Probiotics and Antimicrobial Proteins (2022) 14:613–619
https://doi.org/10.1007/s12602-022-09954-0

Anti-adhesion and Anti-inflammatory Potential of the Leaderless Class IIb Bacteriocin Enterocin DD14

Radja Teiar[1] · Adrián Pérez-Ramos[1] · Hassan Zgheib[1] · Benoit Cudennec[1] · Yanath Belguesmia[1] · Djamel Drider[1]

Accepted: 12 May 2022 / Published online: 23 May 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract

In this study, we investigate the interactions between the leaderless class IIb bacteriocin, enterocin DD14 (EntDD14), or the methicillin or the combination of these antibacterials, and two methicillin-resistant *Staphylococcus aureus* strains (MRSA-S1 and USA 300) which are respectively a clinical strain and a reference strain. The results obtained showed that EntDD14 alone or in combination with the antibiotic could significantly prevent the adhesion of these pathogenic bacteria to human cells. On the other hand, we investigated the anti-inflammatory effect of EntDD14 on the secretion of pro-inflammatory interleukins, including IL-6 and IL-8. The results show that EntDD14 is able to decrease significantly the secretion of both interleukins on Caco-2 cells following their treatments with lipopolysaccharides. These novel data provide insightful informations to support applications of bacteriocins as therapeutic agents capable as well to defeat pathogenic bacteria and concomitantly limit their inflammatory reactions.

Keywords Leaderless class IIb bacteriocin enterocin DD14 · Methicillin-resistant *Staphylococcus aureus* · Synergy · Human cell line · Anti-adhesion

Introduction

Bacteriocins are antimicrobial peptides produced by unicellular organisms, such as bacteria and Archaea [1, 2]. In bacteria, bacteriocins are produced by both Gram-negative and Gram-positive bacteria [1]. Currently, there are more bacteriocins produced by Gram-positive bacteria than by Gram-negative bacteria [3]. Bacteriocins produced by Gram-positive bacteria, such as those produced by lactic acid bacteria (LAB), act by membrane pore forming [4] or on particular targets such as lipid II, which is an intermediate of peptidoglycan [5]. Lipid II is also known to be the target of antibiotics of the glycopeptide family. Bacteriocins produced by LAB, designed therefore LAB-bacteriocins, continue to be subjected to intensive studies in both academic and applied fields. LAB-bacteriocins have been described to have either a narrow or a broad spectrum of action [6, 7]. Despite the number of bacteriocins so far characterized, no common classification is approved by the scientific community. Indeed, several classifications have been proposed, and these classifications have been updated according to recent advances and achievements. The most recent classification includes two main classes: class I containing ribosomally synthesized and post-translationally modified peptides (RiPPs), and class II containing unmodified bacteriocins [8]. Nevertheless, it would be desirable that the scientific community working on bacteriocins agree on a single universally accepted classification.

Synthesis of bacteriocins is known to take place in the form of a pre-peptide, i.e., an association between a signal peptide and the active molecule. During transport and then externalization to the extracellular environment, the signal peptide is cleaved from the active part, becoming the active bacteriocin [9]. Remarkably, bacteriocins in the form of pre-peptide are not active or only very weakly active in the intracellular cell compartment [10]. Bacteriocins without the signal peptide have been reported and called leaderless bacteriocins. Among these bacteriocins, the enterocin DD14 (Ent DD14), which is produced by *Enterococcus faecalis*

Radja Teiar and Adrián Pérez-Ramos contributed equally to this work

✉ Yanath Belguesmia
yanath.belguesmia@univ-lille.fr

[1] UMR Transfrontalière BioEcoAgro No. 1158, Univ. Lille, INRAE, Univ. Liège, UPJV, YNCREA, Univ. Artois, Univ. Littoral Côte d'Opale, ICV – Institut Charles Viollette, F-59000 Lille, France

14 strain isolated from newborn's meconium, exerts a wide spectrum of antibacterial activities, specially in combination with other molecules including alginate nanoparticules and antibiotics [11–14]. Interestingly, a recent study by Pérez-Ramos et al. [15] showed that EntDD14 requires for its transport and consequently externalization an ABC transport system and noteworthy a transmembrane canal composed of two pleckstrin-homology domain-containing proteins, named DdE and DdF, in a stoichiometric ratio that remains to be determined. According to the same study, the intracellular accumulation of EntDD14 could become lethal to the producing strain [15].

For medical applications, it is essential to investigate other parameters induced by the addition of bacteriocins, such as the effect on the intestinal barrier, the interaction with macrophages, and the immune responses. Bacteriocins appeared to trigger interleukins' secretion and mitigate inflammation [16, 17]. The inflammatory reaction sometimes can lead to unexpected effects like immunity disorders, autoimmune diseases, or anaphylaxis [18, 19]. Infectious agents can cause inflammation, as inert foreign substances, physical agents, or post-traumatic injuries do [20]. Cytokines act as mediators of the inflammatory response [20, 21] and are implied in the recognition, activation, or effector phases of an immune response, modulating the balance between humoral and cell-based immune responses. They also regulate the maturation, the growth, and the functional activities of particular cell populations (T cells, B cells, and myeloid cells) [22, 23]. Of note, infectious pathologies due to viruses were known to induce sometimes "cytokine storm," in severe cases leading to irreversible damages as described for influenza, Ebola, or HCoV virus infection complications [24–26] and even as consequence of herpesviruses infections in some cases [27].

To understand this interaction with the methicillin-resistant *S. aureus* S1 (MRSA-S1) and USA300 clinical strains, we conducted an in situ study using human cell lines and enumerated the MRSA-S1 and USA300 clinical strains using conventional and molecular methods, including qPCR, before concluding with an anti-adhesion effect. In addition, the anti-inflammatory potential of EntDD14 on the secretion and genes expression of interleukins implied in inflammation using in vitro inflamed Caco-2 cells are discussed.

Material and Methods

Bacterial Strains

Enterococcus faecalis 14 isolated from meconium [11] was grown in M17 medium supplemented with 0.5% glucose (Sigma-Aldrich, St Louis, MO, USA), at 37 °C for 16 to 18 h. Methicillin-resistant *S. aureus* USA300 and S1 strains (kindly provided by Dr. Gilles Prévost, Strasbourg University, France) were grown in Brain Heart Infusion (BHI, Sigma-Aldrich, St Louis, MO, USA) broth at 37 °C for appropriate experimental time, as indicated below.

Purification of Enterocin DD14 (EntDD14)

EntDD14 was purified to homogeneity, using a two-step procedure described by Zgheib et al. [14]. *E. faecalis* 14 was grown in 100 mL of M17 broth (Sigma-Aldrich, St Louis, MO, USA), supplemented with 0.5% of glucose and buffered with 60 mM sodium phosphate (pH 6.3), at 37 °C for 24 h. After harvesting by centrifugation (8,000 rpm, 4 °C, 10 min), the cell-free supernatant was incubated 24 h with 160 rpm continuous shaking at room temperature with 3 g of CM Sephadex® C-25 resin (GE Healthcare Life Sciences, Chicago, IL, USA) previously suspended in 30 mL of distilled water and equilibrated for 30 min. The resin was then washed with 5 bed volumes (BV) of distilled water and 1 BV of 0.5 M NaCl. The resin-bound EntDD14 was eluted with 2 BV of 1.5 M NaCl. Salts and reagent used in this experiment were provided by Sigma-Aldrich (St Louis, MO, USA) The removal of the salt from the solution containing EntDD14 was achieved by passing 1 mL of the solution into each column of PD MidiTrap G-10 columns (GE Healthcare Life Sciences, Chicago, IL, USA), following the manufacturer's instructions. The protocol was repeated several times to obtain a sufficient quantity of EntDD14, which was kept at 4 °C for further uses.

Anti-adhesion Activity of EntDD14 Treatments on MRSA Strains to Caco-2 Cells

Briefly, Caco-2 cells seeded at 4.10^4 cells per well were in 24-well tissue culture plate, then incubated Dulbecco Modified Eagle Medium (DMEM, Gibco, Thermofisher, Waltham, MA, USA), supplemented with fetal bovine serum (FBS) and antibiotics (penicillin G and streptomycin) from the same supplier, for 15 days at 37 °C (5% CO_2 and 95% air). *S. aureus* MRSA-S1 and USA300 strains were grown in BHI broth medium for 24 h at 37 °C. Then, the bacterial cells were harvested by centrifugation (8,000 g, 10 min), and resuspended in DMEM, without FBS and antibiotics, to a final concentration of 10^7 CFU/well. Next, caco-2 cells were washed with fresh DMEM without FBS and antibiotics and *S. aureus* strains were added to the wells. After 1 h of incubation at 37 °C (5% CO_2 and 95% air), the treatments with EntDD14, methicillin, and a combination of EntDD14 + methicillin were added to the wells at their minimal inhibitory concentrations (MIC = 0.5 μg/mL for MRSA-S1 and 4 μg/mL for USA300), which were determined by checkerboard assay as described previously [13].

The incubation of Caco-2 cells was subsequently prolonged for additional 2 h in the same conditions. Controls without treatment were also performed. All treatments were made in triplicate. After incubation, each well was washed twice with sterile NaCl (0.9%) to remove non-adherent cells of *S. aureus*. The adherent cells were recovered after incubation with trypsin/EDTA for 10 min. The attached *S. aureus* cells were determined by plate counting method after serial dilution and incubation for 18 h at 37 °C.

Alternatively, counting was also performed by qPCR method. For this purpose, specific primers described by Graber et al. [28] (F: 5′-CCTGAAGCAAGTGCATTTACGA-3′, and R: 5′-CTTTAGCCAAGCCTTGACGAACT-3′) were used and targeted the *S. aureus nuc* gene (SAU) and yielded a 166-bp amplicon. Genomic DNA from adherent *S. aureus* cells pellets was extracted using DNA extraction kit from Macherey Nagel (Düren, Germany). Then, the mix reactions were prepared, as recommended by the manufacturer, using SYBR Green PCR Master Mix (Takyon, Eurogentec, Seraing, Belgium), SAU primers (10 mM), and the extracted DNA from each sample. The thermocycler program consisted of 45 cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s, with an initial cycle of 95 °C for 10 min using CFX Connect TM Real-Time System (Biorad, Hercules, CA, USA). In each cycle, the accumulation of PCR products was detected by monitoring the increase in fluorescence of the fluorophore binding to the double-stranded amplicons generated.

Inflammation of Caco2 Cells and Anti-inflammatory Activity of EntDD14

After the production and purification of EntDD14 (as previously detailed), the enterocin was resuspended in DMEM to study the effect on the inflammation of Caco-2 cells. The Caco-2 cells were grown at 37 °C under 5% CO_2 atmosphere into 96-well tissue culture plates at a density of 15,000 cells/well for 7 days. The culture medium was changed every two days to maintain optimal conditions for cell growth. Then obtained Caco-2 cells monolayers were pre-stimulated with lipopolysaccharide (LPS) from *E. coli* (Sigma-Aldrich, St Louis, MO, USA) (50 µg/mL) for 36 h. Then, the EntDD14 was added, in triplicate, at two different concentrations (60 µg/mL and 240 µg/mL) which were previously tested [13, 14], dexamethasone (20 mM) (Sigma-Aldrich, St Louis, MO, USA) as positive control and DMEM containing LPS (50 µg/mL) as negative control. The plate was then incubated overnight at 37 °C under 5% CO_2 atmosphere.

The next day, the supernatant of each well was removed, and a selection of thirteen interleukins and inflammation markers (GM-CSF, IFNγ, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, and TNFα) were quantified using a Milliplex® HSTCMAG-28SPMX13 kit (Merck, Darmstadt, Germany). Additional complementary dosage for IL-6 using a High Sensitive ELISA kit (Bio-Techne, Minneapolis, MN, USA) was also performed. Both assays were realized according to the kit manufacturer's recommendations.

Statistical Analysis

In the Caco-2 cell adhesion assays of the *S. aureus* strains, the data are expressed as mean ± SD calculated from three independent experiments ($N = 3$). In the Caco-2 cell inflammation assays, the data are expressed as mean ± SD calculated from three independent repetitions ($n = 3$) from one experiment ($N = 1$). The data were subjected to the Shapiro–Wilk test to evaluate the normality distribution of the data. This test gave a p-value < 0.05, indicating a no normal distributed data. Consequently, the Wilcoxon method was employed to determine the significant differences between the variables at p-value < 0.05. The statistical analysis was performed using the SAS software (https://www.sas.com).

Results

EntDD14 Impedes the Adhesion of MRSA Strains to Caco-2 Cells

The results of plate counting of *S. aureus* colonies showed that the samples treated with EntDD14 alone or in combination with methicillin were exempt from any visible growth though the development of colonies, advocating thereof any viable cells in these samples. However, as depicted in Fig. 1A, the control and the methicillin-treated samples exhibited a number of colonies reaching more than 3 Log10 CFU/mL for MRSA-S1 or USA300 strains. Interestingly, treatments with methicillin did not affect the adhesion abilities of MRSA-S1 and USA300 to differentiated Caco-2 cells. Regarding the results obtained in *SAU* gene detection by qPCR, we revealed that EntDD14 impacted strongly the adherent populations of MRSA-S1 and USA300 to Caco-2 cells, by reducing the *SAU* copy numbers by at least two folds (Fig. 1B). On the other hand, but interestingly, methicillin enabled similar effects as EntDD14 with a substantial, but not statistically different, reduction of the number of *SAU* genes in this sample (Fig. 1B).

Interleukin Secretion by Caco-2 Cells Treated with EntDD14

Among all the interleukins tested, we observed that only IL-6 and IL-8 were significantly reduced after inducing inflammation in Caco-2 cells with LPS (50 µg/mL) and treated with EntDD14. Caco-2 cells inflamed with LPS (LC control)

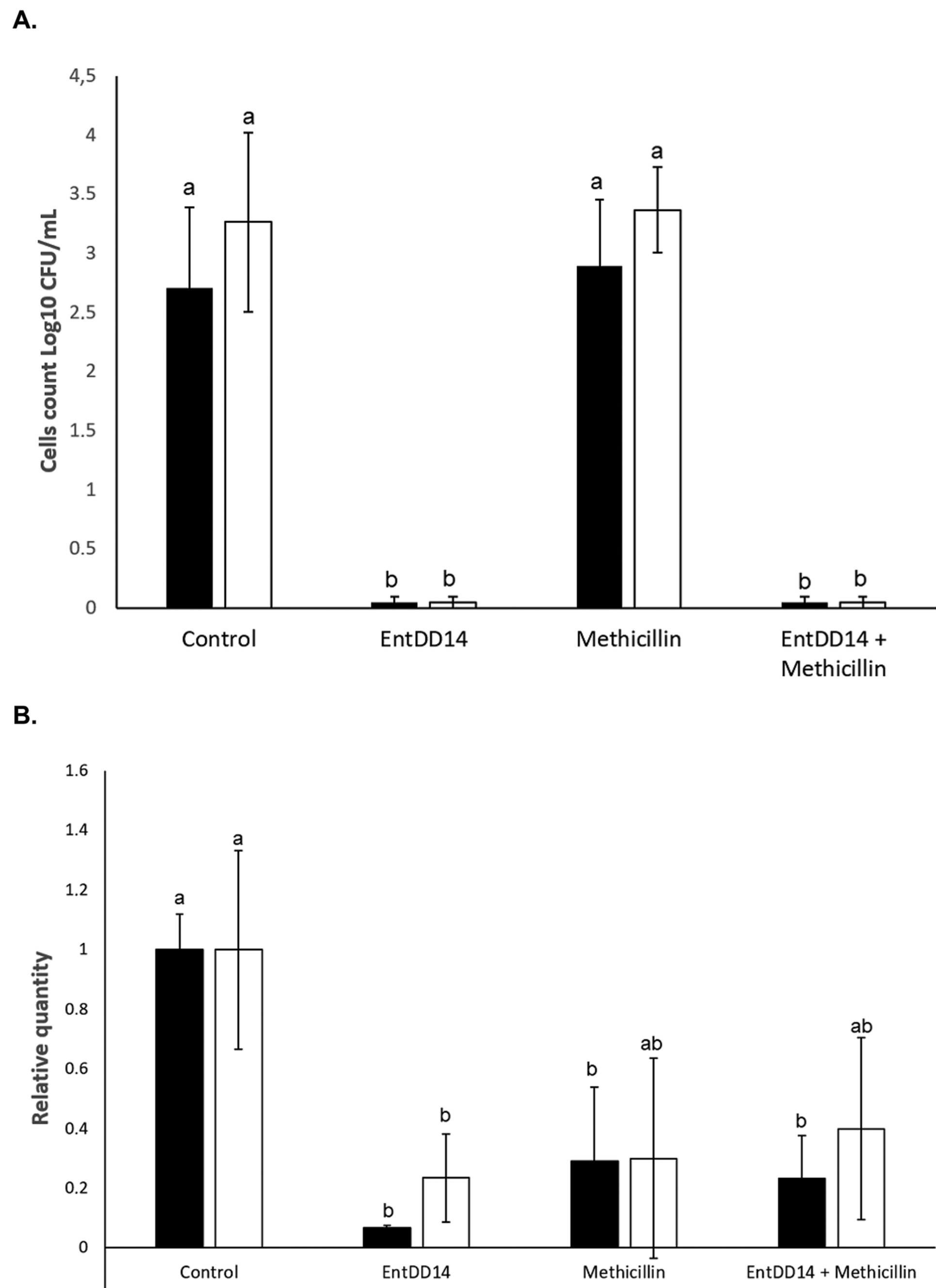

Fig. 1 Quantification of adherent MRSA-S1 "white bars" and USA300 "black bars" populations on Caco-2 cells without treatment and treated with EntDD14, methicillin and EntDD14 + methicillin combination at their calculated MICs, evaluated by (**A**) plate counting on BHI agar expressed in Log_{10} CFU/mL and by (**B**) relative quantity in comparison to untreated control and evaluated by qPCR of a specific gene to *S. aureus* (*SAU* gene). Values are means ± SD, and those without a common letter are significantly different according to statistical analysis ($p < 0.05$)

secreted 2.5 pg/mL of IL-6, whereas it was not detectable in non-inflamed Caco-2 cells (data not shown). It should be noted that the decrease of secreted IL-6 induced by treatments with EntDD14 at 60 and 240 µg/mL was so important that it reached the detection thresholds of the Milliplex assay (data not shown). Results of IL-6 quantification by High Sensitive ELISA assay confirmed the previous results with a significant decrease after treatment with EntDD14 at both concentrations used (1 ×: 60 µg/mL and 4 ×: 240 µg/mL) from 2.4 pg/mL to less than 0.5 pg/mL (Fig. 2). Interestingly, no dose–response was observed with the two concentrations of EntDD14 used (60 and 240 µg/mL), which correspond to one and fourfolds the minimal inhibitory concentration (MIC) toward *Clostridium perfringens* (data not shown).

Regarding IL-8, all the different treatments induced a significant decrease of IL-8 secretion by Caco-2 cells by at least two to threefold the amount obtained with LC control (138.85 pg/mL) (Fig. 3).

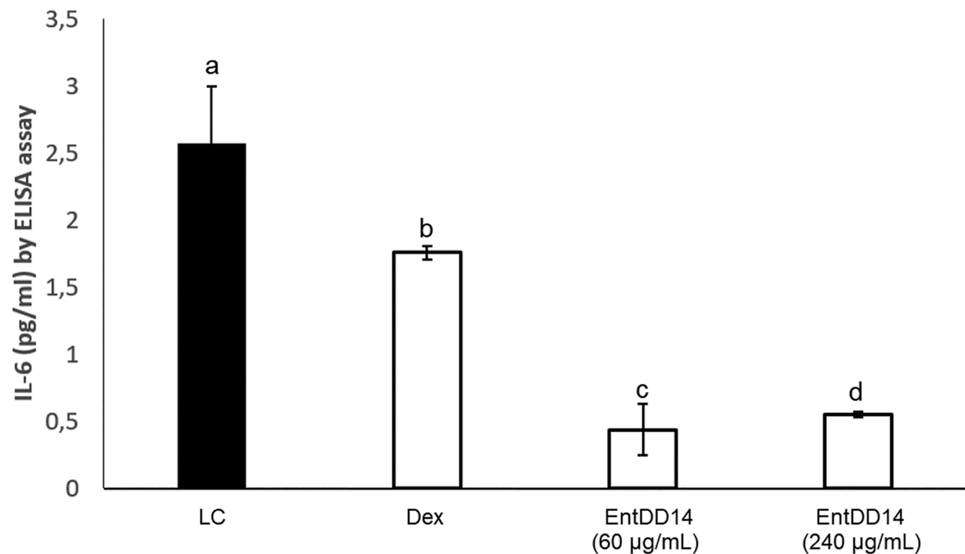

Fig. 2 Quantification of IL-6 cytokine (pg/mL) in the supernatant of LPS-inflamed Caco-2 cultures treated with dexamethasone (Dex), EntDD14 at 60 µg/mL (1×) and 240 µg/mL (4×) by High Sensitivity ELISA assay, in comparison to untreated LPS-inflamed Caco-2 cultures (LC) "black bar". Values are means ± SD, and those without a common letter are significantly different ($p < 0.05$)

Discussion

The adhesion of pathogenic bacteria to the biological surfaces in their host's tissues is a first but a key step before launching the infectious process [29]; a complex process that begins with the recognition of the target areas and ends by the attachment of the pathogens to the tissue [30].

This first step allows the installation of microorganisms, then initiates the invasion process and then the expression of their pathogenesis and virulence backgrounds [29, 30]. The adhesion is a possible target for therapeutic strategies to hamper the effects of pathogenic bacterial strains [31, 32]. Different studies conducted in our laboratory unveiled the potential therapeutic of EntDD14. Indeed, we reported its ability to potentiate in vitro antibiotics such as kanamycin and erythromycin [33] or methicillin [13] against MRSA-S1 or USA300. These studies were supported by new data obtained in vivo in mouse models [34]. It should be noted that EntDD14 is not active against the MRSA-S1 clinical strain [33], but its combination with antibiotics has clearly shown in vitro synergistic effects [13, 33]. Furthermore, we have established that such synergistic formulation allowed cytological protection of the colon, spleen, and liver and the stability of mice's intestinal microbiota following their challenge with MRSA-S1 [34]. Of note, we established that the EntDD14 could exert an anti-adhesive activity on MRSA strains on inert surface like polystyrene [13], and we confirm here its ability of EntDD14 to inhibit the adhesion to Caco-2

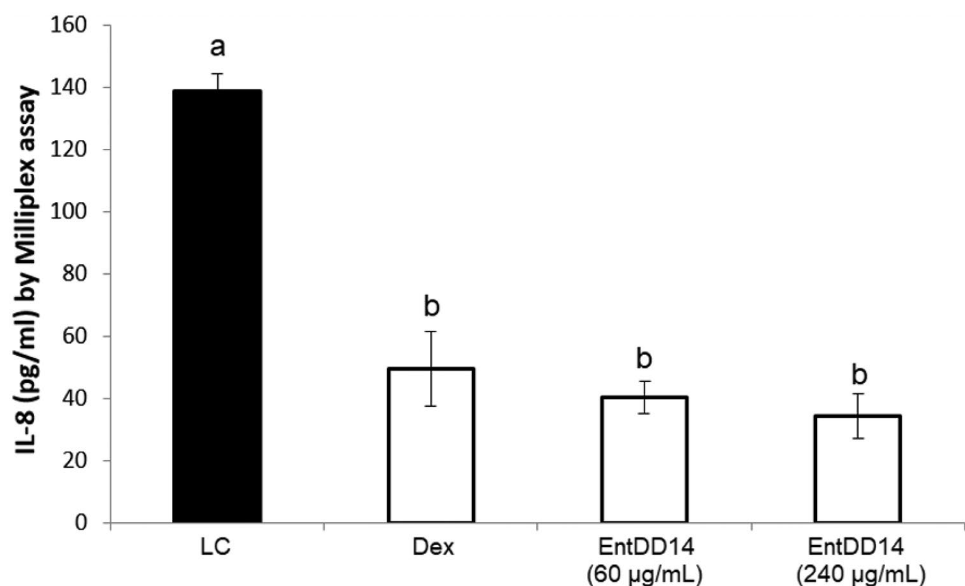

Fig. 3 Quantification of IL-8 cytokine (pg/mL) in the supernatant of LPS-inflamed Caco-2 cultures treated with dexamethasone (Dex), EntDD14 at 60 µg/mL (1×) and 240 µg/mL (4×) by Milliplex assay, in comparison to untreated LPS-inflamed Caco-2 cultures (LC) "black bar". Values are means ± SD, and those without a common letter are significantly different ($p < 0.05$)

cells of MRSA-S1 strain and even that of USA300 (Fig. 1A, B) strain, which is known for its strong adhesion phenotype. These data can strengthen and help to better understand those obtained in vivo when challenging mice with harmful MRSA-S1 [34]. Taken all together, EntDD14 could be humbly considered as an interesting model of bacteriocin that deserves to be further studied from the perspective of medical application as an alternative to replace ageing antibiotics.

In addition to its ability to prevent the adhesion of pathogenic strains to the Caco-2 cell line, we showed in this study that EntDD14 was able to reduce significantly the secretion of IL-6 and IL-8, pro-inflammatory interleukins, by LPS-inflamed Caco-2 cell line (Figs. 2 and 3). Noteworthy, the decrease of these interleukins noted upon adjunction of EntDD14 is either similar or higher in the case of IL-6, than that obtained with the dexamethasone (Fig. 2). These results are particularly interesting because they extend those previously obtained on other classes of bacteriocins and probiotics. Related to that, Yin et al. [16] established the class IIb plantaricin EF's role in reducing colonic tumor necrosis factor-alpha (TNF-α) and IL-6 levels in mice fed with *Lactobacillus plantarum*, a bacteriocin-producing strain. Other bacteriocins are also endowed with anti-inflammatory activities as they reduce the secretion of pro-inflammatory interleukins induced by pathogens. This is the case of plantaricins [17] or nisin [35]. Of note, the IL-6 is produced by different Eukarya cells, including monocytes, fibroblasts, synoviocytes, and osteoblasts… in response to various stimuli like infectious agents or their components [36, 37]. The receptor for IL-6 is expressed on many lymphoid and non-lymphoid cells. IL-6 intervenes by stimulating the hepatocyte production of proteins in the acute phase of inflammation: CRP, SAA, haptoglobin, C3, fibrinogen, a1-antitrypsin, a2-macroglobulin [38, 39]. IL-6 is also involved in the mechanisms of immunity by promoting the differentiation of B lymphocytes into plasma cells, by stimulating the proliferation of T lymphocytes in association with IL-2, and by promoting the generation of cytotoxic T lymphocytes [39–41]. IL-8 is another cytokine implied in acute inflammation and called a chemokine, whose main property is to attract circulating leukocytes to an inflammatory focus [42, 43]. Polymorphonuclear neutrophils are the preferred target of IL-8 via a specific receptor: IL-8-R. IL-8 induces chemotaxis and polymorphonuclear activation with induction of cyclooxygenase, lipooxygenase, and NO-synthase [44–46]. Recently, IL-8 and particularly IL-6 were associated with cytokine storm and recently with COVID-19 disease complications [47, 48].

Conclusion

In the present study, we show the anti-adhesion effect of EntDD14 towards MRSA-S1 and USA300 strains on Caco-2 differentiated cells. This activity was obtained when EntDD14 was tested alone or in combination with methicillin. Furthermore, we establish that EntDD14 also has an anti-inflammatory effect on inflamed Caco-2 cells with LPS. Indeed, the secreted levels of pro-inflammatory IL-6 and IL-8 were significantly decreased in treated Caco-2 cells with EntDD14. These results advocate the potential of EntDD14 as a therapeutic agent. However, the accurate mechanisms impeding adhesion of MRSA strains to Caco-2 cell line upon treatment with EntD14 and its subsequent anti-inflammatory activities constitute our next focus.

Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Drider D, Rebuffat S (2011) Prokaryotic antimicrobial peptides: from genes to applications. Springer-Verlag, New York
2. Hammami R, Zouhir A, Le Lay C et al (2010) BACTIBASE second release: a database and tool platform for bacteriocin characterization. BMC Microbiol 10:22. https://doi.org/10.1186/1471-2180-10-22
3. Zimina M, Babich O, Prosekov A et al (2020) Overview of global trends in classification, methods of preparation and application of bacteriocins. Antibiotics 9:553. https://doi.org/10.3390/antibiotics9090553
4. Kumariya R, Garsa AK, Rajput YS et al (2019) Bacteriocins: classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. Microb Pathog 128:171–177. https://doi.org/10.1016/j.micpath.2019.01.002
5. Bauer R, Dicks LMT (2005) Mode of action of lipid II-targeting lantibiotics. Int J Food Microbiol 101:201–216. https://doi.org/10.1016/j.ijfoodmicro.2004.11.007
6. Mokoena MP (2017) Lactic Acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. Molecules 22. https://doi.org/10.3390/molecules22081255
7. Pérez-Ramos A, Madi-Moussa D, Coucheney F, Drider D (2021) Current knowledge of the mode of action and immunity mechanisms of LAB-bacteriocins. Microorganisms 9:2107. https://doi.org/10.3390/microorganisms9102107
8. Soltani S, Hammami R, Cotter PD et al (2020) Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations. FEMS Microbiol Rev 45:fuaa039. https://doi.org/10.1093/femsre/fuaa039
9. Perez RH, Zendo T, Sonomoto K (2018) Circular and leaderless bacteriocins: biosynthesis, mode of action, applications, and prospects. Front Microbiol 9
10. Simons A, Alhanout K, Duval RE (2020) Bacteriocins, antimicrobial peptides from bacterial origin: overview of their biology and their impact against multidrug-resistant bacteria. Microorganisms 8:639. https://doi.org/10.3390/microorganisms8050639
11. Al Atya AK, Drider-Hadiouche K, Ravallec R et al (2015) Probiotic potential of Enterococcus faecalis strains isolated from meconium. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00227
12. Caly DL, Chevalier M, Flahaut C et al (2017) The safe enterocin DD14 is a leaderless two-peptide bacteriocin with anti-*Clostridium perfringens* activity. Int J Antimicrob Agents 49:282–289. https://doi.org/10.1016/j.ijantimicag.2016.11.016
13. Belguesmia Y, Spano G, Drider D (2021) Potentiating effects of leaderless enterocin DD14 in combination with methicillin on

clinical methicillin-resistant *Staphylococcus aureus* S1 strain. Microbiol Res 252:126864. https://doi.org/10.1016/j.micres.2021.126864

14. Zgheib H, Belguesmia Y, Boukherroub R, Drider D (2021) Alginate nanoparticles enhance anti-*Clostridium perfringens* activity of the leaderless two-peptide enterocin DD14 and affect expression of some virulence factors. Probiotics Antimicrob Proteins. https://doi.org/10.1007/s12602-020-09730-y
15. Pérez-Ramos A, Ladjouzi R, Benachour A, Drider D (2021) Evidence for the involvement of pleckstrin homology domain-containing proteins in the transport of enterocin DD14 (EntDD14); a leaderless two-peptide bacteriocin. Int J Mol Sci 22:12877. https://doi.org/10.3390/ijms222312877
16. Yin X, Heeney D, Srisengfa Y et al (2018) Bacteriocin biosynthesis contributes to the anti-inflammatory capacities of probiotic *Lactobacillus plantarum*. Benef Microbes 9:333–344. https://doi.org/10.3920/BM2017.0096
17. Yoon J-W, Kang S-S (2020) In vitro antibiofilm and anti-inflammatory properties of bacteriocins produced by *Pediococcus acidilactici* against *Enterococcus faecalis*. Foodborne Pathog Dis. https://doi.org/10.1089/fpd.2020.2804
18. Peavy RD, Metcalfe DD (2008) Understanding the mechanisms of anaphylaxis. Curr Opin Allergy Clin Immunol 8:310–315. https://doi.org/10.1097/ACI.0b013e3283036a90
19. Yang L, Wang G, Xia H (2020) Molecular mechanism for impaired suppressive function of Tregs in autoimmune diseases: a summary of cell-intrinsic and cell-extrinsic factors. J Cell Mol Med. https://doi.org/10.1111/jcmm.15743
20. Stone WL, Basit H, Burns B (2020) Pathology, inflammation. In: StatPearls. StatPearls Publishing, Treasure Island (FL)
21. Dinarello CA (2007) Historical review of cytokines. Eur J Immunol 37:S34–S45. https://doi.org/10.1002/eji.200737772
22. Morán GAG, Parra-Medina R, Cardona AG et al (2013) Cytokines, chemokines and growth factors. El Rosario University Press
23. Sivakumar PV, Foster DC, Clegg CH (2004) Interleukin-21 is a T-helper cytokine that regulates humoral immunity and cell-mediated anti-tumour responses. Immunology 112:177–182. https://doi.org/10.1111/j.1365-2567.2004.01886.x
24. Liu Q, Zhou Y, Yang Z (2016) The cytokine storm of severe influenza and development of immunomodulatory therapy. Cell Mol Immunol 13:3–10. https://doi.org/10.1038/cmi.2015.74
25. Wang J, Jiang M, Chen X, Montaner LJ (2020) Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: Review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. J Leukoc Biol. https://doi.org/10.1002/JLB.3COVR0520-272R
26. Younan P, Iampietro M, Nishida A et al (2017) Ebola virus binding to Tim-1 on T lymphocytes induces a cytokine storm. mBio 8. https://doi.org/10.1128/mBio.00845-17
27. Dulek D, Thomsen I (2019) Infectious triggers of cytokine storm syndromes: herpes virus family (non-EBV). In: Cron RQ, Behrens EM (eds) Cytokine Storm Syndrome. Springer International Publishing, Cham, pp 235–252
28. Graber HU, Casey MG, Naskova J et al (2007) Development of a highly sensitive and specific assay to detect *Staphylococcus aureus* in bovine mastitic milk. J Dairy Sci 90:4661–4669. https://doi.org/10.3168/jds.2006-902
29. Pizarro-Cerdá J, Cossart P (2006) Bacterial adhesion and entry into host cells. Cell 124:715–727. https://doi.org/10.1016/j.cell.2006.02.012
30. Josse J, Laurent F, Diot A (2017) Staphylococcal adhesion and host cell invasion: fibronectin-binding and other mechanisms. Front Microbiol 8
31. Krachler AM, Orth K (2013) Targeting the bacteria–host interface. Virulence 4:284–294. https://doi.org/10.4161/viru.24606
32. Asadi A, Razavi S, Talebi M, Gholami M (2019) A review on anti-adhesion therapies of bacterial diseases. Infection 47:13–23. https://doi.org/10.1007/s15010-018-1222-5
33. Al Atya AK, Belguesmia Y, Chataigne G et al (2016) Anti-MRSA activities of enterocins DD28 and DD93 and evidences on their role in the inhibition of biofilm formation. Front Microbiol 7:817. https://doi.org/10.3389/fmicb.2016.00817
34. Bendjeddou K, Hamma-Faradji S, Meddour AA et al (2021) Gut microbiota, body weight and histopathological examinations in experimental infection by methicillin-resistant *Staphylococcus aureus*: antibiotic versus bacteriocin. Benef Microbes 12:295–305. https://doi.org/10.3920/BM2020.0155
35. Małaczewska J, Kaczorek-Łukowska E, Wójcik R et al (2019) In vitro immunomodulatory effect of nisin on porcine leucocytes. J Anim Physiol Anim Nutr (Berl) 103:882–893. https://doi.org/10.1111/jpn.13085
36. Srirangan S, Choy EH (2010) The role of interleukin 6 in the pathophysiology of rheumatoid arthritis. Ther Adv Musculoskelet Dis 2:247–256. https://doi.org/10.1177/1759720X10378372
37. West NR (2019) Coordination of immune-stroma crosstalk by IL-6 family cytokines. Front Immunol 10. https://doi.org/10.3389/fimmu.2019.01093
38. Charlie-Silva I, Klein A, Gomes JMM et al (2019) Acute-phase proteins during inflammatory reaction by bacterial infection: Fish-model. Sci Rep 9:4776. https://doi.org/10.1038/s41598-019-41312-z
39. Tanaka T, Narazaki M, Kishimoto T (2014) IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol 6:a016295. https://doi.org/10.1101/cshperspect.a016295
40. Galandrini R, Cernetti C, Albi N et al (1991) Interleukin-6 is constitutively produced by human CTL clones and is required to maintain their cytolytic function. Cell Immunol 138:11–23. https://doi.org/10.1016/0008-8749(91)90128-x
41. Holsti MA, Raulet DH (1989) IL-6 and IL-1 synergize to stimulate IL-2 production and proliferation of peripheral T cells. J Immunol 143:2514–2519
42. David JM, Dominguez C, Hamilton DH, Palena C (2016) The IL-8/IL-8R axis: a double agent in tumor immune resistance. Vaccines 4:22. https://doi.org/10.3390/vaccines4030022
43. Harada A, Sekido N, Akahoshi T et al (1994) Essential involvement of interleukin-8 (IL-8) in acute inflammation. J Leukoc Biol 56:559–564
44. Fogh K, Larsen CG, Iversen L, Kragballe K (1992) Interleukin-8 stimulates the formation of 15-hydroxy-eicosatetraenoic acid by human neutrophilsin vitro. Agents Actions 35:227–231. https://doi.org/10.1007/BF01997504
45. Nolan S, Dixon R, Norman K et al (2008) Nitric oxide regulates neutrophil migration through microparticle formation. Am J Pathol 172:265–273. https://doi.org/10.2353/ajpath.2008.070069
46. Rossaint J, Nadler JL, Ley K, Zarbock A (2012) Eliminating or blocking 12/15-lipoxygenase reduces neutrophil recruitment in mouse models of acute lung injury. Crit Care 16:R166. https://doi.org/10.1186/cc11518
47. Del Valle DM, Kim-Schulze S, Huang HH et al (2020) An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med 1–8. https://doi.org/10.1038/s41591-020-1051-9
48. Magro G (2020) SARS-CoV-2 and COVID-19: Is interleukin-6 (IL-6) the 'culprit lesion' of ARDS onset? What is there besides Tocilizumab? SGP130Fc. Cytokine: X 2:100029. https://doi.org/10.1016/j.cytox.2020.100029

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.