treatment for all patients because, in these circumstances, the cell cycle arrests in the G1 phase, resulting in apoptosis. However, a number of techniques are employed to eliminate asparaginase from blood plasma, including immunogenic responses and pharmacokinetic restrictions, which are for short half-lives.

Recent advances in biotechnology are being used to lessen these issues. It has been researched to produce asparaginase from several microbiological sources or recombinant forms. It has been extensively researched to use pegylation or immobilisation techniques with nano encapsulation (Linge Wang *et al.*, 2012).

Arginase, arginine deiminase, and arginine decarboxylase, which are found in archaea, bacteria, and eukarya, are the three primary types of arginine degrading enzymes, according to Ebrahimi *et al.*,(2016). Through screening, isolation, and characterization of the microorganisms, new sources for arginine-degrading enzymes may be found. Arginine deiminase (ADI), an arginine-degrading enzyme, has been proposed as a potential anti-tumor agent for the treatment of melanomas and hepatocellular carcinomas (HCCs), both of which are auxotrophic for arginine.

In biology, glutaminases (L-glutamine amidohydrolase, 3.5.1.2) are crucial for both maintaining the glutamine/glutamate equilibrium in living things and for the creation of a variety of nitrogenous compounds. Since glutaminase has demonstrated the capacity to significantly reduce the proliferation of several cancer cell lines, it may one day be used in place of chemotherapy (El-Gendy *et al.*, 2017). Similar to this, it has been demonstrated that the enzyme's recombinant form significantly inhibits HIV replication in vivo (Amobonye *et al.*, 2019).

Recent research has also shown that glutaminases have the potential to scavenge free radicals, which is consistent with earlier results on their antitumor actions. Most antitumor medicines have been shown to have substantial antioxidant capabilities (Mousumi D. and Dayanand A., 2013). In the past ten years, there has been increased interest in the biological synthesis of theanine, a nutraceutical, using glutaminase. Additionally, glutaminases have been used to create biosensors for glutamine and glutamic acid (Rastogi H. and Bhatia S., 2019).

Although the anticancer agent taxol was initially identified in plant metabolites, it has also recently been documented in microbial metabolites (Wall M.E. 1998).

Chen and his research team in China identified 45 moderately halophilic eubacteria strains from the water of the Weihai solar saltern. They were also subjected to screening by Chen's research team to evaluate the productivity of the bioactive agent. Of the 45 strains tested, 23 were antibacterial against B. whereas only one strain hampered the development of E. coli. More significantly, five strains were determined to have an IC50 value below 40 micrograms per litre and crude extracts of 14 strains demonstrated cytoxicity against hepatocellular cancer BEL-7402 cells (Chen *et al.*, 2011).

According to Liu *et al*.,(2013), *Streptomyces* species produced 8-O-Methyltetrangulol and *Naphthomycin A*. Nov. WH26 is a halophilic bacteria that can kill A549, HeLa, BEL-7402, and HT-29 cells.

In a 2013 study by Lie *et al*., 45 moderately halophilic bacteria were examined for their antibacterial and anticancer activity, and 14 of the strains showed anticancer activity. Furthermore, HepG2 cancer cells have been shown to be sensitive to carotenoids from halophile archaeal strains (Abbes *et al*., 2013 and Hou J and Cui HL, 2018).

In this work, Sarvari *et al.* (2015) assessed the effects of nine moderately halophile supernatants on the proliferation of the HUVEC cancer cell line and mesenchymal stem cells. High biodiversity of moderately halophiles has been documented in Iran. Propidium iodide (PI) and DAPI (4',6-diamidino-2-phenylindole) are employed for staining in the MTT experiment in order to determine proliferation rates. Also performed was a preliminary chemical evaluation using TLC chromatography.

In tests on prostate cancer cell lines, Safarpour *et al*., (2018) found that *Halobacterium salinarum* IBRC M10715 supernatant metabolites had the most potent cytotoxic effect on prostate cancer cell lines (IC50 = 0.5 mg/mL) without having any effects on healthy cells. The expression of the SOX2 gene was downregulated, which greatly increased both early and late apoptosis in the androgen-dependent PC3 cell line and decreased the capacity of DU145 and PC3 cells to form spheres.

Additionally, Safarpour *et al.*, (2018) examined the supernatant metabolites (SM) of at least eight halophilic archaea strains that they had recovered from an Iranian hypersaline lake. According to their preliminary screening, the SM of *Halobacterium salinarum* decreased the viability of prostate cancer cell lines at lower doses but had no effect on healthy cells (HFF-5).

This implied that their effects might vary depending on the type of cancer cell, which was also previously documented by Sagar (Sagar *et al.*, 2013 and Sagar *et al*., 2013), who noticed that halophilic bacteria extracts had a stronger effect on HeLa, MCF-7, and DU145 cells (Sagar *et al.*, 2013 and Sagar *et al.*, 2013). The rate of apoptosis was evaluated to determine the mechanism of *Halobacterium salinarum's* SM on prostate cancer cells.

MTT assay, Sphere formation assay, Apoptosis test, Quantitative real-time RT-PCR (qRT-PCR), Immuno histochemistry, and statistical analysis (One Way ANOVA test) were the techniques used to estimate the antitumor activity (Safarpour *et al.*, 2018). LC/MS analysis for Metabolic Profiling of Crude Extracts from Chloroform Extractions (Daz-Cárdenas *et al.* 2020).It does have some restrictions, though. According to Shikha *et al.* (2014), the primary disadvantage of liposomes is their chemical instability. UV/Vis spectrophotometer for Activity Determination of the Arginine Degrading Enzymes (Ebrahimi *et al.*, 2016). L-asparginase and L-glutaminase production can be optimised using the one-factor-at-a-time method and response surface methodology (Shirazain *et al.*, 2016). Most of the techniques used to produce glutaminases have also been proven to be compatible with following downstream processes and end applications (Singh P and Banik R., 2013).

According to research by Dáz-Cárdenas *et al*., in 2020, the Rhodobacteraceae family has been isolated mostly from maritime settings, in conjunction with marine invertebrates like corals, colonising the surfaces of oysters and shells, and in the rhizosphere of halophytes plants from coastal areas (Camacho *et al*., 2016 and Rodrigues *et al.*, 2018). A member of this family, *Labrenzia* spp., has biosynthetic genes for the manufacture of terpenoids, bacteriocins, polyketide synthases (PKS), and non-ribosomal peptides (NRPS), however few secondary metabolites have been discovered.The mentioned chemicals were discovered in a free-living marine *Labrenzia* sp. polyketidepederin analogue. Cancer cell lines A549 (ATCC CCL-185) (lung carcinoma, NSCLC), HT-29 (ATCC HTB-38) (colon adenocarcinoma), MDA-MB-231 (ATCC HTB-26) (breast adenocarcinoma), and PSN-1 (ATCC CRL-3211) (pancreatic adenocarcinoma) are all susceptible to PHM005 (Schleissner *et al.*, 2017).

L-glutaminase from *Bacillus* sp.DV2-37 was optimised, purified, and characterised by Gomaa in 2022. L-glutaminase generated by *Bacillus* sp. has an anticancer impact on human breast, hepatocellular, and colon carcinoma cell lines. All of the studied cell lines were very sensitive to the cytotoxic effects of DV2-37, which were dose-dependent.

Due to their comparatively high salt tolerance, marine bacteria like *Pseudomonas fluorescens, Vibrio cholerae*, and *V. Costi-cola, Micrococcus luteus K-3*, are highly prized sources of glutaminase, as demonstrated by Amobonye*et al.* 2019 study on glutaminase enzyme in marine bacteria. A bacterial glutaminase's maximum specific activity was 325 U/mg protein in *S.maltophilia*NYW-8.

Enhancing the product requires the isolation and characterisation of halophilic bacteria for anticancer investigations. After the fermentation procedure was optimised, Mostafa *et al.*, 2021 who researched the enzymatic productivity of anticancer enzymes, calculated that the enzyme productivity of *Halomonas meridiana's* L-glutaminase increased 2.68-fold.

Comparing *Halomonas meridian* to other bacteria, the quick fermentation duration for enzyme synthesis showed potential productivity in a cheap technique. As the more active L-glutaminase-producing marine bacterium, *Halomonas meridian*, was chosen, it was isolated from the Red Sea. An L-glutaminase producer called *Halomona smeridiana* is employed as a colon cancer preventative.

Alcaligenes faecalis L-glutaminase was found to have anti-tumor activity against HeLa cell lines (Pandia *et al.*, 2014) and Bacillus cereus MTCC 1305 was found to have anti-tumor activity against hepatocellular carcinoma (Hep-G2) cell lines (Singh P and Banik R, 2013), according to Mostafa *et al.*, 2021. The antiviral effect of *Pseudomonas* 7A L-glutaminase against retroviral illnesses is caused by mRNA translation disruption and viral replication inhibition.

According to Zolfaghar *et al.*, 2019, strain GBPx3 (*Vibrio* sp.) at 1.0 IU/ml, strain R2s25 (*Rhodococcus* sp.) for L-glutaminase at 0.6 IU/ml, and stain GAAy3 (*Planococcus* sp.) for L-arginase at 3.1 IU/ml were tested to determine the maximum enzymatic activity.

Halomonas elongate L-asparginase coding gene was identified and expressed in E. coli, according to a study by Ghasemi *et al*. from 2017. E.coli, which Safarpour *et al.*, in 2018, purified and characterised. The enzyme's 1510 U/mg specific activity for L-asparagine was found.

CONCLUSION:

Halophiles have recently been studied for their potential to be used in cancer treatment. Halophiles are a type of microorganism that can tolerate living in high salt concentrations, making them a potential source of new cancer treatments.Various species of halophiles, including bacteria, archaea, and fungi, produce a variety of compounds that could potentially be used to treat and prevent cancer. Halophiles produce compounds that can prevent cancer cell growth and even kill cancer cells. The compounds can also reduce the side effects of chemotherapy, such as nausea, vomiting, and fatigue. Halophiles can also be used to create targeted drugs that can attack cancer cells without damaging healthy cells around them. This means that halophiles could be used to create more effective and less damaging drugs for cancer treatment. There is still more research being done on halophiles and their potential to be used in cancer treatments. However, the current findings are promising, and it appears that halophiles could be a new, effective way to treat cancer.

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