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Determination of the Growth and Bacteriocin Production Kinetics associated with *Bacillus pumilus* and *Bacillus subitlis* Isolated from Rastrineobola argentea (Omena)

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Abstract**—*****This study aimed to determine of the growth and bacteriocin production kinetics of Bacillus pumilus and Bacillus subitlis isolated from Rastrineobola argentea. The study accomplished this goal by focusing on the findings of the existing literature. The findings of the existing literature reveal that B. subtilis and B. pumilus possess a broad-spectrum antimicrobial action against numerous microorganisms including S. aureus, L. plantarían, C. perfringens, L. monocytogenes, B. cereus, E. coli, and L. mesenteroides. The existing literature also reveal that bacteriocins from the two bacteria can survive high temperature of up to 80 ^{0} C for B and 120 ^{0}C for B. subtilis. Besides, the existing literature reveal that the microbial action of bacteriocins from the B. subtilis and B. pumilus are not affected is not affected by proteolytic enzymes such as lipase, proteinase, and trypsin among others. While these bacteriocins operate well over a range of pH conditions (pH = 4.0 to 9.0), their optimum action is realized in alkaline and neutral pH conditions (pH =7). In addition the findings of the literature review reveal that bacteriocins from B. subtilis and B. pumilus exhibit maximum antimicrobial action when they are subjected to peptone and glucose concentrations of 0.5% and 0.25% respectively in MRS broth. The existing literature also established that bacteriocins from the two bacteria are tolerant to high salt concentrations of up to 5% for both KCl and NaCl. However, the existing literature reveal that these bacteriocins cannot survive long duration of subjection to U.V light or rays. The researcher recommends the employment of bacteriocins produced by Bacillus pumilus and Bacillus subtilis extracted from Rastrineobola argentea as bio-preservatives, as they are effective.*

Keywords**— *****Bacillus pumilus, Bacillus subitlis, Rastrineobola argentea, Bacteriocins, Growth.***

I. INTRODUCTION

Rastreneobola argentea, which is locally acknowledged as Omena, contributes approximately 50% of the total catch of fish of above 160, 000 tonnes in Lake Victoria, in Kenya. About 30% of the catch is employed for domestic consumption and fortification of food owing to its rich minerals, proteins, and vitamins source. Apart from contributing significantly to human diet, researchers believe that *Rastreneobola argentea* can be an essential contributor to bio-preservation. According to Luan, bio-preservation involves targeting of spoilage-causing microorganisms or pathogens with the assistance of their metabolites or the prokaryotes themselves, thereby resulting into safety of food or longevity (1). Bacteriocin, which is a proteinaceous compound of low molecular mass produced by bacteria, continues to receive rapid attention as it is generally regarded as safe and does not result into any adverse impact on food. Bacteriocins are peptides or antimicrobial proteins that are ribosomally secreted and produced. These peptides often inhibit the growth associated with microorganisms of closely related or the same species. Bacteriocins also kills sensitive cells or inhibit their growth by jeopardizing the manufacture of cell wall or even by developing pores within the cell membrane (2). Various microorganisms are recognized to secret bacteriocins, and the most prominent producers among these microorganisms are the Bacillus species and lactic acid bacteria. According to Monell, bacteriocin is considered safe for consumption by humans as it becomes inactive when subjected to the digestive enzymes treatment within the stomach (3). Naturally, every bacterium has the capability of secreting novel bacteritKin. Nevertheless, this capability of microorganisms is unexplored to a larger extent. Until now, only a few bacteriocins are available in the commercial arena.

The only commercially available bacteriocins are Pediocin PA-1 and Nisin, which are produced by Pediococcus acidilactici and Lactococcus lactis strains respectively. Despite being significant contributors to bio-preservation, bacteriocins possess certain shortcoming that hinder their extensive employment in preserving foods. For instance, Nisin has been noted to be unstable under basic and neutral environments (4). The antimicrobial spectrum of this bacteriocin is also not broad enough. Besides, the inhibition potential of Nisin on Listeria monocytogenes is relatively weak. Pediocin PA-1 and Nisin are also unable to hinder the growth of the bacterium B. cereus (4). According to Monell, the Bacillus species produce a range of antimicrobial compounds as well as bacteriocins (3). Most of the Bacillus species such as B. cereus, B. pumulus, B. subtillis, B. megaterium, and B. coagulans produce bacteriocins. According to Li, Rastrineobola argentea happens to be a rich source of B. subtilis and B. pumilus producing bacteriocins (5). This study aims to determine the growth and bacteriocin production kinetics associated with *Bacillus pumilus* and *Bacillus subitlis* isolated from *Rastrineobola argentea* (Omena). The study accomplishes this goal by exploring the findings of the existing literature on the growth and bacteriocin production kinetics associated with *Bacillus pumulus* and *Bacillus subitlis*. The findings of this review will contribute significantly to informing practice on food preservation based on biological microorganisms, as opposed to the use of chemical preservatives, most of which expose humans to the risk of contracting illnesses such as cancer.

II. LITERATURE REVIEW

According to Yang, the potential employment of bacteriocins for different technological applications is presently on the rise (6). Bacteriocins secreted by lactic acid bacteria (LAB) employed in the preservation of food undergoes inactivation by digestive proteases possess an insignificant impact on the microbiota of the gut and are often heat and pH tolerant. Further, the LAB bacteriocins are acknowledged as largely safe, possess a wide spectrum of antimicrobial activity against foodborne spoilage and pathogenic bacteria, and demonstrate a bacteriocidal action mode (7). Studies focusing on bacteriocins demonstrate that the employment of bacteriocin with these characteristics in the food industry prolongs the shelf-life of food products and foods, minimizes the transmission of food-borne pathogens through the food chain, offers superfluous defense during conditions of temperature abuse, permits the employment of less severe heat treatment mechanisms without jeopardizing the safety of food, and moderates the employment of chemical preservatives (6). These characteristics assist in healthier conservation of food nutrients and vitamins, and maintenance of organoleptic characteristics of foods. In the applications of food processing, bacteriocins are added as inoculation with strains of bacteriocins or ex-situ synthesized preparations (7). Bacteriocins, which act as antimicrobial agents within the matrix of food, can then demonstrate their specific action. In several instances, the steps of processing and the natural non-stable and microbiota complex nature. Therefore, bacteriocins possess all the limiting aspects associated with the exertion of their activity. According to WON-IL, bacteria belonging to the genus Bacillus secret a range of arsenal comprising antimicrobial substances like lipopeptide and peptide antibiotics, as well as bacteriocins (8). Majority of the bacteriocins associated with Bacillus such as B. subtilis and B. pumulus belong to a category of post-translationally altered peptides known as lantibiotics. Lantibiotics are increasingly becoming significant owing to their occasionally wider inhibition spectra relative to a majority of LAB bacteriocins. For instance, strains of B. subtilis are employed as a starter culture in fermenting mesquite seeds or soybeans. This extensive review of literature focuses on critical assessment of the findings of a range of researches in articles associated with the lantibiotics group of bacteriocins of which B. pumilus and B. subtilis extracted from Rastrineobola Argentea are part of. The literature review determines the growth and bacteriocin production kinetics of B. pumilus and B. subtilis by focusing on various areas.

2.1 Growth

Sharma et al., established that the culture supernatant associated with B. subtilis demonstrated a robust inhibition or hindrance against a range of pathogens or microorganism that result into serious spoilage of food including L. plantarían, L. monocytogenes, B. subtilis, S. aureus, B. cereus, C. perfringens, L. mesenteroides, and E. coli (9). The researchers also observed a broad spectrum of clearance established by crude bacteriocin acquired from B. subtilis R75 which ranged up to 5 mm on the Petri dishes that contained the indicator bacteria within well diffusion

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assay. The findings of the researcher revealed activity of $10 \cdot 10^5$ AU/mL, as shown in table I below:

Table.1: complete and partial purifications of bacteriocin secreted by B. subtilis R75

	V mL	Activity AU/mL	Total activity	γ(protein) mg/mL	Specific activity (10^4)	Purification fold	Recovery %
Culture supernatant	1000	$10 \cdot 10^5$	$1 \cdot 10^9$	4.651	2.15	1.00	100.00
Partially purified bacteriocin (ammonium sulphate precipitation)	20	$40 \cdot 10^5$	$8 \cdot 10^7$	3.023	13.23	6.09	64.90
Purified bacteriocin (washing+SDS)	10	$5 \cdot 10^6$	$5 \cdot 10^7$	1.031	48.49	22.30	22.10

The researchers argued that the robust antagonism against several challenging and serious foodborne spoilage-causing microorganisms or pathogens highlighted the high likelihood of employing this bacteriocin as a suitable food preservative. According to Lanhua[1], bacteriocins exhibit high levels of sensitivity to closely related species thereby limiting their employment in preserving food (10). Nonetheless, recent findings have showed their broad-spectrum action against a range of microorganisms (10). For instance, a newly extracted bacteriocin from B. mycoides can inhibit the growth of S. aureus and L. monocytogenes. In like manner, paenibacillin, which is bacteriocin acquired from Paenibacillus sp., has been established to demonstrate activity against a number of bacteria including Clostridium sporogenes, Bacillus sp., Listeria sp., S. aureus, and iMctobacillus sp. As such, these findings justifies the fact that bacteriocins produced from various bacteria behave contrarily and possess their specific spectra of inhibition. Listerosis resulting from L. monocytogenes presence in foods preserved at low temperatures in refrigerators has been noted to cause many deaths across the globe. However, bacteriocin secreted by B. subtilis R75 has the capability of regulating this form of pathogenicity when employed in the preservation of food (9). As such, this bacteriocin can meet the severe challenge of regulating the decomposition of refrigerated food. B. subtilis is considered completely safe for consumption by humans as it originates from food grade bacteria (9).

In their study, Sharma et al. determined the pattern of producing bacteriocin during the cycle of growth of B. subtilis by measuring the inhibition zones' size against indicator bacteria L. mesenteroides and L. monocytogenes. The measurement of the isolate's absorbance was done up to 96 h and the outcomes demonstrated a constant rise in absorbance from 0 to 72 h followed by a decrease afterwards. The researchers established that the production of the bacteriocin followed the trend depicted by the growth of B. subtilis (9).There was a variation in the zone of inhibition from 2 mm to 4 mm for L. mesenteroides and from 2 mm to 3 mm for L. monocytogenes at 24h. The measurement for maximum bacteriocin production was done at 72 h with 5 mm being the widest zones established against the indicator bacteria as shown in figure I below:

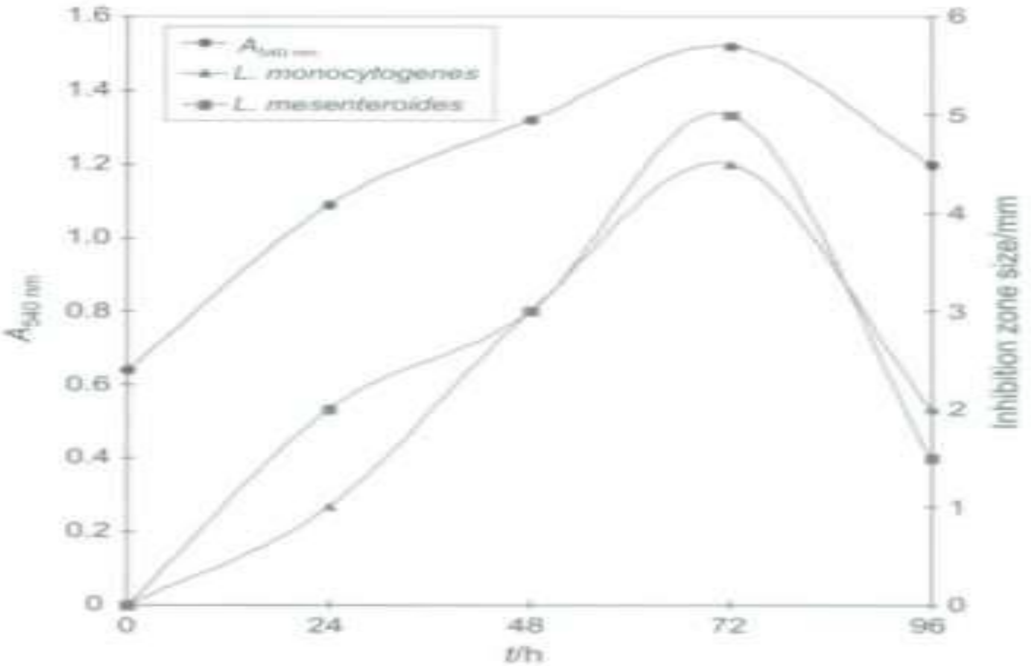

Fig.1: Bacteriocin production during the cycle of growth of B. subtilis

Source: Sharma et al., (2011)

Other researchers have also focused on studying the bacteriocin production kinetics and growth of B. subtilis. In a study conducted by Liu et al., the researchers established that the antibacterial activity associated with B (11). Subtilis EMD4 demonstrated the highest level when the bacteriocin was cultured or grown in TSB as shown in Figure II below:

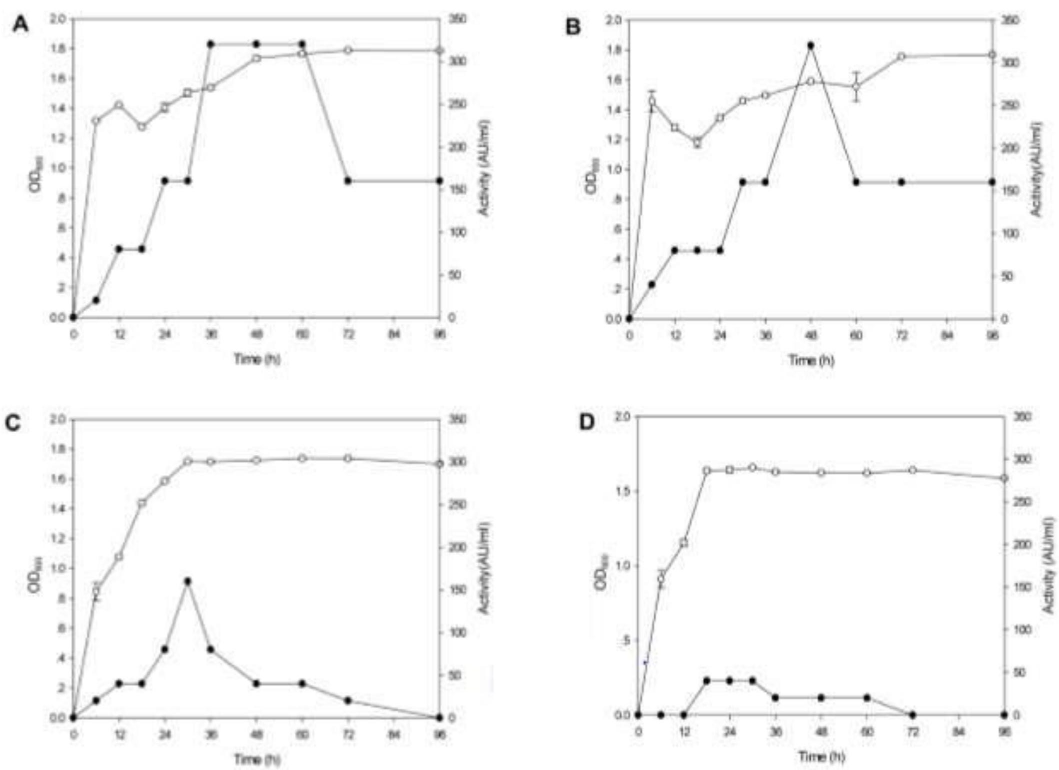

Fig.2: Bacterial activity and growth of B. subtilis

Source: (Liu et al., 2015)

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The observation of the highest activity which was320 AU/ml, was done at 36 h within the TSB broth and it remained at this level until 60 h. At 72 h, the activity of the bacteriocin declined to 160 AU/ml and stayed at this level until 96 h (11). The highest activity of 320 Au/ml was observed in the BHI medium at 48 h. The activity of the bacteriocin within this medium then remained at this level up to 96 h. The bacteriocin's activity attained the highest level of 160 AU/ml at 30 h within the LB medium and the declined slowly, leading to it not being detected at 96 h (11). Among the media employed in this study, the NB was noted to be the worst for the activity of the bacteriocin. This medium recorded the highest bacteriocin activity at only 40 AU/ml at approximately 18 h, which then declined gradually (11). Even through different media exhibited different antibacterial activities, the researchers noted that all of the media facilitated the growth of Bacillus subtilis EMD4.

In the study conducted by Kivanc et al., the findings of the study revealed that B. pumilus PCA 9.4 and B. pumilus PCA 4.2 exhibited antibacterial action against methicillin-resistant S. epidermidis 14.1 and S. epidermidis KA 11.1[2] (12). In a different study, B. pumilus and B. subtilis were noted to exhibit antibacterial activity against a range of Gram positive and Gram negative bacteria. These findings can be applied to the bacteriocins of B. pumilus and B. subtilis extracted from *Rastrineobola argentea*. Rajesh et al determined the growth of B. pumulus. The researchers established that B (13). pumilus DR2 demonstrated a doubling period of 30 min within the medium (that is LB), and that the growth attained the stationery stage at 20 h, as observed by optical density set at 600 nm as shown in figure III below:

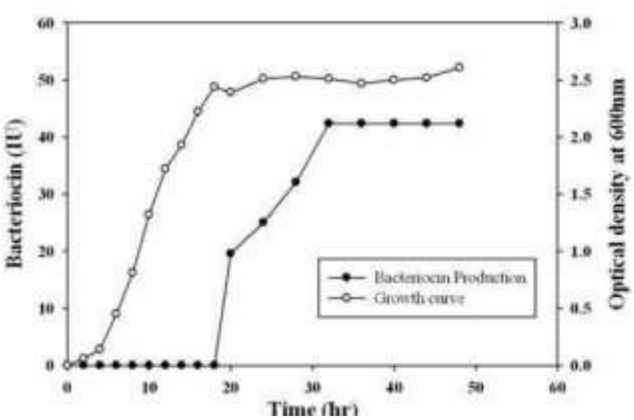

Fig.3: Relationship between bacteriocin production (Pumiviticin) and cell growth in B. pumilus.

Source: Rajesh et al., (2012)

Rajesh et al., monitored the production of bacteriocin (Pumiviticin) by B. pumilus. The outcomes revealed that significant microbial activity only occurred after the stationery stage (i.e. greater than the growth witnessed at 20 h) as shown in figure III above (13). This finding reveals that bacteriocins secreted by B. pumilus extracted from *Rastrineobola argentea* are secondary metabolism's products.

2.2 Temperature

Sharma et al performed a study to purify and characterize bacteriocin secreted by B. subtilis R75, which was extracted from *Phaseolus radiates* also known as the mung bean (9). Well diffusion assay was employed in the measurement of the bacteriocin activity in relation to temperature. The researchers characterized the pure bacteriocin with the aim of assessing its ability to function under an environmental condition which is different from the perspective of food preservation. The findings of the study revealed that B. subtilis was thermostable in nature since it can tolerate high temperature of up to 120 ^{0}C[9]. However, the researchers established a partial loss in the bacteriocin's activity with a constant increase in temperature (9). Thus, these findings reveals that the thermostability of the bacteriocins produced by B. subtilis and B. pumilus ensures that sterilization can be accomplished.

In a study executed by Maina et al the researchers focused on heating crude bacteriocins at various temperatures until a temperature of 80 ^{0}C was attained (14). The bacteriocins were then tested against pathogens from bovine mastitis. The researchers established that there was no significant decrease in the bacteriocin's antimicrobial activity at temperatures below 80 ^{0}C. Nonetheless, the subjection of the bacteriocins to temperatures exceeding 100 ^{0}C to 121 ^{0}C led to a more than 50 percent reduction in antimicrobial activity, which was considered significant. These findings revealed that crude bacteriocins from B. pumulus and B. subtilis extracted from *Rastreneobola argentea* are only effective up to a temperature of 80 ^{0}C. Nevertheless, these Bacillus bacteriocins are still considered thermostable.

2.3 Enzyme

Maina et al subjected bacteriocins from the isolates of Bacillus to lipase, trypsin, and proteinase enzymes, and established a significant reduction of the inhibition zones (14). Crude bacteriocins that were subjected to trypsin were established to have lost up to 40 percent of their antimicrobial activity, whereas those that were subjected to proteinase K did not demonstrate any inhibition zone (14). Nonetheless, lipase was established to have lost only 15 percent of the antimicrobial activity of the bacteriocin (14). In relation to this, Maina et al[14] deduced that proteolytic

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enzymes has an effect on the microbial action of crude bacteriocin, as they have a proteinaceous form (14). It is also vital to note that the bacteriocins may possess a lipid moiety that does not exhibit a substantial loss of the activity of crude bacteriocins. As such, it can be noted that the activity of both B. pumilus and B. subtilis are affected by proteolytic enzymes. Sharma et al., recommend the storage of B. subtilis for about 2.5 months, as it losses activity after 3 months[9]. The loss of activity of this bacteriocin is attributed to the fact that it commences to establish novel interactions with the surrounding when isolated from its natural congenial setting.

2.4 pH

B. subtilis and B. pumilus have been established to demonstrate similar patterns of activity when subjected to various pH ranges. In a study conducted by Sharma et al the researchers determined the effect of pH on the bacteriocin production kinetics of B subtilis by adding a volume of purified bacteriocin (0.5 ml) into nutrient broth (4.5 ml) at various values of pH that ranged from 3.0 to 11.0[9] (9) The sample was then incubated at 37 0C for 30 minutes. Each of the sample subjected to treatment at various values of pH was assayed using the well-diffusion technique against indicator bacteria. Sharma et al. established that B. subtilis thrived in a range of pH, but significant antimicrobial activity was noted at pH 7 and pH 6. When the pH shifted to acidic (i.e. from 4.0 to 6.0), the researchers observed that the bacteriocin partially retained its antimicrobial activity (9).

Bacillus subtilis R75 was noted work well in the extended pH range but the significant activity was observed at pH=6.0 and 7.0 (Fig. 8). Bacteriocin could retain its antimicrobial activity partially when there was a shift to acidic (from 4.0 to 6.0) or basic (from 11.0 to 8.0) range (9). Stability of bacteriocin at different pH scale is a limiting factor for recommending its use in food items.

Maina et al focused on assaying the capability of crude bacteriocins from isolates of Bacillus to the growth of Staphylococcus aureus ATCC 25923 and E. coli ATCC at pH range of between 3 and 9 (14). The researchers established that no inhibition zone was seen at pH range below 5 against the pathogens associated with bovine mastitis. Nevertheless, the findings of this study revealed a mean inhibition zone of 19.57 mm for the range of pH between 7 and 9. These outcomes further demonstrated that these bacteriocins extracted from the isolates of Bacillus are impacted by acidic pH settings and operate well in alkaline and neutral conditions. This finding applies also to the B. pumulus obtained from *Rastrineobola argentea*. Other findings have also revealed that bacteriocins acquired from isolates exhibit stability at pH range between 3 and 9 for about 24 hours. Nonetheless, limited inactivation is experienced at pH range of between 10 and 11 (14). In a study executed by Rajesh et al researchers focused on the identification as well as the secretion of antimicrobial substance by B. pumilus (13). The findings of the study revealed that B. pumulus can grow at the range of pH of between 4.0 and 9.0[14] (13).

2.5 Sugar

B. subtilis and B. pumilus have also been noted to demonstrate similar property when exposed to sugar. Different studies have established that the highest antimicrobial activity of bacteriocin such as B. pumilus and B. subtilis is witnessed when peptone and glucose are subjected to a variation of 0.5% and 0.25% respectively within the constituted broth of MRS (15) (16).

2.6 Salts

Rajesh et al studied the salt tolerance capability of B. pumilus in relation to the production of bacteriocin (i.e. as activity measure) and organism growth (measurement taken at OD 600 nm) within the range of salt concentration of 0 to 2 M (13). Researchers observed a constant rise in the production of biomass at 600 nm until 1.0 M NaCl (). At 1.0 M NaCl concentration, Rajesh et al., observed that the bacterial growth was approximately 6 times faster relative to the growth rate when salt was absent (13). The researchers argued that the strain of B. pumulus DR2 isolated in their study had the ability to grow under high salt concentration levels, thereby highlighting its halotolerant mannerism. Moreover, Rajesh et al established that the production of bacteriocin by B (13). pumilus was not significantly decreased at lower concentrations of salt (0-1). Biomass production was noted to be proportional to the activity of the bacteriocin at higher concentration of salt. As such, it can be noted that the bacteriocin secreted by B. pumilus extracted from *Rastrineobola argentea* has the ability of tolerating higher concentrations of salt.

In a study executed by Caputo et al the researchers established a lack of sensitivity of partially purified bacteriocin to Triton X-100, Tween 20, Tween 80, and NaCl (16). However, the findings of this study revealed that the activity of bacteriocin is reduced by Urea and SDS. As such, it can be noted that the antimicrobial activities of B. subtilis and B. pumilus extracted from *Rastrineobola argentea* are decreased by SDS and UREA. Bacteriocins from these bacteria lack sensitivity to NaCl, Tween 20, Tween 80, and Triton X-100 when the bacteriocins are partially purified[18]. Other studies report that the activity of partially bacteriocin mixed with EDTA is more robust relative to that of EDTA or bacteriocin alone (17) (18) (19).

Bacteriocins from Bacillus groups such as B. pumilus and B. subtilis demonstrate an increase in antimicrobial activity when they are subjected to increased concentrations of KCl and NaCl up to about 5% (13). However, it is vital to note that the activity of these bacteriocins is more enhanced when they are mixed with EDTA, as opposed to working alone.

2.7 UV Light

Researchers have focused on the study of the antimicrobial activity of bacteriocin using bacteriocins extracted from different organisms including L. plantarum and P. Pentosaceus. In a study conducted by Minor-Perez et al, the findings revealed total damaging of a bacteriocin from P. pentosaceus was established after 74 minutes of subjection to UV light (18). Nonetheless, after the same duration of UV light exposure, L. plantarum bacteriocin was noted to be stable.

III. CONCLUSIONS AND RECOMMENDATIONS

This study aimed at determining the growth and bacteriocin production kinetics of *Bacillus pumilus* and *Bacillus subitlis* isolated from *Rastrineobola argentea* by focusing on the findings of the existing literature. The findings from the literature review reveal that B. subtilis and B. pumilus possess a broad-spectrum antimicrobial action against numerous microorganisms including S. aureus, L. plantarían, C. perfringens, L. monocytogenes, B. cereus, E. coli, and L. mesenteroides. The results also indicate that bacteriocins from the two bacteria can survive high temperature of up to 80 ^{0} C for B and 120 ^{0}C for B. subtilis. The outcomes of the review of literature also indicate that the microbial action of bacteriocins from the B. subtilis and B. pumilus are not affected is not affected by proteolytic enzymes such as lipase, proteinase, and trypsin among others. While these bacteriocins operate well over a range of pH conditions (pH = 4.0 to 9.0), their optimum action is realized in alkaline and neutral pH conditions (pH =7). In addition the findings of the literature review reveal that bacteriocins from B. subtilis and B. pumilus exhibit maximum antimicrobial action when they are subjected to peptone and glucose concentrations of 0.5% and 0.25% respectively in MRS broth. The literature review outcomes also reveal that bacteriocins from the two bacteria are tolerant to high salt concentrations of up to 5% for both KCl and NaCl. However, the findings reveal that these bacteriocins cannot survive long duration of subjection to U.V light or rays. In relation to these findings, it can be noted that the bacteriocins produced by *Bacillus pumilus* and *Bacillus subtilis extracted from Rastrineobola argentea are effective when employed as bio-preservatives*. Bacteriocins from the B. subtilis and B. pumilus should be employed in bio-preservation process involving sterilization as they can withstand high temperatures. When used with U.V rays, care should be taken to avoid long exposure duration. Bio-preservation using bacteriocins from the two bacteria should also focus majorly on foods with alkaline or neutral pH conditions.

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