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Discovery

Effect of Plantaricin FF1 on the Mycelia of Aspergillus Niger

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ABSTRACT

Fermented foods are largely consumed in Nigeria where they constitute a bulk of the diet especially fungi, because of their high moisture content. Biocontrol, especially using bacteriocin is a good antidote to this. Bacteriocin is the most potent antimicrobial compound produced by lactic acid bacteria. Its mechanism of antagonism differs among species. Plantaricin FF1, a broad spectrum class II bacteriocin produced by Lactobacillus species isolated from 'fufu', a traditional Nigerian fermented food, was investigated for its effect on fungal cells. Scanning Electron Microscopy (SEM) analysis revealed that it caused lysis, destruction and vacuolation of the fungal cell. The bacteriocin was small (6.5kDa), heat stable, sensitive to proteolytic enzymes and exhibited a cytotoxic effect on the mycelia of A. niger. It was also stable for at least 60 and 120 days when stored at room (25^{o}C) and cold (0^{o}C, 4^{o}C) temperature respectively. It was active at a long range of pH (pH 1-10) and high temperature (121^{0}C). The molecular mass, heat stability and strong antimycotic action of plantaricin FF1 suggested that it was a novel class II bacteriocin produced by L. plantarum. When incorporated into eba, a Nigerian fermented food; it retarded fungal colonization for 10 days. Plantaricin FF1 was active both in-vitro and in-vivo, potentiating its practical application in the food industry to control fungal contamination and other against organisms of foods especially, fermented foods.

Key words: Bacteriocin; Plantaricin; Biocontrol; Fermented Food; Antimycotic action; Fufu, Lactobacillus plantarum.

1. INTRODUCTION

Microbial safety of food is an important public health concern globally. This is because illness could result from microbial contamination of food especially mycotoxicoses. Aside from the problem of ill health, it could also cause a reduction in the food values/nutrients. It is imperative therefore to control microbial contamination and extend the shelf life of food. Incidence of fungal contamination of food is high in Nigeria because of the tropical climate that favours fungal growth (Adebayo *et al.*, 2014). Aside from physical/chemical methods, there is the need to also try biological means of food preservation to combat this because it is more safe and eco-friendly.

Fufu, a staple food in Nigeria is usually prepared from submerged fermentation of cassava. Fufu was used in this study because it is a natural habitat for many bacterial flora, especially acidophiles like lactic acid bacteria (LAB) due to its low pH (4.5-5.6). *Lactobacillus plantarum*, a gram-positive, non-catalase, non-spore producing rod, usually predominate during cassava fermentation because it is the most aciduric of all LAB and most strains produce bacteriocin.

Bacteriocins are antimicrobial peptides or proteins that are generally active against closely related species. Recent studies have established that some are active against some other groups of microorganisms (Magnusson and Schnurer, 2001). The majority of bacteriocins produced by *Lactobacillus* species belong to class II group; generally they are small (<10KDa), moderately to highly (80^{o}C to 120^{o}C) heat-stable and membrane active with amphiphilic characteristics (Tahara and Kanatani, 1996). A common mechanism of action of LAB bacteriocins is the alteration of cytoplasmic membrane permeability (Taokami *et al.*, 2009).

This study reports on the efficacy of an antimicrobial product, plantaricin, from *Lactobacillus plantarum* isolated from fufu on the mycelia of *Aspergillus niger*.

2. MATERIALS AND METHODS

A. *Microorganisms and Growth Media*

The bacteriocin, plantaricin FF1, was obtained from *L. plantarum* FF1 isolated from 'fufu' in our previous work (Adebayo and Aderiye, 2010). Partial characterization of Plantaricin FF1 indicates that its action was sensitive to treatment with proteolytic enzymes but resistant to organic solvents and surfactants. It has a molecular mass of 6.8 kDa and possesses a plasmid. The fungal strain was *Aspergillus niger* obtained from stored fermented foods. The *L. plantarum* FF1 was cultured in de Mann, Rogosa and Sharpe (MRS) broth at 30^{o}C and the test fungus in Potato Dextrose Broth (PDB) at ambient temperature. All theexperiments in this study were performed in triplicate using dual cultures.

B. *Stability of Plantaricin*

The pH stability of the bacteriocin: This was determined by assaying over the pH range 1-10 using either 1mol 1^{-1}NaOH or HCl. After incubation for 1h, it was readjusted to pH 7 and then assayed for the residual activity.

Determination of Storage Temperature: This was done by storing PlantaricinFF1 at various temperatures (0^{0}C, 4^{0}C and 25^{0}C) and assaying the activity by the agar well diffusion.

Heat stability: This was assayed by subjecting plantaricin FF1 to various temperatures before testing for residual activity.

C. *Effect on the Mycelia of Aspergillus niger*

The Scanning electron microscopy of samples was used to determine the inhibitory effect and the mode of action of Plantaricin FF1 on mycelia of *A. niger.*

D. *Effect of PlantaricinFF1 on food sample*

This was by exposing eba treated with bacteriocin to air for 2h and later incubated for 10 days.

3. RESULTS

The result in Fig. 1 showed that the stability of PlantaricinFF1 is high as it was effective over a wide pH range (1-10). The action was optimum at pH values 5.0 to 7.0 but became reduced at extremes of pH (1.0, 9.0 and 10.0). The bacteriocin also maintained full antifungal activity for at least 30days at all temperatures. This now decreased gradually at ambient temperature (25^{0}C) till the 60th day, while the activity still remain high at both the 0^{0}C and 4^{0}C even after 120days (Fig. 2). Plantaricin FF1 retained full activity when exposed to heat even at autoclaving temperature for 5mins (Fig. 3).

The SEM revealed that there was a critical hyphal damage of *A. niger* cell, as evidenced by cytoplasmic extrusion, lysis and vacuolation when co-cultured with plantaricin FF1 (Fig. 4). While the control cell has a normal protoplasm, the hypha of the treated became shortened, dried and empty to the extent that it did not absorb the lactophenol stain (Fig. 5).

Treatment of eba samples with Plantaricin FF1 before exposure to air and incubation for 10days resulted in total protection from fungal colonization. Whereas the untreated eba sample (control) was completely colonized by fungal growth after 10days incubation (Fig. 6). Indicating that the bacteriocin was not only active *in-vitro,* but also potent *in-vivo.* Retained activity means the residual biological activity of the treated in comparison with the control.

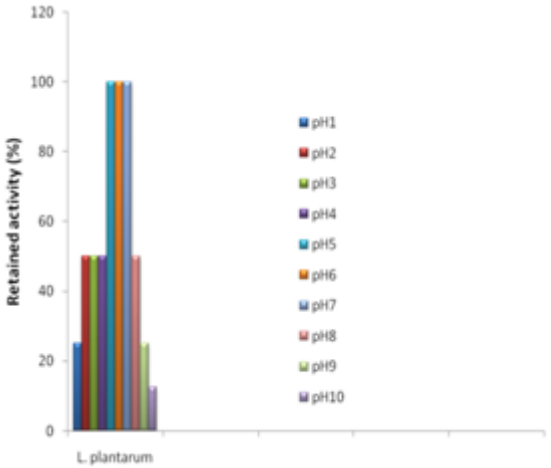

Figure 1 Stability of bacteriocin at different pH values

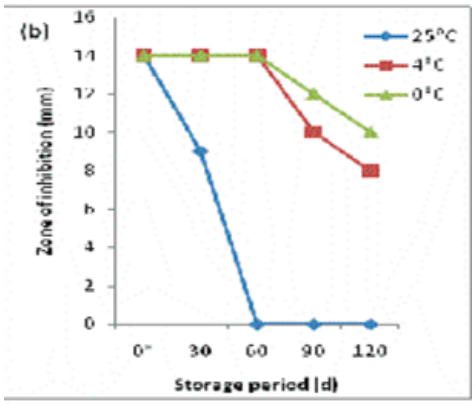

Figure 2 Effects of storage temperature on the activity of Plantaricin FFI

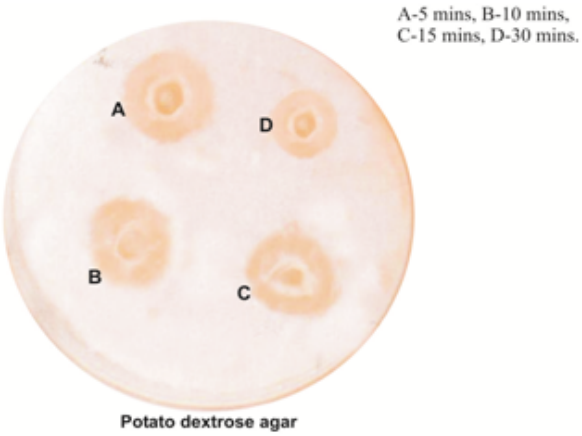

Figure 3 Effect of heating at 121^{0}C on the antifungal activity of Plantaricin FFI

(a): Untreated (b): Treated

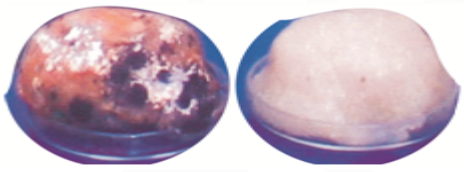

Figure 4 Inhibition of fungal spoilage in 'eba' treated with PlantaricinFF1 and exposed for 2 hours after storage for 10 days

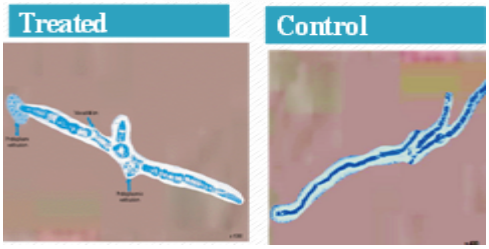

Figure 5 SEM of the effect of Plantaricin FF1 on the mycelia of *A. niger*

(a) Control (b): Treated

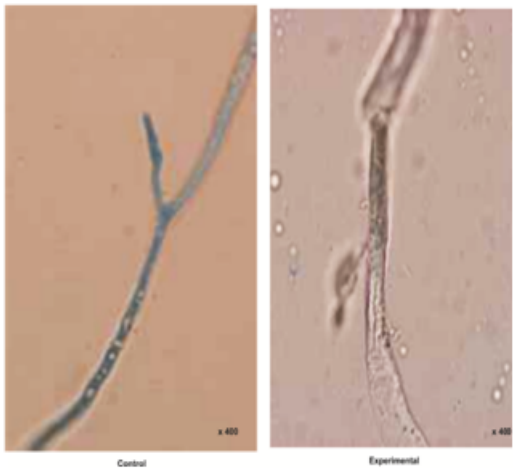

Figure 6 SEM of the effect of Plantaricin FF1 on A. niger; Showing: (a) hypha with normal protoplasm, (b): an empty, disintegrated hyphal cell (strand)

4. DISCUSSION

The activity of plantaricin FF1 at an acidic and neutral pH range is not surprising as bacteriocin produced by LAB are usually highly stable at acidic or neutral conditions but some of them, are easily inactivated under alkaline conditions (Soomro *et al.*, 2002). This result was similar to that of other bacteriocins of LAB (Tahara *et al.*, 1996; Magnusson and Schnurer, 2001). However it is a novel bacteriocin as it has a different molecular mass to previous ones.

Stability at alkaline pH confirmed that the biocidic activity by the producer organism was not due to the influence of organic acid. Activity of the bacteriocin at a broad pH indicated that it is well adapted to the environment of producer organism. It also indicated that plantaricin FF1 has potential value in a wide range of food. Activity at a long pH range, high heat and ambient temperature portends the future applicability of plantaricinFF1 for food preservation. The properties of resistance to surfactants and solvents/thermostability would enhance its application as a food preservative as most foods are processed at high heat. The heat stability revealed that it is a class II bacteriocin.

Storage at room temperature also confers a high advantage on the bacteriocin. The inhibitory action of the bacteriocin was not only *in-vivo* but also *in -vitro*. This may offer an essential promise for it application in the processing and conservation of various foods. *L. plantarum* species have been widely used as starters in many fermentation processes and is regarded as safe strains used in the food industry (Soomro *et al.*, 2002).

The vacuolation and hyphal destructive action of plantaricin FF1 on *A. niger cell* was probably due to its effect on cytochrome P-450 or direct interaction with membrane lipids leading to membrane damage (Benyagoul *et al*, 1996). The former causes an inhibition of ergosterol biosynthesis that result in accumulation of 14 -& - methyl sterols in plasma membran. Sterols are known for their ability to buffer stress-induced elevations in membrane fluidity and low sterol content will thus increase fungal sensitivity to antifungal compounds. Avris and Belanger (2002) reported that a low proportion of sterols was responsible for the higher sensitivity of *Phytophthorainfestans,*

Pythium aphanidermatum and *Pseudozyma flocculosa* to antifungal fatty acids.

The mycelia disintegration by the bacteriocins could also be attributed to cytoplasmic disintegration and electrolyte leakage due to an increased elevation of membrane fluidity. Previous study had propounded that antifungal compounds do naturally insert themselves into the membrane bilayer of fungal membrane and cause a physical (or mechanical) disruption that induces elevated fluidity. Thus causing cytotoxic manifestations such as electrolyte leakage and cytoplasm disintegration. The inability of *A. niger* cells to cope with the excessive electron in membrane fluidity probably caused a generalized disorganization of the membrane that leads to the leakage of essential intracellular components, vacuolation, cytoplasmic disorder and eventually cell disintegration as observed in this study.

The result of this study is in agreement with the reports of several authors that some LAB have pore-forming abilities. Tahara *et al.* (1996) reported that the inhibitory action of acidocin 912 against sensitive *Lact. casei* is caused by the dissipation of the proton-motive force and by pore formation in the cytoplasm. While Savadogo *et al* (2006) revealed that pore formation by plantaricin SA6 on cells of sensitive indicators were reported to cause a leakage of cellular materials and efflux of potassium ions leading to cell lysis. Loss of ions and consequent lysis of treated sensitive cells by bacteriocins of LAB had been reported by Abee, *et al. (*1994). Yurong *et al* also attributed the pore formation and the cell disintegration of *E.* coli when treated with sakacinC2 to depolarization of trans-membrane electrical potential, thus leading to efflux and cell membrane fragmentation.

In summary, this study established that plantaricinFF1 caused a cytotoxic destruction of the mycelia of *A. niger.*

5. CONCLUSION

This study revealed that treatment of the cell of *A. niger* with plantaricin FF1 destroyed the mycelia, caused a lytic effect, extrusion and vacuolation of the protoplasmic content of the fungus. The high activity may be due to its possessing plasmids which increase the potency of the bacteriocin. Inhibition of *A. niger* by the bacteriocin is significant as this fungus is a prevalent cause of food spoilage. The bacteriocin was active both *in-vitro* and *in-vivo* potentating its use for food preservation. Due to the high antimycotic action, storage at ambient temperature, heat/ pH stability, plantaricin FF1 can effectively be used as bio preservative in a wide number/range of foods. The usage of the bactericin as food additive or the usage of *L.plantarum* as starter culture portends no danger as the organism is a natural flora of fermented foods. These studies might lay the preliminary interest for a future application of plantaricinFF1 and its producer, as starter culture and bio-preservative in the food industry. To our knowledge this is the first report on the mechanism of antimycotic action of bacteriocin produced by *L. plantarum* from fufu.

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