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Synergistic Antimicrobial Activities of Bacteriocin from *Lactococcus lactis* and Pyocyanin from *Pseudomonas aeruginosa* Against Selected Microorganisms

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Abstract
Bacteriocin fluids (4274 AU/ml) were produced by *Lactococcus lactis* RCM21 using a modified de Man, Rogosa and Sharpe (MRS) media. Pyocyanin (70µg/ml) was synthesised by *Pseudomonas aeruginosa* OB11 in culture extracts using glycerol-supplemented cetrimide media, extracted and confirmed by FT-IR spectroscopy. Agar diffusion method was adopted and pyocyanin/bacteriocin time-based single and combined applications had varying antimicrobial effects against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10261. Zone of clearing measurements (mm) on agar plates showed that combined use led to the highest inhibition (28.3±0.38 mm) against *S. aureus* when pyocyanin/bacteriocin combination of 50µl/50µl at 72h contact time, while the lowest inhibition (13.8±0.33 mm) was obtained against *E. coli* when a combination of 30µl/70µl at 24h contact time was used. In single use, no inhibition was observed when 100µl of bacteriocin fluid was applied against *C. albicans*. However, values ranging from between 2.5±0.61mm and 14.5±0.81mm were observed when pyocyanin and bacteriocin were used singly on the different test organisms at varying contact times, thus showing a less potent activity of the single when compared with the combined. Antimicrobial values for the singly applied bacteriocin or pyocyanin were lower than values obtained for the combinations used in all experiments, implying synergism. Physiologically, flame photometry also showed that the co-application of the two antimicrobials led to a higher effect of sodium and potassium leakage from cells of the test organisms than single application. Pyocyanin and bacteriocins possess good potentials for further efficacious development especially as combined therapy.

1. Introduction

The concept of antimicrobial science is rapidly evolving and newer antimicrobial compounds that are synthesized from various sources are being tested against a great

diversity of microbial life to establish an antimicrobial basis for their application. Pyocyanin and bacteriocins are two known antimicrobial compounds with potentials for exploitation in antimicrobial medicine [1] [2] [3].

Pyocyanin (N-methyl-1-hydroxyphenazine) is a water – soluble pigment usually blue to green in colour secreted in copious amounts by growing cultures of *Pseudomonas aeruginosa* strains [4] [9]. Biosynthetic studies have shown that pyocyanin is a part of a group of compounds known as phenazines which are natural heterocyclic compounds with varying chemistry and pigmentations and are mostly secreted by *Pseudomonas* species with over 100 different phenazines and structurally related compounds being identified so far [5]. Various antimicrobial studies of pyocyanin and pyocyanin-secreting *P. aeruginosa* have been carried out [6] [7] [8].

The antagonism of pyocyanin against microbial species can be traced to its redox activity and its ability to reduce oxygen in univalent terms to generate superoxide radicals and reactive oxygen species (ROS) [9]. This form of antimicrobial effect can be broad spectrum based given the effect of ROS on cellular life generally. The potency of the pyocyanin compound can be exploited in terms of modes of delivery and subsequently applications. Bacteriocins are a group of high molecular mass antimicrobial substances produced by a variety of food-grade bacteria especially within the group of lactic acid bacteria [10]. They can be described as proteinaceous antimicrobial compounds with activity against closely related strains, but with some exceptional broad spectrum activity against a range of microbial life with the producer cell being immune to the activity [11] [12]. *Lactococcus lactis* is a member of the lactic acid bacteria group and it is a known producer of bacteriocins – lactococcin and nisin, which are a set of one of the most potent bacteriocins known to man [13]. Bacteriocins act by cell envelope pore formation, by crossing cell wall barrier and making contact with the cytosol, leading to subsequent loss of ATP within the target cells [14] and reduction in proton motive force [15].

The individual application of pyocyanin and bacteriocins against microbial species have been chronicled, however, there still remains the option of combinatorial application. This is hypothetically valued, given the different modes of action of the two compounds. There are possible complementarities in synergistic terms that can occur within their combined use. This study therefore tends to determine the combined effect of bacteriocins and pyocyanin extracts produced by their secreting microorganisms on test isolates.

2. Materials and Methods

2.1. Microorganisms and Media

A high pyocyanin producing strain *Pseudomonas* sp. was isolated from a fresh water sample obtained from a site in Lagos, Nigeria. Cetrimide agar supplemented with 5% glycerol was used for the isolation and pyocyanin pigment production was visually detected by observing a blue-green colouration on the culture plate. Isolate identity was confirmed using 16s rRNA analyses as *P. aeruginosa* and it was coded OB11. *Lactococcus lactis* RCM 21 an isolate from previous work [16] was subcultured unto de Man, Rogosa and Sharpe (MRS) media and subjected to further analyses. Non-bacteriocinogenic *Lactococcus* sp RD12 (negative control for bacteriocin production) and bacteriocin sensitive *Lactobacillus casei* 049 (for bacteriocin titre determination) both obtained from the Federal Institute of Industrial Research, Oshodi (FIIRO) Nigeria, were used. Standard test isolates *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10261 were used in the antimicrobial tests.

2.2. Pyocyanin Production, Quantification and Extraction

P. aeruginosa OB11 was cultured on glycerol-supplemented cetrimide broth for 72h at 37°C, after which the broth was centrifuged at 10,000 x 25mins. The (bluish green) supernantant was decanted and the cell pellets were removed. Pyocyanin production and quantification was spectrophotometrically determined after it was extracted from the supernatant according to the procedures of [17] and [18], with slight modifications. Chloroform (3ml) was added to 5ml of culture supernatant and re-extracted into 1 ml of 0.2 N HCl, giving off a reddish solution in the process. Pyocyanin concentration within the supernatant was determined by checking the absorbance at 520nm and the concentration of pyocyanin produced was determined by multiplying the value of the absorbance at 520nm by 17.072. Pure pyocyanin was also used to standardise the experiments and the value were determined in µg/ml.

2.3. FT-IR Confirmation of Pyocyanin Extract

FTIR analysis of the pyocyanin extract was carried out according to the method of [7]. About 3-5 drops of the sample were dripped unto a Thallium bromide aperture plate and the sample was then sandwiched with another aperture plate carefully to avoid bubble formation. With careful insertion of the aperture plates into an IR spectroscopy machine (Shimadzu, Japan), the IR spectra readings with Fourier transformation were observed. IR peak values were compared with published values of standard pyocyanin [7].

2.4. Bacteriocin Production and Quantification

Lactococcus lactis RCM 21 was grown on a modified MRS broth (without sodium acetate) containing peptone (0.5%w/v) and glucose (0.25%w/v) at 30°C for 72 hours. Anaerobic conditions were employed during incubation to minimize production of hydrogen peroxide and acetic acid. Cells were removed from the stationary phase of the growth broth medium by centrifugation at 10,000 r.p.m for 10

minutes at 4°C. The supernatant fluid was adjusted to pH 6.5, treated with 5mg/ml catalase, and then filter-sterilized through a 0.45µm pore size filter. The product was designated as crude bacteriocin. To quantify the amount of bacteriocin produced, an adaptation of the well diffusion assay was used [19]. The crude bacteriocin sample was serially diluted 2 fold and measured aliquots for each dilution was used. Wells of 5mm diameter were bored into solid medium with seeded indicator organism (*Lactobacillus casei* 049). Aliquots of the bacteriocin was introduced into the wells and incubated aerobically at 37°C. Plates were examined for definite zones of inhibition around the inoculated wells, and the bacteriocin titre was quantitatively defined as the reciprocal of the highest dilution showing definite inhibition of the indicator lawn. This was expressed in activity units per ml ($AUml^{-1}$). Filtered supernatant of the culture of bacteriocin-non-producer cells (*Lactococcus* sp RD12) was used as a negative control. Samples of the extracted bacteriocin fluid were subjected to the action of Proteinase K (0.5 mg/ml) and re-tested to ascertain the protein nature of the fluids [20] [16].

2.5. Standardization of Test Organisms

To standardize the test isolates, the method of [21] was adopted as one loopful of the organism was inoculated into nutrient broth and incubated for 24 hours. After incubation, 0.1ml of each of the cultures was obtained and suspended into 10ml sterile nutrient broth (for bacteria) and Sabouraud's dextrose broth (for yeast) and incubated for 6 hours. This was done to standardize the culture to 0.5 McFarland standards. This value yielded approximately 10^6 cfu/ml, and was used for further studies.

2.6. Application of Bacteriocin and Pyocyanin Extracts on Test Organisms

The agar diffusion method was also adopted as for the antimicrobial experiments against the test organisms (*Staphylococcus aureus* ATCC 29213*, Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10261). The standardized bacteria isolates were inoculated unto freshly prepared Muller Hinton agar media, while the yeast was inoculated unto fresh Sabouraud's dextrose agar media using pour plate method. A sterile cork borer was used to make wells on the plates. Measured volumes in microliters of the quantitatively determined bacteriocin and pyocyanin extracts (in single and combined doses) were introduced aseptically into the wells and then the plates were incubated at 37°C for the different contact times (24,48 and 72h).

2.7. Assessment of Microbial Inhibition and Determination of Sodium and Potassium Ion Leakage

To determine antimicrobial activity, the zones of inhibition (in millimeters) of the different combinations applied against the test organisms were then measured after incubations at the different contact times. To gain an insight into the physiological effects of antimicrobial action and the mechanism of inhibition, determination of sodium and potassium leakage from the cells was assessed. Known volumes (1ml) of the standardized cultures were introduced into 10ml of earlier determined concentrations of single (10ml) and combined ratios (2ml/8ml; 3ml/7ml; 5ml/5ml; 8ml/2ml and 7ml/3ml) of pyocyanin/bacteriocin and incubated for 24h. The setups were subsequently centrifuged at 5,000 r.p.m for 7mins, and with the aid of KCl and NaCl standards, flame photometric determination of the presence of leached potassium and sodium ions (at 766nm and 589nm respectively) within the supernatants was carried out [22].

3. Results

The isolate *P. aeruginosa* OB11 was identified molecularly using 16s rRNA analyses and the phylogenetic relationship among closely related strains to the strain are shown in a phylogentic tree (Figure 1). Prior to the inhibitory tests, the pyocyanin produced by the strain was quantified and it was observed that after 72h of incubation, the strain *P. aeruginosa* OB11 synthesised up to 70µg/ml in the culture extracts using glycerol-supplemented cetrimide media. Extracts of pyocyanin when subjected to FT-IR, showed direct spectra similarity with standard pyocyanin with compound peaks of H-O, CH-aromatic, C=N, C=C aromatic, C=O among other peaks (Figure 2, Table 1). Bacteriocin production by an earlier characterized *Lactococcus lactis* RCM21 yielded a bacteriocin with activity value of 4274 AU/ml.

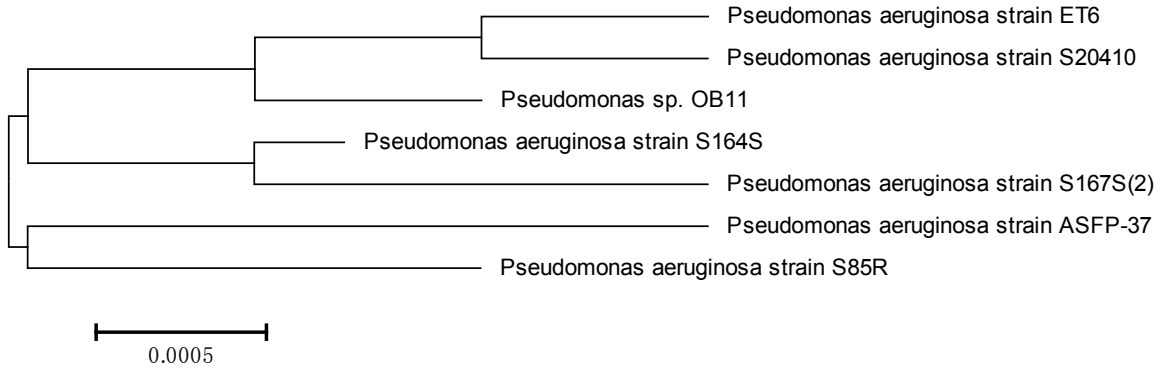

Figure 1. *Phylogenetic tree showing relationship between isolate P. aeruginosa OB11 and other closely related strains.*

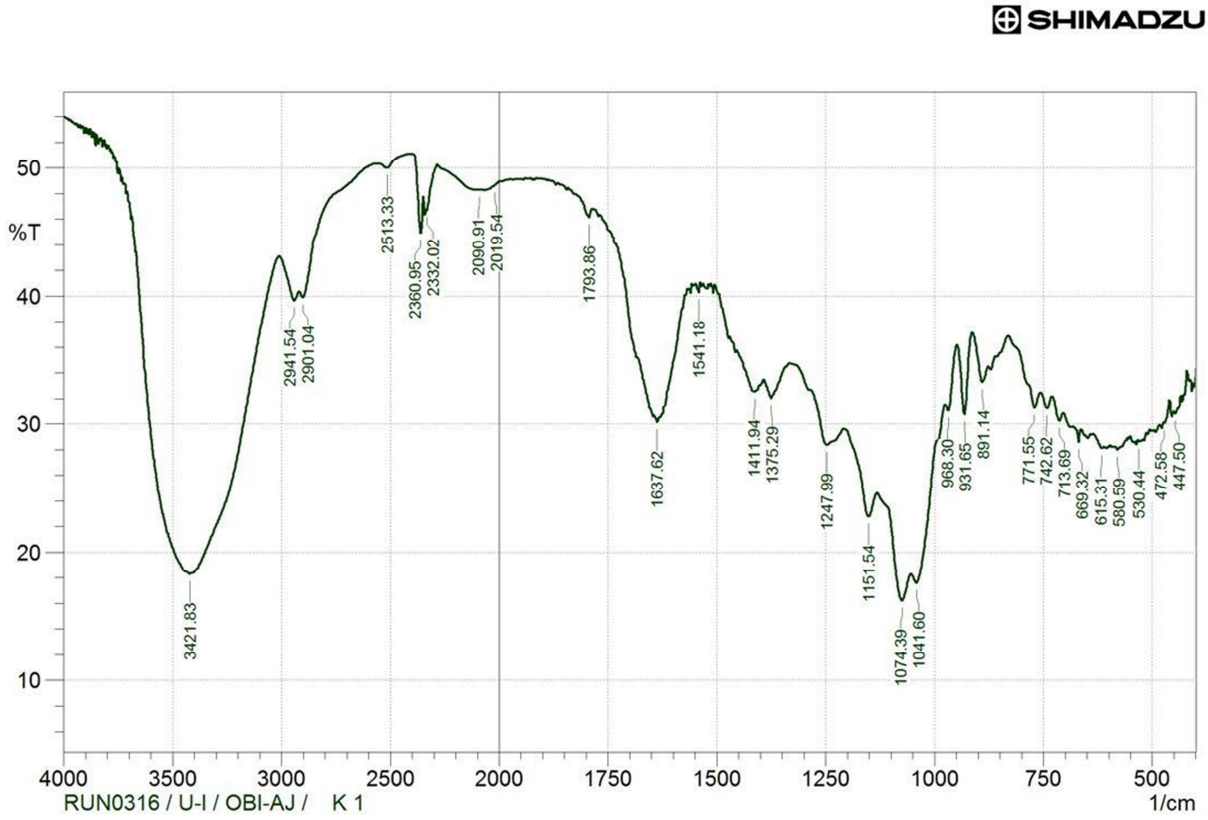

Figure 2. *FTIR measurements for pyocyain extract from P. aeruginosa OB11.*

Table 1. *Comparative absorption bands of FTIR for sample with standard pyocyanin.*

Compound	H-O	C-H – aromatic	C=N	C=C aromatic	C=O	C-O	C-N	ip O-H	ip C-H aromatic	oop C-H aromatic
Standard	3475.73	3059.10	1627.90	1554.63	1469.76	1323.17	1284.59	1107.44	856.39	748.38
Sample	3421.83	2941.54	1637.62	1541.16	1411.94	1375.29	1247.99	1151.54	891.14	742.62

After inhibitory tests in combined and single application of bacteriocin and pyocyanin, results obtained showed a general time dependent inhibitory pattern of the two combinatory compounds against the test isolates of *S. aureus*, *E. coli* and *C. albicans* based on contact time with the highest inhibition obtained after 72 h of incubation for all treatments (single/combined) and test isolates. Generally, there was also a comparatively more efficient activity of the combined treatments over the single treatments as obtained from the results as single treatments of bacteriocin and pyocyanin gave lower zones of inhibition against test isolates (Table 2, 3 and 4). In single treatments, it was however generally observed that pyocyanin treatment was more effective when compared with bacteriocin against all test isolates (Table 2, 3 and 4).

A variable inhibitory pattern based on the combined application of pyocyanin and bacteriocin on each test isolate was observed.

For *S. aureus* ATCC 29213, the combined treatment of 50µl pyocyanin/50µl bacteriocin led to the highest zone of inhibition (28.3 ± 0.38mm) after 72h of contact., while the least value for combined treatment (17.9 ± 0.76 mm) was obtained with 20µl pyocyanin/80µl bacteriocin applied after 24h contact time (Table 2). Single application of bacteriocin and pyocyanin yielded highest values of 13.7 ± 1.12mm and 5 ± 0.21mm respectively after 72h.

In the case of *E. coli* ATCC 25922, the antimicrobial treatments of 80µl pyocyanin /20µl bacteriocin gave the highest inhibition with 17.8±0.98mm after 72h incubation, while the lowest zone of inhibition of 13.8±0.33mm for the combined treatments was obtained with 30µl pyocyanin/70µl bacteriocin combination after 24h contact time (Table 3). When single application of bacteriocin and pyocyanin was tested, respective values of 12.1±2mm and 4.1±1.68mm were recorded as highest inhibition values after 72h contact time

In table 4, it was shown that a combination of 80µl pyocyanin/20µl bacteriocin yielded the highest zone of inhibition of 20.9±1.43mm against *C. albicans* ATCC 10261 after 72h contact time, while the least value in combination based inhibition (20.1±0.67) was also obtained with same combination at 24h contact time. With *C. albicans*, the highest inhibition (14.5±0.81mm) recorded for single application was obtained when pyocyanin alone was used at 72h contact time. There was however no inhibition of *C. albicans* when bacteriocin alone was used at all the contact times tested.

Table 2. *Antimicrobial activity of treatments of pyocyanin and bacteriocin combinations and single application on S. aureus ATCC 29213.*

Antimicrobial treatment	Zone of inhibition (mm) at different contact times		
	24h	48h	72h
20µl pyocyanin + 80µl bacteriocin	17.9 ± 0.76	23.5 ± 1.32	27 ± 1.44
30µl pyocyanin + 70µl bacteriocin	18.6 ±.23	22.3 ± 0.31	26.5 ± 0.62
50µl pyocyanin + 50µl bacteriocin	20.9 ± 0.33	25.5 ± 1.81	28.3 ± 0.38
70µl pyocyanin + 30µl bacteriocin	20.5 ± 0.32	22.2 ± 1.33	26.5 ± 0.34
80µl pyocyanin + 20µl bacteriocin	20.6 ± 2.18	23.3 ± 1.21	27.6 ± 2.27
100µl pyocyanin	12.1 ± 1.03	13.2 ± 0.45	13.7 ± 1.12
100µl bacteriocin	5 ± 1.22	5 ± 0.92	5 ± 0.21

Table 3. *Antimicrobial activity of treatments of pyocyanin and bacteriocin combinations and single application on E. coli ATCC 25922.*

Antimicrobial treatment	Zone of inhibition (mm) at different contact times		
	24h	48h	72h
20µl pyocyanin + 80µl bacteriocin	14.2±0.58	15.6±0.63	17.6±1.11
30µl pyocyanin + 70µl bacteriocin	13.8±0.33	15.9±0.88	17.3±0.96
50µl pyocyanin + 50µl bacteriocin	14.1±0.32	16.9±1.03	17.7±1.34
70µl pyocyanin + 30µl bacteriocin	14.1±0.42	16.5±1.15	17.3±0.63
80µl pyocyanin + 20µl bacteriocin	13.9±0.65	16.8±1.2	17.8±0.98
100µl pyocyanin	10.3±0.52	11.2±1.08	12.1±2.00
100µl bacteriocin	2.5±0.61	3.2±2.04	4.1±1.61

Table 4. *Antimicrobial activity of treatments of pyocyanin and bacteriocin combinations and single application on C. albicans ATCC 10261.*

Antimicrobial treatment	Zone of inhibition at different contact times		
	24h	48h	72h
20µl pyocyanin + 80µl bacteriocin	20.5±0.66	20.5±0.88	20.7±0.55
30µl pyocyanin + 70µl bacteriocin	20.3±0.76	20.4±0.92	20.8±0.74
50µl pyocyanin + 50µl bacteriocin	20.3±0.31	20.4±1.32	20.9±2.18
70µl pyocyanin + 30µl bacteriocin	20.2±0.53	20.2±1.09	20.7±1.65
80µl pyocyanin + 20µl bacteriocin	20.1±0.67	20.6±2.04	20.9±1.43
100µl pyocyanin	14.3±1.13	14.1±0.73	14.5±0.81
100µl bacteriocin	0	0	0

Table 5. *Amount of Potassium and Sodium ions leaked from cells of test isolates by antimicrobial treatments.*

	20µl pyocyanin + 80µl bacteriocin	30µl pyocyanin + 70µl bacteriocin	50µl pyocyanin + 50µl bacteriocin	70µl pyocyanin + 30µl bacteriocin	80µl pyocyanin + 20µl bacteriocin	100µl pyocyanin	100µl bacteriocin
S. aureus ATCC 29213							
K^+	7.76	7.74	7.36	9.11	10.03	5.37	3.35
Na^+	9.37	9.11	9.24	11.00	11.07	7.36	4.59
E. coli ATCC 25922							
K^+	7.32	6.37	7.37	7.43	7.25	3.74	3.35
Na^+	8.34	7.71	8.86	11.00	8.14	5.00	4.59
C. albicans ATCC10261							
K^+	6.36	6.48	6.88	7.26	5.22	2.37	0.08
Na^+	7.21	7.44	8.13	8.35	5.89	3.27	0.06

Flame photometric confirmation of the effect of the inhibitory substances applied on the leakage of sodium and potassium ions from the cells tested showed a correlative effect against the test organisms in line with results of inhibition. Generally quantity of Sodium and Potassium released from cells of all test isolates were more in combined application of pyocyanin and bacteriocin than in single application of each of them (Table 5). The results also showed that values for Sodium ion leakage were generally more than for Potassium ion leakage in all test conditions.

4. Discussion

The identified isolates used for the production of pyocyanin and bacteriocin were known species responsible for the production of such metabolites. A number of earlier research work have focused on the production and antimicrobial activities of these metabolites specifically from *P. aeruginosa* and *L. lactis* [7] [23] [24] [25] [26] [27], thus corroborating the isolates' characteristics as observed in this work. The product concentrations (4274 AU/ml- bacteriocin and 70µg/ml- pyocyanin) as biosynthesized by the respective producing isolates were also in line with concentrations earlier reported in previous research [16] [28].

The functional chemical characteristics of pyocyanin produced as determined in this work by FT-IR was in line with standard pyocyanin also reported by [7]. The presence of H-O, C-H – aromatic, C-O, C=O, C=C aromatic, C=N, C-N, ipO-H, ipC-H and oopC-H groups were all determined thus confirming pyocyanin extracted. These characteristic IR peaks

uniquely define the organic nature of the pyocyanin extract. The bioactivity of pyocyanin was observed as in all three test organisms and was ascribed to be because of the toxic redox property of pyocyanin based on electrochemical transfers in and out of the affected cells in such a way that it is inimical to cellular respiration [25]. Earlier works have proven pyocyanin based inhibition of bacteria and fungi [24] [25] [27]; which are in line with results obtained in this work.

Bacteriocin-mediated inhibition observed in this work was also in line with reports by [23] [26] and [29] as there was substantial inhibition against test organisms when bacteriocins were applied singly. The ability of Lactococcal bacteriocins to facilitate pore formation on cellular membranes based on ionic interactions thereby depleting proton motive force and ATP [15] could be the mode of activity of the bacteriocin applied. This was implied when there was seepage of sodium and potassium ions from the affected cells when bacteriocin alone was applied. However, further physiological experiments will aid in fully confirming the biomechanism of action. Chemical speciation of the bacteriocin produced will also aid in more precise biomachanistic assessment, as different bacteriocins possess different mode of activity and target sites. *Lactococcus lactis* produces a number of bacteriocins including nisin [30], lactococcin [31] and [23] and their target modes of activity are slightly variable. Further assessment on type of bacteriocin produced by *L. lactis* RCM21 will further explain the observed antimicrobial effect. From the results, there was however no inhibition against the yeast *C. albicans* tested. This could be due to the narrow spectrum of antimicrobial activity elicited by the bacteriocin, as bacteriocins have that a major drawback in application [13]. From this work, it was observed that when applied singly, pyocyanin showed a higher inhibitory action against test isolates than the bacteriocin applied. The possible reason for this observation may also be tied to the narrow spectrum of activity of bacteriocins as state above. It is also hypothesized that the concentrations used might have played a role as the work did not target to prove concentration dependent inhibition of the single compounds. The focus was to prove the efficacy increase in combined application as against single application.

It was clearly shown that there was a synergy in activity between pyocyanin and bacteriocin as the inhibition against all test organisms was increased in all the combination treatments in comparison with the single treatment. In increasing the efficacy of antimicrobial agents especially bacteriocins and antibiotics, researchers have involved the combined approach where double efficacy is achieved primarily because of the different modes of action utilized. [3] worked on the synergistic effect between colistin and bacteriocins in reducing the effect of Gram-negative pathogenic bacteria which showed similar results with increased efficacy. [32] also went further to elucidate the synergy observed between natural antimicrobial agents and antibiotics targeted against *Staphylococcus epidermidis*. Other researchers have targeted drug resistant strains via a combined antimicrobial approach as exhibited by [33] when working against Methicillin-resistant *Staphylococcus aureus* using vancomycin combined with other antimicrobials. The effects of the combined application of pyocyanin and bacteriocin was also ratio dependent as the varying ratio in the combination of both agents led to varying results. The reason for the varying degree of inhibition (by the different ratios tested) as determined by the sizes if zones of inhibition were not immediately ascertained in this work. However, the reason can be further explained when a concentration based study of each of the bioactive agents is carried out. The combination was effective against gram negative bacteria, gram positive bacteria and yeast test isolates used increasing the scope of narrow spectrum bacteriocin which was not effective against yeast tested (in single application).

The effect of contact time was also felt as the higher contact time of 72h proved most effective. This means that the antimicrobial agents were basically working in line with microbial time dependent growth and metabolism. This could assist in designing an effective application method for the combinatorial/synergistic application of the two compounds, these findngs are in line with an earlier report by [29] that showed there was an increased activity of antimicrobiosis in increased contact time.

To further elucidate the physiological effect of the single and combined treatments of the antimicrobial agents against test isolates, the assessment of leakage of essential ions Potassium and Sodium from the cell of target organisms was carried out and it was shown that there was a correlation between the leakage and the antimicrobial activity as earlier observed. This gave a peek into the mechanisms of activity of the compounds. The value of Sodium leakage compared with Potassium was more - this could be ascribed to the fact that Sodium ions are comparatively smaller in weight than Potassium ions [34]. The cells generally lost ions in all antimicrobial treatments as compared with the control (results not shown) thus implying a general cell wall degradation and release of essential ionic compounds as a mode of antimicrobiosis in pyocyanin and bacteriocin. Similar ionic concentration loss from cells treated with antimicrobial agents have also been earlier reported [22].

5. Conclusion

The antimicrobial effect of both pyocyanin and bacteriocins can be enhanced by their combined application in such a way that synergizes their efficacies. This stands as a veritable tool and further extends the value of combined approach to antimicrobiosis especially in the expanding challenge of antibiotic resistance. Deeper insight into their mechanisms of activity in synergy will aid in final drug design and application.

References

[1] Saha, S. Thavasi, R. and Jayalakshmi, S. (2008). Phenazine Pigments from *Pseudomonas aeruginosa* and Their Application as Antibacterial Agent and Food Colourants. *Research Journal of Microbiology*, 3(3): 122- 128.

[2] Liang, H., Duan, J., Sibley, C. D., Surette, M. G., and Duan, K. (2011). Identification of mutants with altered phenazine production in *Pseudomonas aeruginosa*. *Journal of Medical Microbiology*, 60, 22e34.

[3] Naghmouchi, K., Baah, J., Hober, D., Jouy, E., Rubrecht, C., Sane, F. and Drider, D. (2013). Synergistic effect between colistic and bacteriocins in controlling Gram-Negative pathogens and their Potential to reduce Antibiotic Toxicity in Mammalian Epithelial Cells. *Antimicrobial Agents and Chemotherapy* 57(6): 2719-2725.

[4] Makarand, R., Prashant, D., Bhushan, L. and Sudhir, B. (2007). Detection, isolation and identification of phenazine-1-carboxylic acid produced by biocontrol strains of *P. aeruginosa. Journal of Scientific and Industrial Research*, 66: 627-631.

[5] Mavrodi, D., Blankenfeldt, W. and Thomashow, L. (2006). Phenazine Compounds in Fluorescent *Pseudomonas* Spp. Biosynthesis and Regulation. *Annual Review of Phytopathology* 44(1): 417-445.

[6] Onbasli, D. and Aslim, B. (2008). Determination of antimicrobial activity and production of some metabolites by *Pseudomonas aeruginosa* B1 and B2 in sugar beet molasses. *African Journal of Biotechnology* 7(24): 4614-4619.

[7] Aziz, L. M., Hamza, S. J. and Abdul-Rahman, I. A. (2012). Isolation and characterization of phenazine produced from mutant *Pseudomonas aeruginosa. Al-Anbar Journal of Veterinary Science* 5(1): 42-53.

[8] Sudhakar, T., Karpagam, S. and Shiyama, S. (2013). Analysis of pyocyanin compound and its antagonistic activity against phytopathogens. *International Journal of Chemical Technology and Research* 5(3) 1101-1106.

[9] Priyaja, A. (2013). Pyocyanin (5-methyl-1-hydroxyphenazine) produced by *Pseudomonas aeruginosa* as antagonist to vibrios in aquaculture: Overexpression, downstream process and toxicity. Ph. D thesis, Cochin Univ. of Science and Technology, India.

[10] Zacharof, M. P. and Lovitt, R. W. (2012). Bacteriocins Produced by Lactic Acid Bacteria: A Review Article. *APCBEE Proceedia,* 2: 50-56.

[11] Chen, H., and Hoover, D. G. 2003. Bacteriocins and their food applications. *Comprehensive Review of Food Science and Food Safety.* 2: 82-100. http://dx.doi.org/10.1111/j.1541-4337.2003.tb00016.x

[12] De Vuyst, L., and Leroy, F. 2007. Bacteriocins from lactic acid bacteria: Production, purification, and food applications. *Journal of Molecular Microbiology and Biotechnology* 13: 194-199.

[13] Suskovic, J., Blazenka, K., Beganovic, J., Pavunc, A. L., Habjanic, K., and Matosic, S. (2010). Antimicrobial activity the most important property of probiotic and starter Lactic acid bacteria. *Food Technology and Biotechnology* 48 (3): 296 – 307.

[14] Chung, H. J., Montville, T. D, and Chikindas, M. L. 2000. Nisin depletes ATP and proton motive force in mycobacteria. *Letters in Applied Microbiology*, 31: 416-420.

[15] Venema, K., Venema, G., and Jok, K. (1995) Lactococcal bacteriocins: mode of action and immunity. *Trends in Microbiology* 3: 299-304.

[16] Ajunwa O. M., Odeniyi O. A., Adeleke A. J., Nwanekwu K. E., and Obiukwu C. E. (2014). Variation in the presence of plasmids associated with proteinase and bacteriocin production of *Lactococcus lactis* isolated from naturally fermented milk. *Suranaree J. Sci. Technol*. 21(4):381-388.

[17] Essar D. W., Eberly L., Hadero A., Crawford I. P. (1990). Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: interchangeability of the two anthranilate synthases and evolutionary implications. *Journal of Bacteriology,* 172(2), 884-900.

[18] O'Malley Y. Q., Reszka K. J., Spitz D. R., Denning G. M., Britigan B. E. (2004). *Pseudomonas aeruginosa* pyocyanin directly oxidizes glutathione and decreases its levels in airway epithelial cells. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 287(1), 94-103.

[19] Tagg, J. R. and McGiven, A. R. 1971. Assay system for bacteriocins. *Journal of Applied Bacteriology* 21: 943-948.

[20] Omole, O. A., 2000. Immunogenicity of the Bacteriocins produced by *Lactobacillus plantarum* from fermented cereal gruels. M. Sc Dissertation, University of Ibadan, Ibadan, Nigeria.

[21] Oyeleke, S. B., Dauda, B. E. N., Boye, O. A. (2008). Antibacterial activity of *Ficus capensis*. *African Journal of Biotechnology* 7(10): 1414-1417.

[22] Oladunmoye, M. K, Adetuyi F. C. and Akinyosoye, F. A. (2007). Release of Sodium and Potassium Ions by Aqueous and Ethanolic Extract of *Cassia occidentalis* on Some Selected Bacteria *Trends in Applied Sciences Research, 2: 85-87.*

[23] Rodriguez, E., Calzada, J., Arapes, J. L., Rodriguez, J. M., Nunez, M., Medina, M. (2004). Antimicrobial activity of Pediocin-producing *Lactococcus lactis* on *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* 0157:H7 in cheese. *International Dairy Journal* 15(1): 51-57.

[24] De Vleesschauwer, D., Cornelis, P. and Hofte, M. (2006). Redox-active pyocyanin secreted by *Pseudomonas aeruginosa* TNSK2 triggers systemic resistance to *Magnaporte grisea* but enhances *Rhizoctonia solani* susceptibility in rice. *Molecular Plant-Microbe Interactions* 19(2): 1406-1419.

[25] El-Shouny, W. A., Al-Baidan, A. R. H. and Hamza, W. T. (2011). Antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from surgical wound-infections. International Journal of Pharmacy and Medical Sciences 1(1): 1-7.

[26] Rashid, F., Sharif, N., Ali, I., Naz, S., Sharif, S. and Nisa, F. (2013). Antimicrobial potential of *Lactococcus lactis* bacteriocin against *Salmonella typhi. African Journal of Veterinary Science* 5(1): 42-53.

[27] Karpagam, S., Sudhahar, T. and Lakshmipathy, M. (2013). Microbial response to pyocyanin produced by *P. aeruginosa* toward clinical isolates of fungi. *International Journal of Pharmacy and Pharmaceutical Sciences* 5(3): 870-873.

[28] El-Fouly, M. Z., Sharaf, A. M., Shahin, A. A. M., El-Bialy, H. A. and Omara, A. M. A. (2015). Biosynthesis of pyocyanin pigment by *Pseudomonas aeruginosa*. *Journal of Radiation and Applied Science* 8: 36-48.

[29] Adebayo, C. O. and Aderiye, B. I. (2010). Antifungal activity of bacteriocins of lactic acid bacteria from some Nigerian fermented foods. *Research Journal of Microbiology* 5(11): 1070-1082.

[30] Sanlibaba, P., Akkoc, N. and Akcelik, M. (2009). Identification and characterization of antimicrobial activity of Nisin A produced by *Lactococcus lactis* subsp. *lactis* LL27. *Czech Journal of Food Science* 27(1): 55-64.

[31] Holo, H., Nilssen, O. and Nes, I. F. (1991). Lactococcin A, a new bacteriocin from *Lactococcus lactis* subsp. cremoris: isolation and characterization of the protein and its gene. *Journal of Bacteriology* 173(12): 3879-3887.

[32] Abidi, S. H., Ahmed, K., Sherwani, S. K. and Kazani, S. U. (2015). Synergy between antibiotics and natural agents results in increased antimicrobial activity against *Staphylococcus epidermidis*. *The Journal of Infection in Developing Countries* 9(9): 925-929.

[33] Da Silva, L. V., Araujo, M. T., dos Santos, K. R. N. and Nunes, A. P. F. (2011). Evaluation of the synergistic potential of vancomycin combined with other antimicrobial agents against methicillin-resistant *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp strain. *Mem Inst Oswaldo Cruz* 106 (1): 44-50.

[34] Trease, G. E. and Evan, C. W. (2002). A textbook of pharmacognosy. 15th Edition, Sands Edinburgh, U. K., pp: 595.