EXTRACTION OF ANTIBACTERIAL SUBSTANCES FROM *Artrhospiraplatensis*BIOMASS AGAINST ANTIBIOTIC-RESISTANT *Staphylococcus* sp. ISOLATED FROM BOVINE MASTITIS

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ABSTRACT – Mastitis is an inflammation of the udder which may be caused by bacteria and cause economic losses by produce harmful substances that result in inflammation, reduced milk production, and altered milk quality. *Arthrospiraplatensis* extract in two different solvents, water (W) and sodium acetate buffer (SAB), was tested for its antibacterial activity against 14 differents antibiotic-resistant *Staphylococcus* sp. isolated from bovine mastitis. Both *Arthrospiraplatensis* extract exhibited antimicrobial activity against all *Staphylococcus* sp., but SAB was most effective than W. The minimum inhibitory concentration of the *A. platensis* extracts at 100mg/ml in SAB was obtained in 29% of isolated and in W was 21%. More than 50% of inhibition was observed at 6 mg/ml in SAB and 12 mg/ml in W. Results suggest that *Arthorspiraplatensis* extracts in SAB has the potential to be evaluated as an alternative or adjunct to antibiotics as intramammary infusion to treat bovine mastitis.

1. INTRODUCTION

Bovine mastitis is a highly prevalent disease in dairy cattle, and one of the most important diseases affecting the world's dairy industry; it places a heavy economic burden on milk producers all over the world (Bennett et al., 1999). Worldwide, annual losses due to mastitis have been estimated to be approximately 35 billion US dollar. In the US, the annual costs of mastitis treatment have been estimated to be 1.5–2.0 billion US dollar (Wells et al., 1998).

Mastitis is defined as an inflammatory reaction of the parenchyma of the mammary gland that can be of an infectious, traumatic or toxic nature. Mastitis is characterized by physical, chemical and microbiology changes in the milk and by pathological changes in the glandular udder tissue. The diagnosis of mastitis is based on clinical signs, e.g. swelling of the udder, tender to the touch, fever, and depression. In many cases, a reduced milk production can be observed. Mastitis-causing pathogens include mycoplasms, fungi, yeasts, and bacteria (Radostits et al., 1994). These pathogens infect the udder generally through the ductuspapillaris, which is the only opening of the udder to the outside world.

Staphylococcus sp. is the predominant pathogen isolated from cases of mastitis and it causes one of the most common types of chronic mastitis.Antimicrobial are commonly used to trat clinical mastitis and occasionally cases of subclinical mastitites (Mc Dougall et al., 2014). Mc Dougall et al. (2014) relatated that resistance to some beta-lactam antimicrobials and trimethoprim/sulfamethoxazole were found in *Staphylococcus sp.*isolates from cases of bovine mastitis.

Staphylococcal species isolated of goat milk were *S. epidermidis, S. aureus, S. caprae, S. lentus, S. simulans, S. capitis, S. lugdunensis, S. xylosus, S. chromogenes, S. hominis, S. arlettae, S. warneri, S. sciuri, and S. saprophyticus*. Highest somatic cell count (SCC) in milk and the highest prevalence of clinical udder alterations were associated with coagulase-positive *S. aureus (*Deinhofer and Pernthaner, 1995)

Natural substances offer interesting pharmacological perspectives for antibacterial drug development with regard to broad spectrum antiviral properties and novel modes of action. Although extremely effective, antibiotics are able to induce resistance in bacteria. For 450 years, bacterial resistance has been the main factor responsible for the increase of morbidity, mortality and health care costs of bacterial infections (Abd El-Baky, *et al*, 2006)

Cyanobacteriumis rich sources of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interests in the pharmaceutical industry.

A nontoxic cyanobacterium*Arthrospira* strain can be new source of natural antimicrobial (Shafar et al., 2013). Several studies have focused on physiological properties of some valuable antimicrobial compounds in these cyanobacteria. The search for cyanobacteria with antimicrobial activity hasgained importance in recent years due to growing worldwide concern about alarming increase in the rate of infection by antibiotic-resistant microorganisms.

The present work aimed to study the antimicrobial activity from*A. platensis*extract disrupted with water (W) or sodium acetate buffer (SAB) against *Staphylococcus* sp. conventional antibiotic-resistantisolates of bovine mastitis.

2. MATERIALSAND METHODS

2.1Microorganisms

Arthrospiraplatensis (UTEX 1926) was obtained from the culture collection of the University of Texas (UTEX 2011). Prof.RinaldoAparecido (UFRPE) provided 14 different *Staphylococcus* sp.isolated from bovine mastitis.

2.2. Growth and Culture Conditions

Arthrospiraplatensis was cultivated inSchlössermedium (1982) whose composition per liter of distilled water is: $NaHCO_3$ 13.61 g; Na_2CO_3, 4.03 g; K_2HPO_4, 0.50 g; 2.50 g $NaNO_3$; K_2SO_4, 1.00 g; NaCl, 1.00 g; $MgSO_4.7H_2O$, 0.20 g; $CaCl_2.2H_2O$, 0.04 g; * PIV metal solution, 6 mL; Chu ** micronutrients solution, 1mL; vitamin B12 (15 g/100mL H_2O), 1 ml; * PIV metal solution (in 1 liter of distilled water): Na_2EDTA, 750 mg; $FeCl_3.6H_2O$, 97 mg;

$MnCl_2.4H_2O$, 41mg; $ZnCl_2$, 5 mg; $CoCl_2.6H_2O$, 2 mg; $Na_2MoO_4.2H_2O$, 4 mg; Chu ** micronutrients solution (in 1 liter of distilled water): Na_2EDTA, 50 mg; H_3BO_3, 618 mg; $CuSO_4.5H_2O$, 19.6 mg; $ZnSO_4.7H_2O$, 44 mg; $CoCl_2.6H_2O$, 20 mg.

Cells for the cultivation were grown in 250mL Erlenmeyer flasks containing 100mL of medium, kept on rotary shaker at $100min^{-1}$(Ferraz 1986), temperature of 27◦C, light intensity of 14 μmolsphotonsm^{-2} s^{-1} and initial cell concentration of 50 mg L^{-1}. At the end of cultivation, the cells were centrifuged,lyophilized and stored at -18 º C for further analysis.

2.3. Cell Disruption

Cell lyophilized was suspending in distilled water (W) or sodium acetate buffer (SAB) at concentration of 100mg/mland subsequently sonicatedfor 1 min pulse during10 minutes in an ice bath. After sonication, the sample is centrifuged at 2500xg at 4 ° C for 5 min. The supernatant was utilized for assessment of biological activities.

2.4. Determination of Minimum Inhibitory Concentration using plate microdilution

The minimal inhibitory concentration (MIC) was determined by the microdilution method described by NCCLS (2003) using 96-well standard microtiter plates. A series of dilutions with concentrations ranging from 100 to 3 mg/ml for extract was used in the experiment against every microorganism tested. Briefly, 50 μL of twofold serial dilutions of examined samples was added to 50 μL microbial suspension adjusted to yield approximately 1.0×10^5 CFU mL^{-1}. MIC was encountered as the lowest concentration of examined sample that inhibits the microbial growth after 24h incubation at 37 °C. Negative controls were included too. Bacterial growth is determined by measuring the absorbance at 630 nm.

3. RESULTS AND DISCUSSION

The water (W) or sodium acetate buffer (SAB) extracts were tested for antibacterial activity against 14 different antibiotic-resistant *Staphylococcus* sp. isolated from bovine mastitis. The degree of activity was varied according to concentration and type of *A.platensis* extracts. The SAB extract showed the antibacterial activity with MIC at 100mg/ml against four different pathogens, corresponding to 29% of all *Staphylococcus* sp. analyzed.With decrease of cell extracts concentration to 50 mg/ml, the inhibition degree decrease and was not observed MIC in concentration among 50 – 3 mg/ml. However, cell extracted at concentration of 12 mg/ml obtained more than 50% of inhibition in 14% of *Staphylococcus* sp and only one bacteria obtained inhibition higher than 50%. At 3 mg/ml of cell concentration, all *Staphylococcus* sp. was resistant (Table 1).

In relation to cell extracts with water (W), it was observed that there was an inhibition of 100% of the antibiotic-resistant *Staphylococcus* sp. isolated from bovine mastitisat a concentration of 100mg/ml, but only in 7% of the pathogens was observed MIC and 86%, IC50. At 50mg/ml, 50% of the *Staphylococcus* sp. obtained inhibition more than 50% (IC50), at 25mg/ml, only 3% of the *Staphylococcus* sp. obtained IC50 and in the others concentration (12, 6 and 3 mg/ml), the IC50 was less that 2% In the same way as observed with water extract, at 3 mg/ml, all *Staphylococcus* sp. was resistant (Table 1).

Table 1 – Percentage of inhibited *Staphylococcus* sp.isolates of bovine mastitis in different *A. platensis* extracts

	Cell concentration					
	100mg/mL	50mg/mL	25mg/mL	12mg/mL	6mg/mL	3mg/ml
Sodium acetate buffer extracts (SAB)	29%[1]	43%[2]	36%[2]	14%[2]	7%[2]	*
Water extracts (W)	7%[1]	36%[2]	14%[2]	7%[2]	*	*

[1] MIC = minimal inhibitory concentration considering 100% of inhibition; [2] IC_{50} = inhibition with value above to 50%.

Water extracts of cyanobacteria*Gloeocapsa* sp, *Synechocystis* sp. and *Nostoc* sp. was active against *S. aureus*, *S. pyogenes*, *B. cereus*, *E. coli*, *P. aeruginosa* and *C. albicans* with MIC values varying from 1.56 to 6.25 mg mL^{-1} (Najdenski et al., 2013) This value is lower than our because the MIC was done with dry residues after centrifugation and freeze-dried, while our results was obtained after only centrifugation.

Shafar et al. (2013) related that cold water extract, hot water extract and phosphate buffer extract of *Arthrospira* strain inhibited herpes simplex infectivity by 54.9%, 64.6%, and 99.8%, respectively, in a dose-dependent manner, obtained the higher inhibition using phosphate buffer, as observed in our work.

Many fresh water cyanobacteria have been recognized as potential source of antibacterial substances. It is evident that microorganisms, living in an environment where competition and predation are the maximum without physical-defence structure, defend themselves by production chemical to survive. There, the exploration of the cyanobacterium represents a promising strategy in the search for active compounds, while there is a need for new medicines, due to the appearance of resistance to available treatments in many microorganisms, specifically for antifungal, antiprotozoal, antibacterial and antiviral activities (Yasuhara-Bell et al., 2010).

Kaushi and Chauhan (2008) related that *A. platensis* extract concentrated using organic solvents such as hexane, ethyl acetate, dichloromethane exhibited different degree of antibacterial activity against one Gram-positive bacterium (*Staphylococcusaureus*) and four Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Klebsiellapneumoniae*). The results showed that the MICs of methanolic and dichloro-methane extract were 128 µg/ml and 512 µg/ml against *S. aureus*. This value is above to obtainedin our work because we not used *A. platensis* extract concentrated.

The results concerning activities of the crude extracts are not surprising. Similarly, aqueous cyanobactria extracts of *Arthrospira maxima*, *Nostoc*, *Synechocystis*, *Gloeothece* sp, were found antagonistic against different pathogenic microorganisms (excepting *Salmonella* sp.) in a large studies (Guedes *et al*., 2011; Medina-Jaritz *et al*., 2011). Phycobiliproteins (PBPs) (phycocyanin, allophycocyanin and phycoerythrin) are a family of coloured photosynthetic accessory pigments of cyanobacteria

and red algae. C-PC from *Spirulinaplatensis* was identified as a potent agent against *E. coli, Klebsiellapneumoniae, P. aeruginosa* and *S. aureus* (Saradaet *al*., 2011). Besides, the PBPs of the two red microalgae (*P. aerugineum* and *P. cruentum*) were active against *S. aureus* and*S. pyogenes*. The growth inhibition activity towards antibiotic-resistant *Staphylococcus sp.* from *A. platensis*extractswere an interesting finding, due to a lack of reports on such activity in the literature.

Numerous green alga, such as*Desmococcusolivaceous, Chlorococcumhumicola, Chlorella vulgaris, Ulvafasciata, Enteromorphaintestinalis, Chaetomorphaaerea* were screened for antimicrobial activity in search of the new antimicrobial agents (Seenivasan et al., 2010; Uma et al., 2011), but in this study, for the first time, the antimicrobial activity of the sodium acetate buffer extract of *A. platensis* was investigated. In correlation with results obtained in experiments with other algae, we noticed that the SAB of *A. platensis* showed relatively antimicrobial activity

The present study results revealed that the SAB extracts of *A. platensis* showed the antibacterial activity against antibiotic-resistant *Staphylococcus* sp. isolated from bovine mastitis. It suggests that in addition to the available drugs, alternatively we can use the extracts of *A. platensis* against bovine mastitis. The results of the present study suggest that the SAB extracts of *A. platensis*may be used to bovine mastitis.

4. CONCLUSION

In conclusion, the performed comparative study allows us to determine that sodium acetate buffer extracts of *A. platensis* is the most promising which show a relatively high level of antimicrobial potency and wider range of substances, active against the most antibiotic-resistant *Staphylococcus* sp. isolated from bovine mastitis**.**

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