MODESTUM
OPEN ACCESS

Eurasian Journal of Analytical Chemistry, 2018, 13(5), em53
ISSN:1306-3057
Research Paper https://doi.org/10.29333/ejac/95169

Microbicide Action of IAA and IAA/HRP on Staphylococcus Aureus

Andreia C. N. Vaz [1], Silvana M. P. Pugine [1], Mariza Pires De Melo [1*]

[1] University of São Paulo, Faculty of Animal Science and Food Engineering, Department of Basic Sciences, Pirassununga, SP, BRAZIL

Received 19 April 2018 ▪ Revised 2 August 2018 ▪ Accepted 8 August 2018

ABSTRACT

Action of indole-3-acetic acid (IAA) in absence and presence of horseradish peroxidase (HRP) was evaluated on the virulence factors produced by *Staphylococcus aureus*: biofilm producer; enterotoxins producer (*seb*-gene, SEB); methicillin resistance (mecA) and control-stain by: i) dose-response on microorganism growth and minimum inhibitory concentration (MIC) by spectrophotometry at 625nm; ii) membrane damage by flow cytometry using fluorescence detector at 610nm and 530nm; iii) biofilm production by spectrophotometry at 570 nm; iii) antioxidant enzyme activity as catalase and superoxide dismutase by spectrophotometry at respective 240nm and 550nm. Dose-response on microorganism growth was seen and MIC by IAA alone was 40mM for biofilm, 50mM for SEB and control-stain, and 60mM for *mec*A; HRP potentiated the IAA action on biofilm, SEB and *mec*A. Subinhibitory IAA concentration increased membrane damage for only biofilm and control-strain; however in presence of HRP the injury was potentiate in all stains. Except for biofilm, all strains showed similar increase in catalase activity by IAA and IAA/HRP. Superoxide dismutase activity was similarly increased in presence of IAA and IAA/HRP for all stains. IAA alone or combined with HRP presents microbicide action on *S. aureus* with different pathological characteristics; probably involving reactive oxygen species since increased activity of antioxidant enzymes.

Keywords: auxin, enterotoxins, food poisoning, methicillin resistance, virulence factors

INTRODUCTION

Staphylococcus aureus is a pathogenic microorganism capable of causing a range of life-threatening and mild diseases, including septicaemia, meningitis, toxic shock syndrome, food poisoning and skin abscess [1]. The virulence factors produced by *S. aureus* include biofilm production, enterotoxin production and resistance to antibiotics such as methicillin. The production of biofilm may be responsible for the persistence of bacterial infections, by increasing adherence of the microorganism and providing protection against antimicrobial substances [2]. *S. aureus* produces a variety of extracellular protein toxins including enterotoxins related to food poisoning [3]. Various types of staphylococcal enterotoxin (SE) are produced by *S. aureus*; types A (SEA), B (SEB), C (SEC) and D (SED) are commonly implicated in staphylococcal food poisoning [3]. The protein SEB, a potent toxin which has also gained the status of a biological warfare agent, is expressed by the *seb* gene of *S. aureus* [3]. Virulence-related resistance to methicillin in *Staphylococcus* spp. is commonly conferred by the *mec*A gene that encodes a cell wall protein with low affinity for this class of antimicrobial [4, 5]. Studies have identified new compounds for use in possible alternative therapeutic strategies for pathogen combat.

Indole-3-acetic acid (IAA), an auxin, and other indole compounds act in vitro against various pathological microorganisms [6,7]. Combination of this auxin with horseradish peroxidase (HRP), a plant hemeprotein, showed a microbicidal effect on *S. aureus* and *Prototheca zopfii* obtained from subclinical isolates [8,9] and on *P. zopfii* ATCC 1653 [10]. Specifically, IAA/HRP causes a drastic reduction in colony formation and in cell viability.

Diverse mechanisms have been reported for cytotoxicity from the oxidation of IAA by HRP enzyme, including the involvement of indolyl, skatolyl and peroxyl radicals and reactive oxygen species (ROS) such as superoxide

✉ acvaz@usp.br ✉ spiccoli@usp.br ✉ mpmelo@usp.br **(*Correspondence)**

anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) [11,12]. Reactive species act on microbial targets causing multiple concurrent disruptive events, thereby preventing development of resistance, and allowing inactivation of a wide spectrum of microorganisms, independent of their profiles of resistance to classical antimicrobial agents [8, 12].

The aim of this study was to evaluate the action of IAA and IAA/HRP on *S. aureus* strains with different characteristics conferred by biofilm production, enterotoxins production by *seb*-gene presence (food poisoning) and methicillin resistance by *mec*A gene presence, and a strain negative for these virulence characteristics.

MATERIALS AND METHODS

Materials and Reagents

All reagents and enzymes were of analytical grade and were obtained from Sigma Chemical Company (St. Louis, MO, USA). Culture medium was obtained from Oxoid (Basingstoke, Hampshire, England).

IAA and HRP Preparation

A stock solution of IAA (100 mM; 17.5 mg/mL) was prepared in tryptic soy broth (TSB) and NaOH (5 M) was added for complete solubility of this acid, and thereafter the pH value was adjusted to 7.4 by using HCl (25%, v/v) [13]. HRP solution (1 μM) was prepared in Phosphate Buffer Saline (PBS) and the pH value was adjusted to 7.4, from a stock solution of HRP (1000 μM) [10].

Microorganism Preparation for Assays

Staphylococcus aureus ATCC 6538 (biofilm producer) [14, 15], ATCC 14458 (*seb* gene presence = SEB) [16–20], ATCC 43300 (*mec*A gene presence = mecA) [21–24] and ATCC 29213 (absence characteristic to biofilm production, *seb* gene and *mec*A gene = control stain) [22, 24, 25] were used in present study. Microorganism were inoculated into brain heart infusion (BHI) broth and cultivated aerobically under continual rotation overnight at 37°C. They were then harvested and washed once in sterile phosphate-buffered saline (PBS). *S. aureus* were suspended in PBS at a concentration of 3.0×10^8 colony forming units per milliliters (CFU/mL) (optical density at 625 nm of 0.2).

Microorganism (3.0×10^3 CFU/mL) had been cultured in Tryptic soy broth (TSB) for 18 hour, aerobically with constant agitation at 37°C, in absence (microorganism alone or control assay) and presence of IAA, HRP or IAA/HRP. The HRP concentration was 1 μM for all assays [26] and IAA concentration was described below. After incubation time, microorganism washed three times in PBS and was used to dose-response determination and MIC determinations, membrane damage determination, biofilm production and antioxidant enzymes activities.

Dose-response of IAA on *S. Aureus* Growth

Microorganism growth was available by optic density (OD) value at 625 nm and by a qualitative technique using resazurin assay [27] with some modifications. To both methods the microorganism (3.0×10^3 CFU/mL) was placed in polystyrene plate using TSB and 0.5, 4, 10, 20, 30, 40, 50, 60 and 70 mM of IAA in absence or presence of HRP 1 μM. Negative control incubations without the microorganism were performed. For OD assay, after incubation of microorganism at 37°C under agitation for 18 hours, microplates OD was determinate using a microplate reader at 625 nm (Beckman Coulter DU-800 (UV/Vis). The results were expressed as OD values *versus* IAA concentration. Inhibition percentage of microbial growth was determinate to each IAA concentration in relation with microorganism in absence of IAA. For resazurin assay, a resazurin solution (6.76 mg/mL) was placed in each well and the plate incubated at 37°C under agitation for 18 hours. The change of purple (oxidized state of resazurin) to pink color (reduced state of resorufin) is recorded as positive (growth of bacteria).

Biofilm Formation

The biofilm production was assessed by the OD value from adhering bacteria and stained with violet crystal evaluated at 570 nm using a microplate reader (Multiskan FC -Thermo Scientific) [28]. The *S. aureus* biofilm-producer (ATCC 6538) and control stain (ATCC 29213) were used. The microorganism (3.0×10^3 CFU/mL) was placed in polystyrene plate using TSB supplemented with 0.25% glucose in presence of 30 mM (subinhitory) and 40 mM (MIC) of IAA in absence or presence of HRP 1 μM. Negative control incubations without the microorganism were performed. Bacterial suspension was incubated for 18 hours at 37°C. So, each well was aspirated and was carefully washed three times with PBS and dried at 60 minutes in inverted position. Next, the wells were stained with 0.5% violet crystal solution for one minute. For qualitative assay the plate was photographed and analyzed. The intensity of blue color in polystyrene plate wells indicated bacterial adhesion and has been grouped into three

categories according to the color: no adherent (OD ≤ 0.120), weakly bonded (OD 0.120 to 0.240) and strongly adherent (OD ≥ 0.240).

Membrane Damage

For membrane integrity a sub inhibitory IAA concentration was used. The IAA concentration was 30 mM to stain biofilm and SEB and 40 mM to mecA and control stains. Cell membrane integrity was analyzed by FACSAria flow cytometry equipment (BD Biosciences, USA). This instrument is equipped with an argon ion laser for excitation of the fluorescent dyes, providing 15 mW at 488 nm. Filter set up was standard. In this assay, the suspension of *S. aureus* (3.0×10^4 CFU/mL) was incubated with propidium iodide (PI) (43 μM) and thiazole orange (TO) (420 nM) for 5 minutes at room temperature according to the BD Cell Viability Kit. PI red fluorescence and TO green fluorescence emitted were collected using respective band-pass filters at 610 nm and 530 nm. The data were analyzed using the BD FACSDiva Software (BD Biosciences, USA). To determine differential staining of live (green fluorescence) and dead microorganism (red fluorescence)) in the presence of TO and PI, similar assays were carried out also through a mixture of live microorganism and microorganism that was heat killed by exposure to 70°C for 10 min [29].

Antioxidant Enzyme Activities

Antioxidant enzyme activities were evaluated in *S. aureus* incubated with a sub inhibitory IAA concentration, where 30 mM IAA for biofilm and SEB stains and 40 mM IAA for mecA and negative control stains were used. For enzymes extraction, the microorganism was incubated with lysostafin (20 IU/mL) for 30 minutes at 37°C with constant agitation and was centrifuged at 13,000 x *g* for 15 minutes. Next, the supernatant was kept in the ice to quantify the protein content and enzyme activity using a spectrophotometer (DU-800, Beckman). The catalase activity was measured at 240 nm, following H_2O_2 reduction [30]. The SOD activity was determined based on its inhibitory effect on the rate of superoxide-dependent reduction of nitro blue tetrazolium by xanthine–xanthine oxidase at 550 nm [31]. The protein content was determined by the described method for [32], using bovine serum albumin for construction of the curve.

Expression of Results and Statistical Analysis

Data are expressed as means ± standard deviation. Comparisons between groups were initially performed by analysis of variance (ANOVA). The alpha level (significance level related to the probability of rejecting a true hypothesis) was set at 0.05. Significant differences were then compared using Tukey's test with a significance coefficient of 0.05.

RESULTS

Dose Response of IAA on *S. Aureus* Growth

Inhibition of bacterial growth related to IAA concentration in the absence and presence of HRP was observed (Figure 1). All *S. aureus* strains cultured in the presence of IAA (Figure 1A) or IAA/HRP (Figure 1B) showed normal growth below 10 mM in both cases, evaluated by optical density. However, concentrations up to 20 mM of IAA and IAA/HRP inhibited microorganism growth for all strains except for mecA which was sensitive even at low IAA concentrations.

The growth inhibition percentage of *S. aureus* cultivated in the presence of IAA and IAA/HRP was determined; the values are shown in **Table 1**. Inhibition of microbial growth by IAA in the absence or presence of HRP was evidenced firstly for mecA (10 mM), followed by both biofilm and SEB (20 mM) and finally the control strain (30 mM). An MIC condition, when IAA concentration inhibited 90% of bacterial growth, was determined. Alone, IAA showed an MIC of 40 mM for the biofilm strain, 50 mM for both SEB and control strains and 60 mM for the mecA strain. HRP enzyme potentiated the inhibitory effect of IAA on microorganism growth; IAA MIC of 30 mM for biofilm, 40 mM for SEB and 50 mM for mecA strain were seen. However, for the control strain, MIC was 50 mM IAA for IAA alone or in the presence of HRP.

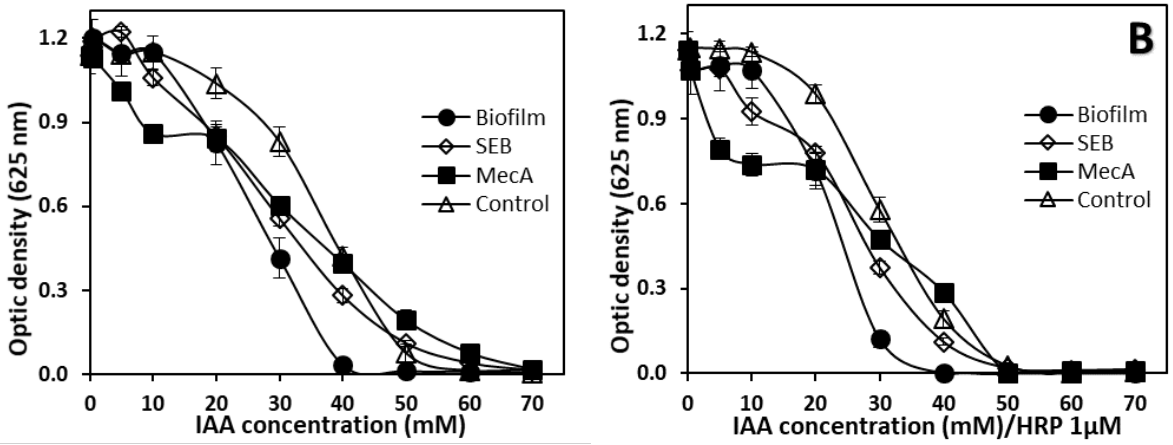

Figure 1. Dose response evaluated by IAA in absence and presence HRP on growth inhibition of *S. aureus* biofilm producer (biofilm), *seb*-gene presence (SEB), *mec*A gene presence (mecA) and control-stain (control = no biofilm produce and absence of *seb*-gene and *mec*A gene). Results as mean and standard error (n = 6)

The results show that the *S. aureus* biofilm-producing strain was most sensitive to exposure to IAA or IAA/HRP, showing 100% inhibition of microorganism growth in the presence of IAA at lower concentrations than for the other strains. The mecA strain was more resistant to IAA exposure than the others, 60 mM IAA being necessary to achieve 94% inhibition of microbial growth, although it presented sensitivity, showing a reduction in microbial growth from 10 mM IAA.

In MIC conditions, the number of viable bacteria was reduced, so for subsequent assays IAA concentrations below the MIC (sub inhibitory concentrations) were used. Thus, in the present study, the sub inhibitory IAA concentration was 30 mM for biofilm and SEB strains, and 40 mM for mecA and control strains; in these conditions, around 50% to 60% bacterial growth inhibition was found.

Table 1. Relative percentage of inhibition growth of *S. aureus* biofilm producer (biofilm), *seb* gene presence (SEB), *mec*A gene presence (mecA) and control stain (control = no biofilm produce and absence of *seb* gene and mecA gene) evaluated in different IAA concentrations in absence (-) and presence (+) of HRP 1µM

IAA (mM)	HRP	Inhibition growth (relative percentage)			
		biofilm	SEB	mecA	control
0.5	-	5.50	3.70	1.30	5.20
	+	5.90	7.20	6.90	0.330
4.0	-	0.70	6.50	11.7	0.110
	+	4.60	6.80	31.4	0.0500
10	-	0.90	7.70	25.3	1.00
	+	6.10	20.1	36.1	0.970
20	-	27.3	26.1	26.7	9.00
	+	37.3	32.7	37.7	14.0
30	-	63.7	51.9	47.6	27.3
	+	**89.4**	68.3	59.3	49.9
40	-	**97.2**	76.2	65.9	63.2
	+	100	**91.3**	75.6	83.5
50	-	99.1	**91.0**	83.8	**93.6**
	+	100	99.5	**99.8**	**98.1**
60	-	99.2	97.2	**94.0**	99.2
	+	100	100	100	100
70	-	98.6	99.5	99.2	99.8
	+	100	99.8	100	100

Inhibition percentage was calculated, in relation to microorganism growth alone, by equation: 100-(ODA/DOB)*100, when ODA = DO in presence of IAA or M/HRP and ODB = OD in absence of IAA or IAA/HRP. Bold values indicate inhibitory concentration of IAA (no highlighted) and IAA/HRP (highlighted). (n=8)

The resazurin assay showed a purple colour in the culture medium for microorganisms grown in the presence of IAA and IAA/HRP which inhibited microbial growth; a similar colour was seen in the presence of gentamicin (9.4 µg/mL). Culture medium for microorganisms cultured in the absence of IAA showed a pink colour, indicating

good microorganism growth (results not shown). Data from the resazurin assay corroborated the results obtained for optical density (OD) at 620 nm.

IAA Action on *S. Aureus* Biofilm-producing Strain

Bacterial growth of the *S. aureus* biofilm-producing strain was more sensitive to IAA exposure; however, biofilm formation by this strain was not affected by IAA. Biofilm production was assessed by the bright blue colour resulting from the interaction between the crystal violet dye and microorganisms adhered to the culture plate. In the present study, a similar colour was observed for the *S. aureus* biofilm-producing strain in the absence and presence of IAA or IAA/HRP (data not shown); this parameter was quantified as an OD of 0.34 ± 0.03 for microorganism alone and as 0.44 ± 0.03 and 0.33 ± 0.03 for microorganism exposed to IAA (30 mM) in the absence and presence of HRP, respectively. In contrast, the control strain showed OD values of 0.03 ± 0.01, 0.03 ± 0.01 and 0.03 ± 0.01, respectively, for the strain incubated alone and in the presence of IAA or IAA/HRP. Values are expressed as mean and standard deviation for six different experiments (n = 6).

IAA Action on Membrane Integrity and Antioxidant Enzyme Activity

S. aureus exposed to IAA and IAA/HRP had reduced membrane integrity (**Table 2**). IAA alone increased damage 1.7 and 2.5 times for the biofilm and control strains, respectively, compared to microorganism alone. However, IAA/HRP treatment increased membrane damage for all strains compared to microorganism alone. Membrane damage was increased 4.0, 3.4, 14.8 and 4.4 times compared to microorganism alone for biofilm, SEB, mecA and control strains, respectively. The presence of HRP potentiated the action of IAA on membrane damage 1.8 times for biofilm, 1.6 times for SEB and 3.6 times for mecA strains. For the control strain, no significant difference was found between IAA and IAA/HRP.

Table 2. Relative percentage of membrane damage from *S. aureus* biofilm producer (biofilm), *seb*-gene presence (SEB), mecA presence (mecA) and control stain (no biofilm produce and absence of *seb* gene and mecA gene). (-) absence of IAA or HRP and (+) presence of IAA or HRP

IAA	HRP	Membrane damage (%)			
		biofilm	SEB	mecA	control
-	-	6.60±1.50^{a}	2.19 ± 0.50^{a}	2.20 ± 0.60^{a}	3.90 ± 1.30^{a}
+	-	17.9± 1.6^{b}	6.10 ± 0.60^{a}	9.70 ± 1.00^{a}	13.5 ± 2.8^{b}
+	+	32.7 ± 2.6^{c}	9.70 ± 1.00^{b}	34.7± 4.1^{b}	19.9 ± 2.5^{b}

Relative percentage of membrane damage calculated in relation to total microorganism (above 1000 cells). Different lowercase letters represent significant difference in the same row (P≤0.05). The values are presented as the mean and standard deviation from six experiments (n=6). IAA = 30 mM to biofilm and SEB stains; IAA = 40 mM to mecA and negative stains. HRP = 1 μM for all tests

An increase in antioxidant enzyme activity was seen for microorganism in presence of IAA and IAA/HRP (**Table 3**). IAA alone showed a significant increase of CAT activity, 1.3, 5.2 and 1.9 times for SEB, mecA and control strains, respectively, compared to microorganism alone. The presence of HRP did not alter the effect of IAA on CAT activity for SEB, mecA and control strains. For the biofilm strain, no alteration in CAT activity was seen for IAA or IAA/HRP. Microorganism alone, incubated in the absence of IAA or IAA/HRP, showed CAT activity for all strains; however, the *S. aureus* biofilm-producing strain had greater CAT activity than the other strains.

SOD activity increased 1.6, 0.8, 0.9 and 0.7 times for biofilm, SEB, mecA and control strains, respectively, incubated in the presence of IAA alone compared to microorganism alone. However, similar values were found for microorganism incubated in the presence of IAA/HRP. All strains showed SOD activity in the absence of IAA or IAA/HRP.

Table 3. Enzyme activity of catalase (CAT) and superoxide dismutase (SOD) from *S. aureus* biofilm producer (biofilm), *seb* gene presence (SEB), *mec*A presence (mecA) and control stain (no biofilm produce and absence of *seb* gene and *mec*A gene) evaluated in absence of IAA (-), presence of IAA (+) and presence of HRP (+)

	IAA	HRP	Enzyme activity			
			biofilm	SEB	mecA	control
CAT	-	-	1.04 ± 0.08[a]	0.165±0.020[a]	0.170 ± 0.010[a]	0.190 ± 0.020[a]
	+	-	1.07 ± 0.10[a]	0.540 ± 0.060[b]	1.05 ± 0.08[b]	0.550 ±0.040[b]
	+	+	0.91 ± 0.08[a]	0.390 ± 0.040[b]	1.08 ± 0.03[b]	0.530 ± 0.040[b]
SOD	-	-	103.2 ± 8.2[a]	146.6 ± 8.2[a]	148.5±10.4[a]	113.6± 5.6[a]
	+	-	226.8 ± 12.2[b]	218.9±18.4[b]	289.2±26.5[b]	193.8±18.7[b]
	+	+	268.1±31.8[b]	262.9±24.3[b]	340.2±53.6[b]	225.0±35.6[b]

Different letters in the same row represent significant difference (P≤0.05) for each enzyme. The values are presented as the mean and standard error from nine experiments (n=9). CAT is expressed as mmol/min per mg of protein and SOD is expressed as U/mg of protein. IAA = 30 mM to biofilm and SEB, IAA = 40 mM to mecA and control stain. HRP = 1 μM for all tests

DISCUSSION

Antimicrobial agents from natural compounds have been studied as alternative therapies due to increasing microbial resistance to current therapies. Cunha et al. (2015) observed that reduction of *P. zopfii* viability was related to IAA concentration. An in vitro study showed antimicrobial activity of auxin on *S. aureus* and other microorganisms [6].

Similar to IAA molecules, indole-3-carbinol and 3-methylene-2-oxindole have a toxic action on prokaryotic and eukaryotic cells [10, 33]. Indole-3-carbinol has antimicrobial activity in vitro by disrupting the structure that makes up the cell membrane in various species of microorganism such as the Gram-positive bacteria *S. aureus*, *S. epidermidis* and *Enterococcus faecium*; Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*; and fungi such as *Saccharomyces cerevisiae*, *Trichosporon beigelii* and *Candida albicans* [34].

In the present study, the results show that IAA alone or combined with HRP reduces *S. aureus* growth for strains with different virulence characteristics; a subinhibitory IAA concentration reduced membrane integrity and increased antioxidant enzyme activity (CAT and SOD), but no DNA fragmentation was seen. These results corroborate those of other studies in which a drastic reduction of *P. zopfii* growth by 1 mM IAA associated with 1 μM HRP was observed without, however, changing DNA integrity [9]. Similar results were found by Pugine et al. (2010) for *S. aureus* from subclinical bovine mastitis exposed to the same concentrations of IAA and HRP. Others studies show IAA as antifungal on *Aspergilus fumigates*, *Candida albicans* and *Rhizopusoryzae* [35].

Antioxidant enzymes such as CAT and SOD have been shown to be sensitive indicators of increased oxidative stress, and increased activity of these antioxidant enzymes is known to serve as a protective response to eliminate ROS [27, 36]. In the present study, the inhibition of *S. aureus* growth by exposure to IAA or IAA/HRP may be due to prooxidant action. In fact, the IAA/HRP system has been explained by several authors as leading to an increase in formation of ROS which present themselves as potent cytotoxic radicals [11, 26, 33]. Studies show that reactive cytotoxic species can cause irreversible damage to molecular microorganism constituents or even destroy them [37]. Furthermore, exogenous microbial metabolites have been shown to reduce CAT activity in *S. aureus*, and the authors suggest that CAT activity depletion could be related to the mechanism of cell death [38, 39]. Moderate oxidative stress conditions increase the antioxidant activity of the enzymes; however, in extreme oxidizing conditions, enzymatic activity is impaired [13]. In the present study, an increase in antioxidant enzyme activity was observed in *S. aureus* exposed to exogenous substances.

For the biofilm-producing strain, CAT activity was not changed by the presence of IAA or IAA/HRP, which seems to be related to the high basal activity of CAT in the *S. aureus* biofilm-producing strain, suggesting that oxidative stress produced by these exogenous substances does not result in increased CAT activity. Even so, the deleterious effects of IAA and IAA/HRP on the *S. aureus* biofilm-producing strain were detected by changes in bacterial growth and by membrane damage.

OD values greater than 0.240 classify the biofilm-producing microorganism as strongly adherent, and OD values smaller than 0.120 classify it as nonadherent [28]. Our results show that the strong adherence of the *S. aureus* biofilm-producing strain was not affected by exposure to IAA or IAA/HRP (respective OD of 0.34 and 0.44). The physiological characteristics of biofilm organisms and biofilm structure can confer inherent resistance to antimicrobial agents [40]. However, in the present study, IAA alone or combined with HRP induced membrane damage and inhibited bacterial growth of the *S. aureus* biofilm-producing strain without affecting biofilm formation.

The spectrophotometric technique employed in the present study to estimate the bacteria number related to OD (625 nm) value is widely used in microbiology studies [3-10, 18]. Recently, OD (570 nm) value produced by a dye

incorporated into bacteria has been used to determine biofilm formation by same microorganisms [14, 15, 28]. In present study, OD determination was shown to be efficient to evaluate the action of IAA and IAA/HRP on the *S. aureus* growth; as well as these compounds action on biofilm formation.

CONCLUSION

Our studies show that IAA alone or combined with HRP has microbicide action on *S. aureus* with different virulence factors; probably involving reactive oxygen species by increasing of antioxidant enzymes activities. The membrane damage of *S. aureus* shows a possibility for growth inhibition this microorganism in presence of IAA. These findings can contribute to the understanding of bacterial cell death, which may be useful for the discovery of new bactericidal and bacteriostatic products.

ACKNOWLEDGMENT

The authors thank the Oswaldo Cruz Foundation/Brazil for the S. aureus strains donation. This research was financed by FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) (grant: 2008/10412-6) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for PhD scholarship to first author.

REFERENCES

1. Chessa D, Ganau G, Mazzarello V. An overview of Staphylococcus epidermidis and Staphylococcus aureus with a focus on developing countries. Journal of infection in developing countries. 2015;9(6):547–550. https://doi.org/10.3855/jidc.6923
2. Barsoumian AE, Mende K, Sanchez CJ, Beckius ML, Wenke JC, Murray CK, Akers KS. Clinical infectious outcomes associated with biofilm-related bacterial infections: a retrospective chart review. BMC Infectious Diseases. 2015;15(1):223. https://doi.org/10.1186/s12879-015-0972-2
3. Schelin J, Wallin-Carlquist N, Cohn MT, Lindqvist R, Barker GC, Rådström P. The formation of Staphylococcus aureus enterotoxin in food environments and advances in risk assessment. Virulence. 2011;2(6):580–592. https://doi.org/10.4161/viru.2.6.18122
4. Purrello SM, Garau J, Giamarellos E, Mazzei T, Pea F, Soriano A, Stefani S. Methicillin-resistant Staphylococcus aureus infections: A review of the currently available treatment options. Journal of Global Antimicrobial Resistance. 2016;7(4): 178–186. https://doi.org/10.1016/j.jgar.2016.07.010
5. Oliveira CJB, Tiao N, de Sousa FGC, de Moura JFP, Santos Filho L, Gebreyes WA. Methicillin-Resistant Staphylococcus aureus from Brazilian Dairy Farms and Identification of Novel Sequence Types. Zoonoses and public health. 2016;63(2): 97–105. https://doi.org/10.1111/zph.12209
6. Lee JH, Cho MH, Lee J. 3-Indolylacetonitrile Decreases Escherichia coli O157:H7 Biofilm Formation and Pseudomonas aeruginosa Virulence. Environmental Microbiology. 2011;13(1):62–73. https://doi.org/10.1111/j.1462-2920.2010.02308.x
7. Minvielle,MJ Eguren K, Melander C. Highly Active Modulators of Indole Signaling Alter Pathogenic Behaviors in Gram-Negative and Gram-Positive Bacteria. Chemistry-A European Journal. 2013;19(51):17595-17602. https://doi.org/10.1002/chem.201303510
8. Pugine SMP, Piza T, Costa E, De Melo MP. Toxicity of indole-3-acetic acid combined with horseradish peroxidase on Staphylococcus aureus. webmedcentral, 2010;1(9): WMC00695.
9. Cunha LT, Pugine SMP, Silva MRM, Costa EJX, De Melo MP. Microbicidal action of indole-3-acetic acid combined with horseradish peroxidase on Prototheca zopfii from bovine mastitis. Mycopathologia. 2010;169(2):99–105. https://doi.org/10.1007/s11046-009-9234-4
10. Cunha LT, Pugine SMP, Lins PG, Brunetti IL, De Melo MP. Induction of Oxidative Stress in Prototheca zopfii by Indole-3-Acetic Acid/HRP or 2,4-Pentanedione/HRP Systems and Their Oxidation Products. Mycopathologia. 2015;179(1–2):73–79. https://doi.org/10.1007/s11046-014-9807-8
11. Kawano T, Kawano N, Hosoya H, Lapeyrie F. Fungal auxin antagonist hypaphorine competitively inhibits indole-3-acetic acid-dependent superoxide generation by horseradish peroxidase. Biochemical and biophysical research communications. 2001;288(3):546–551. https://doi.org/10.1006/bbrc.2001.5800
12. Vatansever F, de Melo WCMA, Avci P, Vecchio D, Sadasivam M, Gupta A, Chandran R, Karimi M, Parizotto NA, Yin R, Tegos GP, Hamblin MR. Antimicrobial strategies centered around reactive oxygen species - bactericidal antibiotics, photodynamic therapy, and beyond. FEMS Microbiology Reviews. 2013;37(6):955–989. https://doi.org/10.1111/1574-6976.12026
13. Mourão LRMB, Santana RSS, Paulo LM, Pugine SMP, Chaible LM, Fukumasu H, Dagli MLZ, De Melo MP. Protective action of indole-3-acetic acid on induced hepatocarcinoma in mice. Cell Biochemistry and Function. 2009;27(1):16–22. https://doi.org/10.1002/cbf.1528

14. McLaughlin RA, Hoogewerf AJ. Interleukin-1β-induced growth enhancement of Staphylococcus aureus occurs in biofilm but not planktonic cultures. Microbial Pathogenesis. 2006;41(2–3):67–79. https://doi.org/10.1016/j.micpath.2006.04.005
15. Toté K, Vanden Berghe D, Maes L, Cos P. A new colorimetric microtitre model for the detection of Staphylococcus aureus biofilms. Letters in Applied Microbiology. 2008;46(2):249–254. https://doi.org/10.1111/j.1472-765X.2007.02298.x
16. Sergeev N, Volokhov D, Chizhikov V, Rasooly A. Simultaneous Analysis of Multiple Staphylococcal Enterotoxin Genes by an Oligonucleotide Microarray Assay. Journal of Clinical Microbiology. 2004;42(5):2134–2143. https://doi.org/10.1128/JCM.42.5.2134-2143.2004
17. Kamboj DV, Nema V, Pandey AK, Goel AK, Singh L. Heterologous expression of staphylococcal enterotoxin B (seb) gene for antibody production. Electronic Journal of Biotechnology. 2006;9(5):551–558. https://doi.org/10.2225/vol9-issue5-fulltext-8
18. Nema V, Agrawal R, Kamboj DV, Goel AK, Singh L. Isolation and characterization of heat resistant enterotoxigenic Staphylococcus aureus from a food poisoning outbreak in Indian subcontinent. International Journal of Food Microbiology. 2007;117(1):29–35. https://doi.org/10.1016/j.ijfoodmicro.2007.01.015
19. Pocsfalvi G, Cacace G, Cuccurullo M, Serluca G, Sorrentino A, Schlosser G, Blaiotta G, Malorni A. Proteomic analysis of exoproteins expressed by enterotoxigenic Staphylococcus aureus strains. Proteomics. 2008;8(12):2462–2476. https://doi.org/10.1002/pmic.200700965
20. Qiu J, Wang D, Xiang H, Feng H, Jiang Y, Xia L, Dong J, Lu J, Yu L, Deng X. Subinhibitory Concentrations of Thymol Reduce Enterotoxins A and B and α-Hemolysin Production in Staphylococcus aureus Isolates. Plos One. 2010;5(3):1–7. https://doi.org/10.1371/journal.pone.0009736
21. Reischl U, Linde HJ, Metz M, Leppmeier B, Lehn N. Rapid identification of methicillin-resistant Staphylococcus aureus and simultaneous species confirmation using real-time fluorescence PCR. Journal of Clinical Microbiology. 2000;38(6):2429–2433.
22. Almer LS, Shortridge VD, Nilius AM, Beyer JM, Soni NB, Bui MH, Stone GG, Flamm RK. Antimicrobial susceptibility and molecular characterization of community-acquired methicillin-resistant Staphylococcus aureus. Diagnostic Microbiology and Infectious Disease. 2002;43(3):225–232. https://doi.org/10.1016/S0732-8893(02)00394-2
23. Palazzo ICV, Rehder A, Darini ALC. Quantitative disk diffusion as a convenient method for determining minimum inhibitory concentrations of oxacillin for staphylococci strains. Journal of Microbiological Methods. 2007;71(3):186–190. https://doi.org/10.1016/j.mimet.2007.08.014
24. Skov R, Smyth R, Yusof A, Karlsson A, Mills K, Frimodt-Moller N, Kahlmeter G. Effects of temperature on the detection of methicillin resistance in Staphylococcus aureus using cefoxitin disc diffusion testing with Iso-Sensitest agar. Journal of Antimicrobial Chemotherapy. 2009;63(4):699–703. https://doi.org/10.1093/jac/dkp019
25. Saiful AJ, Mastura M, Zarizal S, Mazurah MI, Shuhaimi M, Ali A M (2006). Detection of methicillin-resistant Staphylococcus aureus using mecA/nuc genes and antibiotic susceptibility profile of Malaysian clinical isolates. World Journal of Microbiology and Biotechnology. 2006;22(12):1289–1294. https://doi.org/10.1007/s11274-006-9174-9
26. De Melo MP, De Lima TM, Pithon-Curi TC, Curi R. The mechanism of indole acetic acid cytotoxicity. Toxicology Letters. 2004;148(1–2):103–111. https://doi.org/10.1016/j.toxlet.2003.12.067
27. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. 2007;42(4):321–324. https://doi.org/10.1016/j.ymeth.2007.01.006
28. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, Beachey EH. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. Journal of clinical microbiology. 1985;22(6):996–1006.
29. Sträuber H, Müller S. Viability states of bacteria-Specific mechanisms of selected probes. Cytometry Part A. 2010;77(7):623–634. https://doi.org/10.1002/cyto.a.20920
30. Beers RF, Sizer JW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. The Journal of Biological Chemistry. 1952;195(1):133–140.
31. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 1971;44(1):276–287. https://doi.org/10.1016/0003-2697(71)90370-8
32. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72(1–2):248–254. https://doi.org/10.1016/0003-2697(76)90527-3
33. Folkes LK, Wardman P. Oxidative activation of indole-3-acetic acids to cytotoxic species - A potential new role for plant auxins in cancer therapy. Biochemical Pharmacology. 2001;61(2):129–136. https://doi.org/10.1016/S0006-2952(00)00498-6

34. Sung WS, Lee DG. In vitro antimicrobial activity and the mode of action of indole-3-carbinol against human pathogenic microorganisms. Biological & pharmaceutical bulletin. 2007;30(10):1865–9. https://doi.org/10.1248/bpb.30.1865
35. Chitra G, Franklin DS, Sudarsan S, Sakthivel M, Guhanathan S. Indole-3-acetic acid/diol based pH-sensitive biological macromolecule for antibacterial, antifungal and antioxidant applications. International Journal of Biological Macromolecules. 2017;95(2), 363-375.
36. Reiter RJ, Tan DX, Terron MP, Flores LJ, Czarnocki Z. Melatonin and its metabolites: New findings regarding their production and their radical scavenging actions. Acta Biochimica Polonica 2007;54(1):1–9.
37. Li N, Gao C, Peng X, Wang W, Luo M, Fu Y, Zu Y. Aspidin BB, a phloroglucinol derivative, exerts its antibacterial activity against Staphylococcus aureus by inducing the generation of reactive oxygen species. Research in Microbiology. 2014;165(4), 263–272. https://doi.org/10.1016/j.resmic.2014.03.002
38. Bukharin OV, Sgibnev AV, Cherkasov SV, Ivanov IB. The effect of the intra- and extracellular metabolites of microorganisms isolated from various ecotopes on the catalase activity of Staphylococcus aureus 6538P. Microbiology. 2002;71(2):183–186. https://doi.org/10.1023/A:1015185818509
39. Bartolomeu M, Rocha S, Cunha Â, Neves MGPMS, Faustino MAF, Almeida A. Effect of Photodynamic Therapy on the Virulence Factors of Staphylococcus aureus. Frontiers in Microbiology. 2016;7(3):1–11. https://doi.org/10.3389/fmicb.2016.00267
40. Donlan RM, Costerton JW, Donlan RM, Costerton JW. Biofilms : Survival Mechanisms of Clinically Relevant Microorganisms. Clinical Microbiology Reviews. 2002;15(2):167–193. https://doi.org/10.1128/CMR.15.2.167-193.2002