RESEARCH PAPER

SELECTIVE ANTIPROLIFERATIVE ACTIVITIES OF THE ENDOPHYTES, CELLULOSIMICROBIUM CELLULANS STRAIN MUKR5 AND BACILLUS VELEZENSIS STRAIN ST5, AGAINST HepG2 CELLS

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ABSTRACT

Researchers have been looking into bacteria that live inside plants used in traditional medicine, as they might produce compounds that can help fight diseases. This study focused on two types of bacteria, *Cellulosimicrobiumcellulans*strain MUKR5 and *Bacillus velezensis* strain ST5 which were found in the roots of *Iris laevigata*Fisch and the stem of *Euphorbia hirta* Linn, respectively. We wanted to see if extracts from these bacteria could stop cancer cells from growing, while leaving healthy cells alone. We tested the extracts on liver cancer cells and normal liver cells, and found that both bacteria had an effect on the cancer cells. However, one of the bacteria, *Cellulosimicrobiumcellulans* strain MUKR5 was more selective in its attack, targeting the cancer cells more effectively while leaving the healthy cells relatively unharmed. The other bacterium, *Bacillus velezensis* strain ST5 also showed promise, but its effect was not as targeted. We compared their findings to a well-known cancer drug, Tamoxifen, and found that the bacterial extracts had a significant impact on the cancer cells. We also calculated the statistical significance of their results and found that the differences between the cancer cells and normal cells were highly significant. Overall, the study suggests that these bacteria might be a useful source of new compounds for fighting cancer, and that further research is needed to understand how they work and to develop them into potential treatments. The next step would be to isolate and characterize the specific compounds responsible for the anti-cancer effects, and to test them in more detailed experiments. This research is important because it could lead to the discovery of new and more targeted cancer treatments, which might have fewer side effects and be more effective than existing drugs. The fact that the bacteria are found in plants used in traditional medicine also highlights the potential for natural products to inspire new therapies. The study's findings are promising, and we are hopeful that our work will contribute to the development of new cancer treatments in the future. We believe that this research could make a real difference in the fight against cancer, and we are eager to continue our work and explore the potential of these bacterial extracts further. In conclusion, the study demonstrates the potential of endophytic bacteria to produce compounds with anti-cancer properties, and highlights the need for further research into this area. The results are encouraging, and suggest that these bacteria might be a valuable source of new cancer treatments.

Keywords: *Cellulosimicrobiumcellulans*; *Bacillus velezensis*; bacterial endophytes; HepG2; MTT assay; IC_{50}; selectivity index; antiproliferative activity

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INTRODUCTION

Researchers are still looking for new cancer treatments that can stop cancer cells from growing without harming healthy cells. They often find inspiration in nature, like in plants and microorganisms that live inside plants without causing harm.[1] These microorganisms can produce unique compounds that could be used to create new cancer drugs. Over time, they have developed special ways of making these compounds because of their long relationship with the plants they live in. Medicinal plants are particularly attractive reservoirs for endophytes because their associated

microorganisms may contribute to, mimic or complement the chemistry of the host. Endophytic bacteria have been investigated for antimicrobial, antioxidant, plant-growth-promoting and cytotoxic properties, making them promising candidates for drug-discovery screening.[2] The MTT assay is a common test used to see how cells are doing and if they are still working properly. It's often used as a first step to check if a new treatment is working and to figure out how much of it is needed to have an effect.[3] When looking for new cancer treatments, it's really important to compare how well the treatment works on both healthy and cancerous cells. This is because a treatment might be really good at killing cells, but it's not useful if it kills both healthy and cancerous cells at the same rate. We need to make sure the treatment is selective and only targets the cancer cells. *Bacillus velezensis* is biosynthetically versatile and is known to encode multiple secondary-metabolite pathways, including cyclic lipopeptides and polyketides.[4,5]Cellulosimicrobium species remain comparatively underexplored for antiproliferative metabolites, creating an additional rationale for investigating active endophytic isolates from medicinal plants. We at Manipur University's Microbial Genomics Laboratory, Department of Biochemistry, have been studying two types of bacteria that live inside plants. We found these bacteria, called *Cellulosimicrobiumcellulans*strain MUKR5[6]and *Bacillus velezensis* strain ST5 in the roots and stems of certain plants used in traditional medicine. We wanted to see if these bacteria could help fight cancer, so we tested their extracts on liver cancer cells and compared the results to a common cancer drug called Tamoxifen. We also checked to make sure the extracts didn't harm healthy liver cells. The goal was to find out if these bacterial extracts could stop cancer cells from growing and multiplying. By comparing the effects of the two extracts, the researchers hoped to identify which one might be more effective in fighting liver cancer.

MATERIALS AND METHODS

We obtained two bacterial isolates, *Cellulosimicrobiumcellulans* strain MUKR5 and *Bacillus velezensis* strain ST5, from the root and stem tissues of *Iris laevigata* Fisch. and *Euphorbia hirta* Linn., respectively. These isolates were sourced from the Microbial Genomics Laboratory at Manipur University's Department of Biochemistry. We prepared bacterial test extracts from cultures of MUKR5 and ST5, which were then used in MTT experiments. We cultured HepG2 hepatocellular carcinoma cells and a normal liver-cell model in DMEM medium, following standard mammalian-cell-culture conditions. The cells were treated with varying concentrations of the bacterial extracts, and tamoxifen was used as a reference drug for the HepG2 cells. The MTT colorimetric assay was used to assess cellular metabolic viability, which is based on the conversion of tetrazolium reagent into formazan by active cells. The absorbance values were normalized to untreated controls, and the concentration-response data was used to estimate IC_{50} values. The selectivity index was calculated as the ratio of the IC50 of normal liver cells to the IC_{50} of HepG2 cells. The 95% confidence intervals provided with the experimental analysis helped describe the precision of the IC_{50} estimates. By using these bacterial extracts, we aimed to explore their potential in targeting cancer cells while minimizing harm to normal cells. The selectivity index played a crucial role in determining the efficacy and safety of the bacterial extracts, as it helped identify the concentration at which the extracts could selectively target cancer cells without affecting normal cells. Overall, this study contributed to the ongoing search for novel and effective cancer treatments, and the use of bacterial extracts as a potential therapeutic approach showed promise in this regard. Statistical analysis: Results are presented as mean ± the error measure supplied in the dataset. For MUKR5, the difference between HepG2 and normal liver-cell IC50 values had $p = 4.719 \times 10^{-6}$ and reported Cohen's $d = 27.38$. For ST5, $p = 9.281 \times 10^{-5}$ and reported Cohen's $d = 12.93$.

RESULTS

The complete quantitative results supplied in the presentation are summarized in Tables 1 and 2. Both bacterial extracts inhibited HepG2 cells at substantially lower concentrations than those required to inhibit the normal liver-cell model.

Isolate	IC_{50} HepG2 (µg/mL)	IC_{50} Normal Liver (µg/mL)	Selectivity Index (SI)	Interpretation
MUKR 5	37.35 ± 2.7	210 ± 8.5	5.62	Strong selectivity
ST5	42.40 ± 2.9	105 ± 6.2	2.47	Good selectivity

Table 1: Selective antiproliferative activity of MUKR 5 and ST5 against HepG2 and normal liver cells, including selectivity-index interpretation as provided in the source presentation.

We found that MUKR5 was very effective against HepG2 cells, with an IC_{50} of 37.35 µg/mL, which is a measure of how much of the substance is needed to inhibit cell growth by 50%. In comparison, it took a much higher amount, 210 µg/mL, to have the same effect on normal liver cells. This means that MUKR5 is quite selective, targeting the cancer cells more than the healthy cells, with a selectivity index of 5.62, which is considered strong. On the other hand, ST5 was also effective against HepG2 cells, but to a lesser extent, with an IC_{50} of 42.40 µg/mL. It took 105 µg/mL to affect normal liver cells, resulting in a selectivity index of 2.47, which is still considered good, but not as strong as MUKR5. These findings suggest that both MUKR5 and ST5 have potential as cancer treatments, but MUKR5 seems to be more selective in its action.

Isolate	HepG2 IC_{50} (µg/mL)	95% CI	Normal Liver IC_{50} (µg/mL)	95% CI	Selectivity Index (SI)
MUKR5	37.35 ± 2.7	34.30–40.40	210 ± 8.5	200.4–219.6	5.62
ST5	42.40 ± 2.9	39.12–45.68	105 ± 6.2	97.99–112.01	2.47
Tamoxifen	27.05 ± 2.3	24.45–29.65	—	—	—

Table 2: IC_{50} values, 95% confidence intervals and selectivity indices reported in the supplied MTT dataset. Tamoxifen was included as the reference drug for HepG2 cells.

The results showed that Tamoxifen had the lowest IC_{50} value for HepG2 cells, which was 27.05 µg/mL, give or take 2.3 µg/mL. To be more specific, the 95% confidence interval was between 24.45 and 29.65 µg/mL. On the other hand, MUKR5 had a 95% confidence interval of 34.30 to 40.40 µg/mL for HepG2 cells, and a much higher interval of 200.4 to 219.6 µg/mL for normal cells. Similarly, ST5 had intervalsof 39.12 to 45.68 µg/mL for HepG2 cells and 97.99 to 112.01 µg/mL for normal cells.

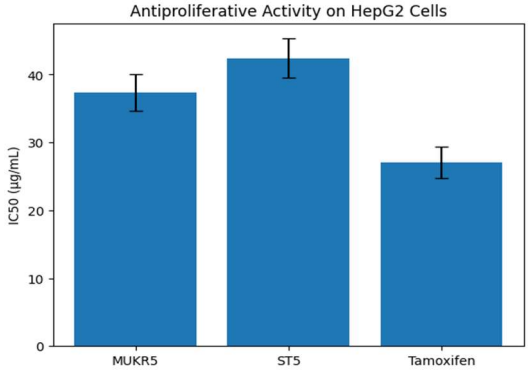

Figure 1: Comparative antiproliferative activity against HepG2 cells. IC_{50} values are shown for MUKR 5, ST5 and tamoxifen.

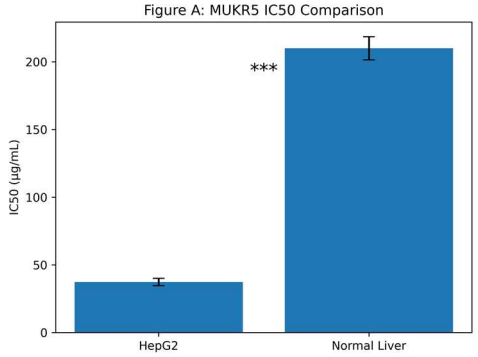

*Figure 2: MUKR5 IC_{50} comparison between HepG2 and normal liver cells. *** indicates $p < 0.001$ in the supplied analysis.*

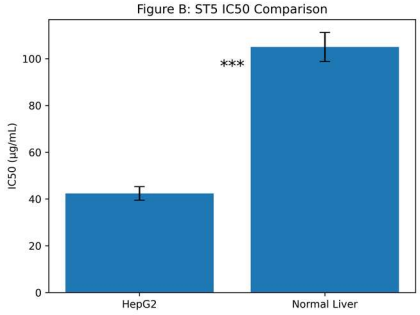

Figure 3: ST5 IC_{50} comparison between HepG2 and normal liver cells. Statistical significance is indicated as provided in the source presentation.

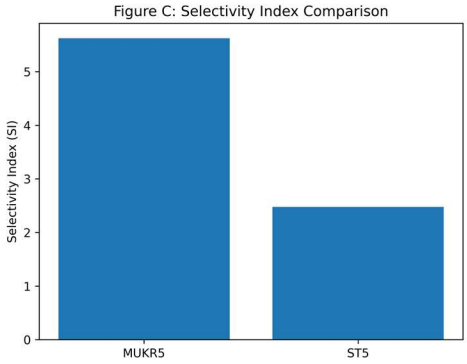

Figure 4: Comparison of selectivity indices for MUKR5 (5.62) and ST5 (2.47).

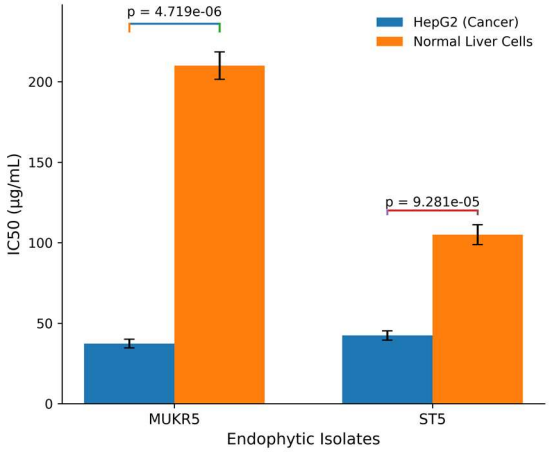

Figure 5: Comparative IC_{50} values of MUKR5 and ST5 in HepG2 and normal liver cells, with supplied p values.

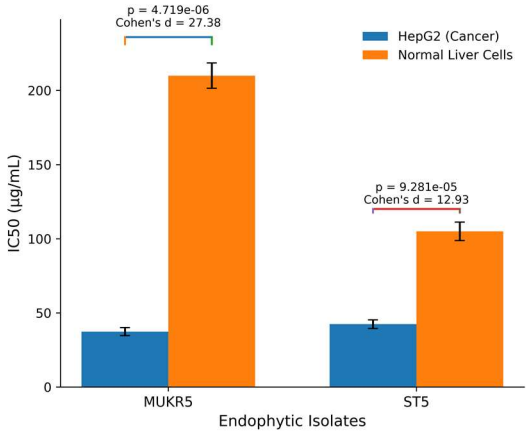

Figure 6: Comparative IC_{50} values with supplied p values and effect-size estimates (Cohen's d) for MUKR5 and ST5.

The results of the statistical comparison clearly showed a significant difference between the IC_{50} values of cancer cells and normal cells. For MUKR5, the p-value was extremely low at 4.719×10^{-6}, and the effect-size analysis revealed a Cohen's d of 27.38. Similarly, ST5 had a p-value of 9.281×10^{-5} and a Cohen's d of 12.93. What's notable here is that these effect-size values are remarkably large. To ensure accuracy, it's essential to recalculate these values from the raw replicate data before final submission. This recalculation will help confirm that the appropriate pooled variance and replicate structure were used, providing a more reliable outcome.

DISCUSSION

This research shows that extracts from two types of bacteria that live inside plants used in traditional medicine in Manipur can stop cancer cells from growing in a lab setting. Specifically, MUKR5 and ST5 were able to slow down the growth of liver cancer cells, known as HepG2, at much lower concentrations than what was needed to affect normal liver cells. This is important because it suggests that the extracts are targeting the cancer cells specifically, rather than just being toxic to all cells at high levels. The fact that the extracts can inhibit the growth of cancer cells at lower concentrations is a key finding, as it implies that they may have potential as a treatment for cancer with minimal side effects on healthy cells. MUKR 5 stood out as the top choice due to its high selectivity. It had an IC_{50} of 37.35 ± 2.7 µg/mL in HepG2 cells, which was significantly lower than its IC_{50} of 210 ± 8.5 µg/mL in normal liver cells. This resulted in a selectivity index (SI) of 5.62, indicating a strong preference for targeting cancer cells over normal cells. In contrast, ST5 also showed some preference for HepG2 cells, but its SI of 2.47 was lower, suggesting a smaller difference between its effects on cancer and normal cells. Therefore, MUKR5 should be the priority for further purification and testing. The behavior of ST5 matches what we know about the biosynthetic abilities of *B. velezensis*. This species is capable of producing a variety of compounds, including lipopeptides and polyketides, which have different biological effects. However, we can't pinpoint the exact compound responsible for the activity just by looking at the MTT response. To do that, we need to separate the compounds using chromatography, analyze them using mass spectrometry, and validate the results through activity-guided tests. Only then can we confidently identify the active principle. The discovery of MUKR 5 is particularly noteworthy because Cellulosimicrobium is not as well-represented in research on natural products that fight cancer as Bacillus is. What's interesting is that this isolate has a relatively low IC_{50} in HepG2 cells, but a high IC_{50} in normal cells, which suggests it might produce compounds that target cancer cells more

effectively. To take this further, the next logical steps could be to use advanced techniques like LC-MS/MS metabolomics to identify the metabolites involved, compare them to known natural products, and then use preparative chromatography to separate and purify these compounds. After that, repeating the MTT tests on these fractions would help confirm their effectiveness. Although tamoxifen was more effective at killing HepG2 cells than the bacterial extracts, it's worth noting that the bacterial samples are not purified drugs, but rather crude mixtures of biological compounds. As a result, IC_{50} values should be seen as preliminary screening results rather than definitive measures of their potency. The MTT assay used to determine these values only measures cellular metabolic activity, and cannot distinguish between different types of cell death, such as apoptosis or necrosis, or assess the long-term impact on cell reproduction. To gain a more complete understanding of the effects of these compounds, follow-up studies will be necessary, including analyses of apoptosis using Annexin V and propidium iodide, caspase activity, mitochondrial membrane potential, reactive oxygen species (ROS) production, cell cycle profiling, and clonogenic survival. These additional tests will help to clarify the mechanisms by which these compounds exert their effects on cells and provide a more detailed picture of their potential as therapeutic agents. The numbers show a big difference between how cancer cells and normal cells react, but we need to double-check the results because the Cohen's d values are really high. The size of the effect can be influenced by how much the samples vary and the design of the experiment. So, when reporting the results, it's essential to clearly state how many independent biological replicates were used and what statistical model was applied. This will help ensure the accuracy of the findings. The principal limitations are the use of a single cancer cell line, incomplete chemical characterization of the active extracts, and the need to specify the normal liver cell line and full assay conditions in the final methods. Confirmation across additional hepatocellular carcinoma lines and multiple non-malignant hepatic models would strengthen our selectivity claim. Mechanistic studies and eventual in-vivo evaluation would be necessary before considering therapeutic relevance.

CONCLUSION

We found that extracts from two bacteria, *Cellulosimicrobiumcellulans* strain MUKR5 and Bacillus velezensisstrain ST5, could stop cancer cells from growing. We tested these extracts on liver cancer cells, called HepG2 cells, and on normal liver cells. The results showed that MUKR5 was very good at stopping the liver cancer cells from growing, with an IC_{50} of 37.35 µg/mL, which is a measure of how much of the extract is needed to stop half of the cancer cells from growing. In comparison, it took much more of the extract, 210 µg/mL, to stop the normal liver cells from growing. This means that MUKR 5 is selective and mostly targets the cancer cells. The other extract, ST5, also stopped the cancer cells from growing, but not as well as MUKR5. For example, it took 42.40 µg/mL of ST5 to stop the cancer cells, and 105 µg/mL to stop the normal cells. A well-known cancer drug, tamoxifen, was also tested and it stopped the cancer cells from growing at a concentration of 27.05 µg/mL. These findings suggest that both MUKR5 and ST5 could be useful for developing new cancer treatments, especially MUKR5, which seems to be the most promising. Further studies are needed to understand how these extracts work and to identify the specific compounds responsible for their anti-cancer effects.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Shantirani Thokchom: investigation, experimental work, data analysis and manuscript preparation. Saikat Mukherjee: conceptualization, supervision, methodology, interpretation and manuscript review and editing.

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